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Editorial

Dr. Shilin N. Shukla
MD, Hon. Director and Hon. Professor of Medical Oncology, GCRI,
Chairman, Scientific Advisory Committee, GCS

Constantly Changing Every Moment is the Pleasant Beauty

क्षणे क्षणे यन्नावतामुपैति तदेव रुपं रमणीयतायाः ।

Kavi Kalidas was a prominent Sanskrit literary person who defined pleasant beauty as a constant change in form and colour that is seen in the Nature. So should change the 'GCS Research Bulletin' also and metamorphose into the 'Gujarat Cancer Society Research Journal'. One may call the later as the re-incarnation of the former, 13 editions of which have already established the style and standard, both scientific and aesthetic. One step further can be a giant leap into the future. It was, and so shall be the present form, the official mouth-piece and the scientific ambassador of the Gujarat Cancer Society (GCS) and the Gujarat Cancer and Research Institute (GCRI) portraying their research and academic pursuits and efforts.

As a part of lifelong learning after formal education and training, a doctor should continue communicating with other doctors, as such communications promote knowledge and happiness. So said learned Vaidya Charak in the 15th stanza of the chapter called Vijnansthanam in the Charak Samhita, the treaties of medical science written by him.

भिषक् भिषजा सह संभाषयेत ।
तद्विद्य संभाषा हि ज्ञानभियोग संहर्षकरी भवति ॥

And hence, one cannot over emphasize need to create platforms for such communications. Thus one more oncology journal in India will only boost science and scientific deliberations. It will also provide a wider and a louder platform to the scientists and doctors to project their work and views more authentically.

The GCS Research Bulletin and now the Journal represent academic as well as basic, clinical and clinico-basic research carried out by the staff of the GCS and GCRI during the preceding period starting from publication of the last issue. It generally comprises of scientific papers, anecdotal reports, summaries of papers and articles published in various journals and other scientific publications, as well as an account of the work presented in the clinical meetings and the journal clubs. It may include invited review articles and publish oration speeches. It will continue to serve as an academic-research bridge between the basic sciences and the applied sciences, viz. various disciplines of medicine within and outside GCS-GCRI.

What is new? (1) Its content is peer-reviewed. Peer review is a scientific exercise of friendly and benevolent, as well as pure and un-envious eyed, balanced review of need, content and presentation of the research material given for publication:

मित्रस्य चक्षुषा समीक्षामहे ।

(2) The frequency of publication is intended to be increased. (3) Its circulation will be enhanced systematically, initially by wider free distribution. Later on the concept of paid subscription will be introduced to ensure quality, spread and sustainability. (4) The present day scope is to publish the work done at GCS and GCRI during a specified period of time but later on it may be expanded to include former GCRIans' work done at GCRI. There can be more developments but the basic fabric and framework will be scientific and academic activities of the parent organizations. It should be able to promote the cause of oncology in the country and also abroad, simultaneously projecting the work, the workers, the work-place and the warehouse.

Key to success is the authenticity, tenacity, precision and meticulousness in evaluating and accepting material that defines, establishes, maintains and raises the standards as per the changing demands. Boldness in rejections on the basis of merit will determine the level of bar.

Content of this very first issue of the journal explains its quality and expanse. Its range includes wisdom of oration awardees, molecular biology, in-silico studies, diagnostics, therapeutics as well as brain feeders and brain teasers.

On behalf of the GCS and GCRI Governing Committees, the Scientific Advisory Committee and myself, I acknowledge the hard work put in by Dr. Asha Anand, Dr. Pariseema Dave and Dr. Nandita Ghosh in preparing and presenting my idea and ideals in a beautiful way. It is said, 'If it is beautiful, it is complete'. So should be this effort. I wish to congratulate the worthy contributors. The last mention of the untiring support of Shri Bankimbhai Desai and Shri Dharmeshbhai Panchal is to show how dear they and their commitment are to me.

May God bless this effort to fulfill its purpose.

तद्विद्यसंभाषापरिषद्

संभाषाविधिमत ऊर्ध्वंव्याख्यास्याम: । भिषक् भिषजा सह संभाषेत । तद्विद्यसंभाषा हि ज्ञानाभियोगसंहृषकरी भवति । वैशारद्यमपि चाभिनिर्वर्तयति । वचनशक्तिमपि चाधते यशश्चाभिदीपयति । पूर्वश्रुते च संदेहवत: पुन: श्रवणाच्छ्रुतसंशयमपकर्षति । श्रुते चासंदेहवतो भूयोऽध्यवसायमभिनिर्वर्तयति । अश्रुतमपि च कञ्चिदर्थं श्रोत्रविषयमापादयति । यच्चाचार्य: शिष्याय शुश्रूषवे प्रसन्न: क्रमेणोपदिशति । गुह्याभिमतमर्थजातं तत् परस्परेण सह जल्पेन् पिण्डेन विजिगीषुराह संहर्षात् तस्मातद्विद्यसंभाषामभिप्रशंसन्ति कुशला: । - चरकसंहिता, विज्ञानस्थानम् १५॥

Seminar/Symposium/Conference/Scientific Communication

Process of Communication through Conferences: After explaining processes of teaching and learning, the process of communication through science-meetings is defined. A doctor should communicate with other doctor. Communication with the subject expert increases knowledge and it is a pleasant experience. It also improves skills. It develpos communication skills also. It spreads prestige. Such communication removes doubts about subjects which were previousy studied or heard about. It generates determined decision making intelligence. Sometimes one becomes acquainted to previously unheard or not studied (new) topics and subjects. Some of these things can be learnt from an expert teacher by serving hard and pleasing him and finding one's place in his good books only. During scientific meetings, they pleasing their own ego and proving their own point of view, demonstrate their expertise and reveal such knowledge and skills. Therefore, what can be attained and obtained after a lot of efforts can be easily learnt during such science meetings. Hence, the experts and the learned praise such science meetings.

- Charak Samhita, Vijnanasthanam, 15

Shri Madanmohan Ramanlal GCRI Lumanary Award 2011-2012

Devendra D. Patel,

MBBS, MS, FRCS (Edinburgh, London),
Hon. Fellow of College of Surgeons of India
Former Hon. Director, The Gujarat Cancer & Research Institute (GCRI)

Achievements in My Journey Through Life

It is my proud privilege to stand before you to deliver this oration. Such orations are equivalent to life time achievement of person's journey through life. This achieves significant attention if one is on his own on podium for oration in front of his own Parivar. I will deliver this oration in two parts.

The first is about my early life education and hard work to attend certain goals. I was born in Bhadran 15.09.1932. You can decide my age as I speak before you. I passed my SSC in 1949 from Vadodara centre. I had to go through routine examination with fever and illness about 100km from my home. I passed this examination in Grade I. I decided for medical profession and thus proceeded to Ahmedabad in 1951. As my two maternal uncle were residing in Ahmedabad. I went to Ahmedabad in 1951. For the first time as I approached Gujarat College, my mind was like Alice in wonderland. I had no idea what was beyond Gujarat College railway crossing. I passed Inter science in 1951 with first class. Those days Government announced that all those who have more than 60% marks may apply for admission to any of the medical colleges in Bombay province. I opted for B J Medical College, Ahmedabad and started my undergraduate medical education on 15th June 1951, day on which I lost my father at my native place. I passed my MBBS in June 1956 and secured highest marks in surgery and University Gold medal. Post graduate surgical training was done at K M School of Post Graduate Medicine and Research, V.S. Hospital from 1956-59. I proceeded to U.K. in June 1960 by ship for a journey to U.K lasting 16days. I have obtained FRCS of England and Edinburgh in 1964. I then proceeded to Lahey clinic Boston, USA for fellowship in surgery with special reference to cancer surgery. I returned back to Ahmedabad in January 1966 as a pool officer of DSTGOI and I joined V.S. Hospital, this completes first part of my life.

The second half now starts with my involvement at GCRI. As the events of second half now opens up, in 1960 Gujarat State was formed out of Bombay State and the other portion being Maharashtra. The first Governor of Gujarat State Shri Mehndi Nawab Jung started efforts to develop cancer complex in the state with active and able involvement of Shri Jitendra Mehta. Through their efforts Gujarat Cancer society was formed. Shri J. Harivallabhdas was first Executive Chairman and Vice President of GCS. The society received its first major donation of sterling pound 55,000 from M.P. Shah Trust in UK, M.P. Shah Cancer Hospital was started in 1965 with the blessings of Ravishankar Maharaj. No activity took place in 1965 because of Indo Pak war in 1965, In early part of 1966, My pool officer ship were transferred to M.P. Shah Cancer Hospital and I was appointed as a Part time Surgeon at M.P. Shah Cancer Hospital in May 1966. At that time there were three persons in the Hospital. Dr. N.L.Patel, Dr. M.T. Bhatia and Matron Suryavanshi. M.P. Shah Cancer Hospital had two floors, The present Administrative block+ Basement, Ground and first floor of the Hospital. This complex was dedicated by late PM Smt.Indira Gandhi in the presence of Shri Hitendra Desai as Chief Minister and started with 40 beds, 25 male and 15 female. These were housed on the first floor where today we have postoperative recovery ward and ICU. There was one Operation Theatre. OPDs were run daily. The first major Operation of Radical parotidectomy was performed on 27th February, 1967 and as God's gift this patient survived for 7years. In order to accelerate development of the Hospital, M.P. Shah Cancer Hospital had converted into Gujarat Cancer and Research Institute in 1972 and Dr. T.B.Patel was appointed as its first Honorary Director. There was exponential growth of GCRI as more buildings were added, more equipments were purchased, more Specialists were appointed and to serve Cancer patients more beds were added. Dr. T. B Patel retired in 1990 at a stage when Hospital has developed into comprehensive Cancer Hospital and recognized as Regional Cancer Hospital. Today under the Directorship of Dr. N. L. Patel, myself, Dr. P. M. Shah and Dr. Shilin N. Shukla, this Hospital is 650 bedded Cancer Hospital with specialists of all branches in oncology. I must specifically mentioned pediatric Cancer center first of its type in the Country,

Neuro oncology first of its type attach to Cancer complex and other super specialties. During my tenure I had considerable academic activity of which are conference with BASO in 1986, Biennial conference of ISO in 1990 where I was elected as President. Conference with European School of Oncology and very successful conferences with Indian Association of Cancer Research the Hospital is a recognized teaching hospital of a super specialty courses in surgery and medical oncology and post graduate courses in Gynecology, Radiology and Radiotherapy services, Gynec Oncology and well established departments of Cancer research. I can continue to write about GCRI for next few hours. However, I retired as Director in January 2003 and during all these year of my journey through Cancer Hospital from 1966 to January 2003 I received unstinted support of all GCRIans and successive office bearers of Cancer society. Further history and development at the GCRI will be narrated by future Shri Madan Mohan GCRI Luminal orators. I have great satisfaction that GCRI is now known nationally and internationally.

My special Thanks and gratitude to Government of Gujarat and Department of Health & Family welfare for full support to GCRI, all these years, Shri J. Harivallabhdas, Shri Jitendra Mehta, Shri Hiralal Bhagwati, Shri Baldevbhai Dosabhai, Shri L.R. Dalal, Shri Arvindbhai, Shri Vadilal Mehta, Shri Thakorbhai Munsa, Shri K. M Kantawala and Shri G.G Vaidya and past and present office bearers of Cancer society. Specially Thanks and gratitude to Shri Hitendra Desai for donation done to GCS through his WILL, to Shri Prashant Kinariwala and Shri K. Madan Mohan, present general secretary and secretary of GCS, Shri N.T. Chavda who was associated with me from 1966 to 2003 as a friend and philosopher and guide, to Shri Jaipal and all the office staff during my tenure from 1966 to 2003. Late Shri M. D. Patel, Late Shri P. B. Patel and staff of PWD with special reference to their cooperation in building Cama Hall in a short period of 9 months so that we can hold BASO Conference in January 1986. All GCRIans who loved me and respected me in spite of my strict nature and in the end I thank all of you for awarding me this Oration.

Shri Ramniklal J. Kinarivala Research Award 2011-2012

Raj K. Puri,

MD, PhD
Director, Division of Cellular & Gene Therapies,
Chief, Tumor Vaccines and Biotechnology Branch,
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Targeting Interleukin Receptors for Cancer Therapy

Despite advances in medical sciences including surgery, radiation, chemo- and small molecule therapy, the 5 year survival rate for cancer patients has not improved significantly in past 25 years. Many patients develop resistance to the first line therapies and their cancers metastasize to vital and other distant organs and die due to disease progression. Therefore, new therapeutic approaches are needed for the treatment of cancer. In past two to three decades, new classes of anti-cancer therapeutic agents such as gene therapy, cancer vaccines, adoptive immunotherapy and targeted fusion proteins have emerged, which offer great promise. Therefore, careful scientific attention is needed in the development of these therapeutic approaches for cancer. We have discovered that the receptors for T_H2 derived cytokines interleukin-4 and interleukin-13 are over expressed on the cell surface of solid human cancer compared to normal immune cells and these receptors may serve as novel targets for cancer therapy. A variety of solid tumor samples and cell lines derived from human renal cell carcinoma, malignant glioma, pancreatic cancer, ovarian carcinoma, squamous cell carcinoma of head and neck and AIDS associated Kaposi's sarcoma express high levels of IL-4 (IL-4R) and IL-13 receptors (IL-13R). By radio-labeled binding, flow cytometry, in-situ immunoflouresc-ence, immuno-histochemistry, PCR, immunoprecipi-tation and reconstitution studies, we have shown that both IL-4R and IL-13R are expressed as three different types in different cell types. Although both IL-4 and IL-13R are expressed on tumor cells, the significance of expression of these receptors on tumor cells is not completely clear. A lot of attention has been focused on the signal transduction through IL-4R and IL-13R in cancer and normal cells. It is shown that IL-4 after binding to IL-4R chain recruits either $IL\text{-}2R_c$ chain or IL-13R1 chain and IL-13 after binding to IL-13R1 chain recruits IL-4R and then mediates signal transduction. In addition, IL-13Rα2 signals by itself by a different pathway. We have recently discovered that IL-13Rα2 is a novel tumor antigen. A cDNA vaccine to this chain can produce immune response against established tumors and cause regression of tumors.

To target IL-4R and IL-13R, recombinant chimeric fusion proteins comprised of human IL-4 and IL-13 and a truncated form of *Pseudomonas* exotoxin has been produced. These chimeric proteins are highly cytotoxic to IL-4R or IL-13R over expressing human tumor cell. Both Iimmunotoxins can induce apoptosis in tumor cells *in vitro* and *in vivo*. In contrast, IL-4-PE and IL-13PE are not cytotoxic to hemopoietic cells such as monocytes, B cells, T cells, bone marrow cells and non-hemopoietic cells such as endothelial cells, and astrocytes. The *in vitro* data were confirmed by *in vivo* studies in a number of human tumor xenograft models. IL-4 and IL-13 cytotoxin mediated dramatic tumor responses in established human tumors (e.g., malignant glioma, head and neck tumors, ovarian tumor, AIDS-Kaposi's sarcoma etc.) in immunodeficient mice.

Various preclinical toxicology studies have also been performed. Based on pre-clinical studies, several Phase I/II clinical trials were initiated to determine safety and tolerability of IL-4-PE and IL-13-PE in patients with malignant brain tumors and other tumors. In these clinical studies, IL-4-PE and IL-13-PE cytotoxin has been administered by convection enhanced delivery (CED) via intra tumoral or peri-tumoral catheters. In the first clinical trial, IL-13-PE was administered directly into the tumor by CED using 1-2 catheters. In second trial, IL-13-PE was administered directly into the tumor followed by resection after 2 weeks. The primary objective of these trials was to determine the MTD in terms of duration of infusion and drug concentration of IL-13-PE delivered by CED via one or two intratumoral catheters into recurrent or progressive malignant glioma prior to surgical resection. In the third trial, the histologically effective concentration of IL-13PE cytotoxin in vivo was studied. In this clinical

trial, IL-13PE cytotoxin was infused for 2 days by intra tumoral CED into recurrent malignant glioma prior to surgical resection. These patients were then infused with IL-13 cytotoxin for 4-5 days by 2-3 cathers placed in normal brain parenchyma adjacent to tumor cavity. The primary objective of this study was to determine the toxicity of the drug infused via catheter into brain adjacent to tumor resection site after surgical resection. All three studies demonstrated that intratumoral and peri-tumoral infusion of IL-13-PE was well tolerated. On the basis of these results, a Phase III PRECISE clinical trial was initiated in which 2-3 catheters were placed peritumorally and IL-13-PE at a concentration of 0.5µg/ml was infused. This multi-center trial was completed and this study will need to be extended to examine the efficacy and tolerability of IL-13-PE in GBM patients.

Stat3 mRNA Expression in Premalignant, Malignant and Adjacent Normal Mucosa of the Oral Cavity

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Summary

The present study investigated the role of Signal transducer and activator of transcription 3 (Stat3) at transcriptional level in oral carcinogenesis. For this, Stat3 mRNA was studied by semi-quantitative RT-PCR in oral premalignant (N=35), malignant (N=70) and tumor adjacent normal mucosa (ANM; N=70). Data analysis revealed up-regulation of Stat3 mRNA in ANM (p=0.034) and tumors (p=0.001) compared to premalignant lesions. Stat3 mRNA in primary tumors directly correlated with tumor size (p=0.048). In ANM, it significantly correlated with lymphatic permeation (p=0.025) and survival. In multivariate analysis, Stat3 mRNA in ANM was a significant predictor of relapse-free survival at step 1 and overall survival at step 2 after stage with a relative hazard ratio of 4.81 and 4.78, respectively. Our observations demonstrate a strong association between Stat3 activation at transcriptional level in oral carcinogenesis, and strengthen our previous study of Stat3 activation is an early event in oral carcinogenesis mediated by tyrosine kinase signaling pathway. Moreover, Stat3 mRNA expression in ANM may be regarded as a marker of early undetectable disease and an important prognostic tool.

Introduction

Oral squamous cell carcinoma (OSCC) affecting the oral cavity is a disfiguring disease that continuous to increase in incidence, even among the young and to an extent that cannot be fully explained by increased exposure to known risk factors. Even in India, it is the leading malignancy with majority of patients presenting with locally advanced disease at the time of diagnosis. With currently available clinical assessment and treatment methods, patients are diagnosed at a late stage of the disease and the overall survival rate has not improved significantly.[1] This highlights the need for continuous efforts to advance our understanding of the molecular basis of oral carcinogenesis to establish parameters for early diagnosis and designing of therapies that specifically target oral tumor cells.

It is well established that oral carcinogenesis is a multistep process involving accumulation of genetic alterations and in many cases involves a phenomenon coined "field cancerization" wherein the genetically altered adjacent normal field might likely provide a soil for facilitating tumor invasion and metastasis. In recent years it has become apparent that a wide array of oncogenic alterations may exert their effects by stimulating a relatively limited number of transcription factors, over activity of which represents the point of convergence of multiple cancer related molecular and signaling aberrations.[2] In this context, Signal transducer and activator of transcription 3 (Stat3) is considered a key signaling molecule in many cytokine and growth factor receptor pathways and in head and neck squamous cell carcinoma (HNSCC) it holds great promise for development of more rational and effective treatments.[3-4] Our earlier studies demonstrated the significant role of Stat3 protein expression in oral carcinogenesis.[5-6] Further, the results of Stat3 protein expression indicated constitutive Stat3 activation to be an early event in OSCC and a potential risk factor for poor prognosis.[7] As the squamous mucosa of oral cancer is 'condemned' or predisposed to deregulation, it would be interesting to explore the biological relevance of Stat3 mRNA expression and whether it contributes to predisposition. To our knowledge there is no report that have investigated Stat3 mRNA in tumor adjacent normal mucosa (ANM) in oral cancer, although there are very few reports of Stat3 mRNA expression in OSCC.[8-9] Apart from OSCC, Stat3 mRNA expression has also been demonstrated in human non-small cell

lung cancer cell lines, colorectal cancer and cervical warts.[10-12] Hence, the present study was undertaken to gain further insight into the role of Stat3 in OSCC, at transcriptional level. For this, Stat3 mRNA expression was studied in premalignant lesions, primary tumors and corresponding ANM of tumors. Further, the expression was correlated with established prognostic factors and survival.

Materials and Methods

Patients: A total of 105 untreated patients with histopathologically confirmed oral premalignant lesions (N=35) and OSCC (N=70) enrolled between 2000 and 2003 were included in this study. Written consent of the patients was obtained, prior to tissue collection. Biopsy tissues from patients with submucous fibrosis (N=15) and leukoplakia (N=20) were collected from Department of Surgical Oncology, The Gujarat Cancer & Research Institute (GCRI), Ahmedabad, while primary tumor tissues (buccal mucosa: N=39; tongue: N=31) and their respective ANM were collected at the time of surgery, from GCRI only. Portions of premalignant biopsy tissue, primary tumor and corresponding ANM of tumor selected by a pathologist was immediately snap frozen in liquid nitrogen and stored at 85°C for total RNA extraction. Postoperative treatment included radiotherapy and chemotherapy, instituted by the Radiotherapy and Medical Oncology units, respectively. The detailed clinical history (age, gender, anatomic site, habit, pTNM stage, histopathological findings, treatment given, appearance of recurrence/metastases, survival time, etc) was noted from the case files maintained at the Medical Record Department of the institute. According to the histopathological type, premalignant lesions were grouped into hyperplasia (N=25) and dysplasia (N=10). In patients with OSCC, the disease was staged according to the criteria of the International Union Against Cancer (UICC) pTNM classification.[12] Thus, the present study included 9 patients of stage I ($T_1N_0M_0$), 25 patients of stage II ($T_2N_0M_0$), 13 patients of stage III ($T_3N_0M_0$, $T_{1-3}N_{1-2}M_0$) and 23 patients of stage IV ($T_4N_0M_0$, $T_{1-4}N_{1-3}M_0$). The tumors were histologically graded as well- (N=29), moderately- (N=39) and poorly- (N=2) differentiated, based on the criteria of Broders.[14] The patient and tumor characteristics included in the study are presented in Table- 1a and 1b. Out of a total of 70 OSCC patients, 66 (94%) patients could be followed for a minimum period of 2 years or until death within that period. Within 2 years, 53% (35/66) of the patients developed recurrent disease and death due to cancer occurred in 44% (29/66) of patients.

Table-1a: Patient and tumor characteristics

Characteristics	Malignant (N=70)	
	N	%
Age (year)		
⩽45	42	60
>45	28	40
Gender		
Male	55	79
Female	15	21
Anatomic site		
Buccal Mucosa	39	56
Tongue	31	44
Tobacco habit		
No	12	17
Yes	58	83
Treatment		
Surgery	23	33
Followed by		
Radiotherapy	33	47
Chemotherapy	02	03
Radiotherapy and chemotherapy	12	17

Semi-quantitative RT-PCR: Total RNA was extracted from premalignant, primary tumor and its corresponding ANM by the single-step acid gaunidinium thiocyanate phenol chloroform extraction method of Chomczynski and Sacchi.[15] Following RNA extraction, the purity of the recovered RNA was measured spectrophotometrically at 260 nm and 280 nm. Further, the quality of the isolated RNA was confirmed by agarose gel electrophoresis. One microgram of total cellular RNA was reverse transcribed to cDNA. RT-PCR was performed using GeneAmp EZ r*Tth* RNA PCR kit (Perkin Elmer, CA, USA) following the kit's protocol. Primers for Stat3 mRNA were similar to that used by Nagpal et al[8] and are as follows: sense (5'- TCT CCT ACT TCT GCT ATC TTT GAG -3') and anti-sense (5'- ATG GGT CTC AGA GAA CAC ATC -3'). The reaction was performed as follows: reverse transcription at 60°C for 30 min, an initial denaturation at 94°C for 1 min, followed by 35 cycles of PCR (94°C for 45 sec, 59°C for 45 sec and 72°C for 1 min), and a final extension at 72°C for 10 min. The amplified products along with the 50 base pair ladder were electrophoresed on 1.8% agarose gel. At the end of the run, the intensity of PCR products (116 base pair) was measured and integrated on Gel Documentation System (Bio-Rad, USA) using the Molecular Analyst Software, in the units of counts/mm^2.

Statistical Analysis: The data were analyzed statistically using SPSS software (release 13; Chicago, IL, USA, 1999). Non parametric Mann-Whitney 'U' test was performed to evaluate

Table-1b: Patient and tumor characteristics

Characteristics	Malignant (N=70)	
	N	%
Tumor size		
1 (<2 cm)	09	13
2 (2-4 cm)	36	52
3 (>4 cm)	08	11
4 (>4cm and invades adjacent structures)	17	24
Stage		
I	09	12
II	25	36
III	13	19
IV	23	33
Nodal status		
Negative	46	66
Positive	24	34
Tumor differentiation		
Well differentiated	29	41
Moderately/ Poorly differentiated	41	59
Keratin		
Absent	05	07
Present	65	93
Lymphatic permeation		
Absent	45	64
Present	25	36
Vascular permeation		
Absent	62	89
Present	08	11

significance of Stat3 mRNA transcript level between the groups. Two-tailed χ^2 test was used to assess association between two parameters. Correlation between two parameters was calculated using Spearman's correlation coefficient (r). To predict prognosis univariate and multivariate survival analysis was performed using Cox proportional hazard regression model. The Wald statistic and hazard ratio (HR) with 95% confidence interval (CI) were used to assess risk for relapse-free survival (RFS) and overall survival (OS). Kaplan-Meier survival curves were performed for RFS and OS. P values $\leqslant 0.05$ were considered significant.

Results

Stat3 mRNA expression in premalignant lesions, primary tumors and ANM of tumor: Data analysis showed that the expression of Stat3 mRNA was significantly up-regulated in ANM and tumors compared to premalignant lesions. Stat3 mRNA expression levels in premalignant, malignant and corresponding ANM of tumors in terms of mean ± standard error were 1405.54 ± 224.82, 2457.84 ± 184.72 and 2088.23 ± 190.63, respectively (Table-2). Their corresponding median levels used as cut-off to differentiate low and high expression of Stat3 mRNA were 1203.0, 2246.0 and 1852.0. Using the Mann-Whitney 'U' test, statistical significance was noted in Stat3 mRNA expression between premalignant and malignant lesions (p=0.001), and between premalignant and ANM of tumors (p=0.034). Stat3 mRNA expression in oral cancer patients are depicted in Figure 1.

Relation of Stat3 mRNA expression with histopathological features: Stat3 mRNA expression in primary tumors and corresponding ANM was correlated with histopathological parameters and is depicted in Table-3 and Table-4, respectively.

Primary tumors: Stat3 mRNA expression in primary tumors demonstrated a significant positive correlation with tumor size (r=+0.237, p=0.048) and a marginal non-significant positive correlation with tumor stage (r=+0.229, p=0.056). No significant correlation was observed with rest of the established histopathological features.

Adjacent normal mucosa: Stat3 mRNA expression in ANM of tumor when correlated with histopathological characteristics showed a significant positive correlation with lymphatic permeation (r=+0.268, p=0.025) and to a lesser extent with tumor stage (r=+0.229, p=0.056). With rest of the histopathological parameters, no significant correlation was observed.

Table-2: Stat3 mRNA expression in oral premalignant malignant and adjacent normal mucosa

	Premalignant (N=35)	Malignant (N=70)	Adjacent normal[$] (N=70)	p value
Range	0.0-4079.0	0.0-5408.0	35.0-5264.0	0.034[@]
Mean ± SE	1405.54±224.82	2457.84±184.72	2088.23±190.63	0.001[#]
Median	1203.0	2246.0	1852.0	

[@]premalignant vs adjacent normal, [#]premalignant vs malignant,

[$]adjacent normal mucosa of tumors

Table-3: Relation between Stat3 mRNA expression in primary tumors and histopathological parameters

Characteristics	N	Stat3 mRNA		r	p value
		<2246.0	⩾2246.0		
		N (%)	N (%)		
Tumor size				+ 0.237	**0.048**
T1/T2	44	26 (59)	18 (41)		
T3/T4	26	09 (35)	17 (65)		
Nodal status				+ 0.060	NS
Negative	46	24 (52)	22 (48)		
Positive	24	11 (46)	13 (54)		
Tumor stage				+ 0.229	0.056
Early stage	34	21 (62)	13 (38)		
Advanced stage	36	14 (39)	22 (61)		
Histologic grade				+ 0.029	NS
I*	29	15 (52)	14 (48)		
II** + III***	41	20 (49)	21 (51)		
Keratin				- 0.055	NS
Absent	05	02 (40)	03 (60)		
Present	65	33 (51)	32 (49)		
Lymphatic permeation				+ 0.209	NS
Absent	45	26 (58)	19 (42)		
Present	25	09 (36)	16 (64)		
Vascular permeation				+ 0.180	NS
Absent	62	33 (53)	29 (47)		
Present	08	02 (25)	06 (75)		

*Well differentiated, ** Moderately differentiated, *** Poorly differentiated
NS: Not significant

Survival analysis**:** Survival analysis was carried out in 94% (66/70) of OSCC patients which could be followed for a period of 2 years.

Relapse-free survival: Univariate survival analysis for RFS indicated that Stat3 mRNA expression in primary tumors was unable to predict RFS (p=0.089, Table-5). However, Stat3 mRNA expression in ANM along with histopathological parameters showed that in total patients the significant risk predictors were tumor size (HR=2.41, p=0.010), nodal status (HR=2.26, p=0.017), tumor stage (HR=3.28, p=0.001), lymphatic permeation (HR=2.84, p=0.002) and Stat3 mRNA (HR=4.81, p=0.0001). Thus, in multivariate analysis, Stat3 mRNA expression emerged as the most significant indicator of RFS (HR=4.81, p=0.0001) followed by tumor stage (HR=2.87, p=0.004). The relative risk for recurrence was 4.81 in patients with Stat3 mRNA expression ⩾ 1852.0 as compared to those with Stat3 mRNA expression <1852.0 (Table-5). Moreover, Kaplan-Meier survival curve for RFS demonstrated that 79% (26/33) of patients with Stat3 mRNA expression ⩾ 1852.0 in ANM showed significantly reduced RFS as compared to 27% (9/33) of patients with Stat3 mRNA expression <1852.0 in ANM (Table-7, Figure 2).

Overall survival: Univariate analysis for OS demonstrated that the significant risk predictors were tumor size (HR=3.81, p=0.0001), nodal status (HR=3.44, p=0.001), tumor stage (HR=6.80, p=0.0001), lymphatic permeation (HR=4.07, p=0.0001), vascular permeation (HR=2.91, p=0.020) and Stat3 mRNA (HR=5.59, p=0.0001). However, in multivariate analysis, tumor stage (HR=6.80, p=0.0001) emerged as the most significant prognosticator followed by Stat3 mRNA expression

Table-4: Relation between Stat3 m RNA expression in adjacent normal and histopathological parameters

Characteristics	N	Stat3 mRNA		r	p value
		< 1852.0	⩾ 1852.0		
		N (%)	N (%)		
Tumor size					
T1/T2	44	25 (57)	19 (43)	+ 0.177	NS
T3/T4	26	10 (38)	16 (62)		
Nodal status				+ 0.120	NS
Negative	46	25 (54)	21 (46)		
Positive	24	10 (42)	14 (58)		
Tumor stage				+ 0.229	0.056
Early stage	34	21 (62)	13 (38)		
Advanced stage	36	14 (39)	22 (61)		
Histologic grade				+ 0.145	NS
I*	29	17 (59)	12 (41)		
II** + III***	41	18 (44)	23 (56)		
Keratin				+ 0.166	NS
Absent	05	04 (80)	01 (20)		
Present	65	31 (48)	34 (52)		
Lymphatic permeation				+ 0.268	**0.025**
Absent	45	27 (60)	18 (40)		
Present	25	08 (32)	17 (68)		
Vascular permeation				+ 0.180	NS
Absent	62	33 (53)	29 (47)		
Present	08	02 (25)	06 (75)		

*Well differentiated, ** Moderately differentiated, *** Poorly differentiated
NS: Not significant

in ANM (HR=4.78, p=0.001; Table- 6). Kaplan-Meier curve for OS indicated that Stat3 mRNA expression in primary tumors ⩾ 2246.0 exhibited a trend towards poor survival (Log rank= 3.39, df=1, p=0.065) (Table-7). Contrary, high level of Stat3 mRNA expression in ANM was associated with inferior OS (Log rank=17.72, df=1, p=0.00001) (Table-7, Figure 3).

Discussion

Our earlier studies using immunohisto-chemistry have demonstrated constitutive activation of Stat3 protein as one of the molecular changes required for oral carcinogenesis and also as a potential risk factor for poor prognosis in OSCC.[7] However, there is very limited data concerning the expression of Stat3 mRNA in OSCC.[8-9] The current study evaluated Stat3 mRNA expression in premalignant lesions, primary tumors and corresponding ANM of tumors. The results indicated significantly increased levels of Stat3 mRNA in OSCC and ANM compared with the levels detected in premalignant lesions, suggesting up-regulation of Stat3 mRNA expression in oral carcinogenesis. Moreover, Stat3 mRNA expression in premalignant lesions supported our earlier finding of Stat3 protein expression in premalignant lesions[5] and provided further evidence that Stat3 activation is an early event in oral carcinogenesis.

In OSCC, increased Stat3 mRNA and results of increased Stat3 protein expression from previous study[5] indicated that both transcription and translation products of this molecule were highly expressed in OSCC. However, this increase in Stat3 mRNA was not paralleled by an increase in Stat3 protein expression. For Stat3 gene, it has been reported that alternate splicing results in 2 isoforms Stat3α and Stat3β.[16] Moreover, Chakraborty et al[17] and Biethahn

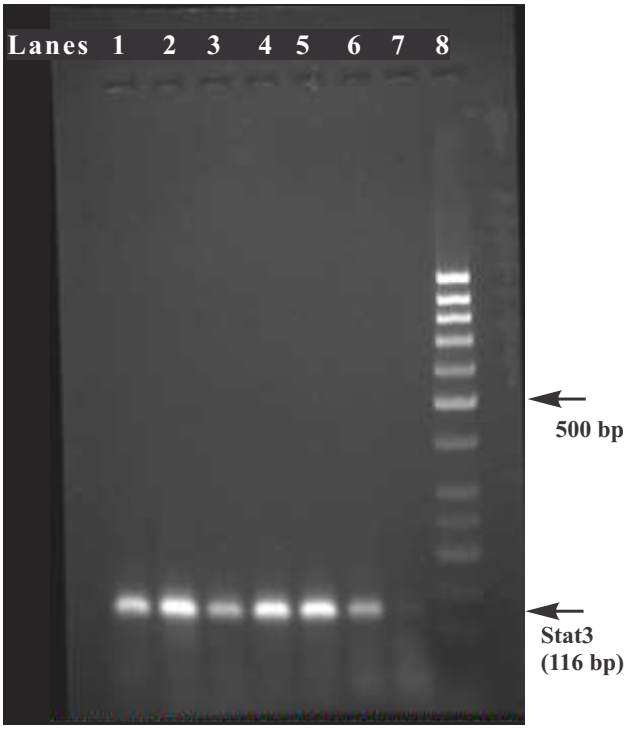

Figure 1: Stat3 mRNA expression in oral squamous cell carcinoma
Lane 1 and 3: Adjacent normal mucosa of tumors
Lane 2, 4-6: Primary oral tumors
Lane 7: Negative control
Lane 8: 50 bp ladder

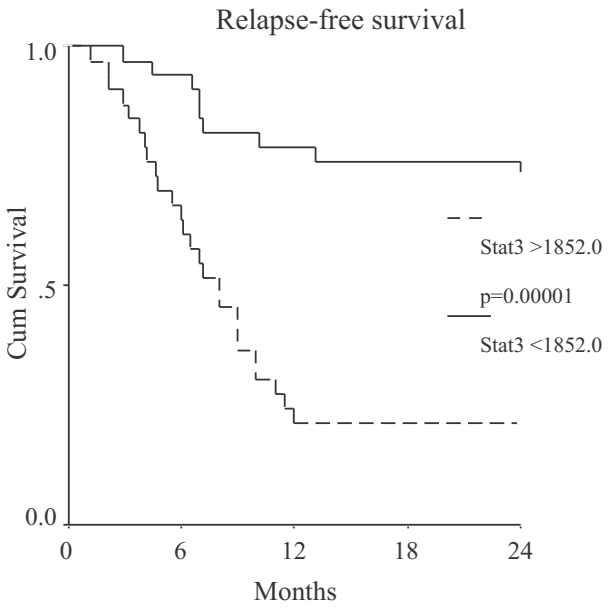

Figure 2: Kaplan-Meier survival curve for RFS demonstrated that patients with Stat3 mRNA expression ⩾ 1852.0 in ANM had significantly reduced RFS as compared to patients with Stat3 mRNA <1852

Table-5: Univariate and multivariate survival analysis for relapse-free survival (N=66)

Variables	Wald	HR	95% CI		p value
			lower	upper	
Univariate analysis					
Tumor size	6.70	2.41	1.23	4.70	**0.010**
Nodal status	5.66	2.26	1.15	4.43	**0.017**
Tumor stage	10.98	3.28	1.62	6.62	**0.001**
Histological grade	2.02	1.67	0.82	3.42	0.155
Keratin	0.18	1.36	0.32	5.69	0.669
Lymphatic permeation	9.36	2.84	1.45	5.54	**0.002**
Vascular permeation	2.65	2.08	0.86	5.02	0.103
Stat3 mRNA in primary tumors	2.88	1.80	0.91	3.54	0.089
Stat3 mRNA in ANM*	16.08	4.81	2.23	10.39	**0.0001**
Multivariate analysis					
Step 1 Stat3 mRNA*	16.0	4.81	2.23	10.39	**0.0001**
Step 2 Tumor stage	8.44	2.87	1.41	5.85	**0.004**

*Adjacent normal mucosa

et al[18] have reported that the ratio of Stat3α and Stat3β varies in cells and tissues, ranging from 3:1 to 10:1 at the mRNA level and 1:3 to 10:1 at the protein level and this variation may have important biologic consequences because the functions of the 2 isoforms do not overlap. In colorectal cancer, Zhang et al[12] have reported 1.97 times higher Stat3 mRNA level in colorectal cancer tissues compared to that of adjacent normal tissue. Similar to our findings, recently, Yin et al[10] have demonstrated significantly higher Stat3 mRNA expression in primary tumors compared to corresponding ANM. In contrast, Lassman et al[19] have reported similar Stat3 mRNA levels in normal and neoplastic colonic epithelial cells and absence of any correlation with Stat3 protein expression. Concerning

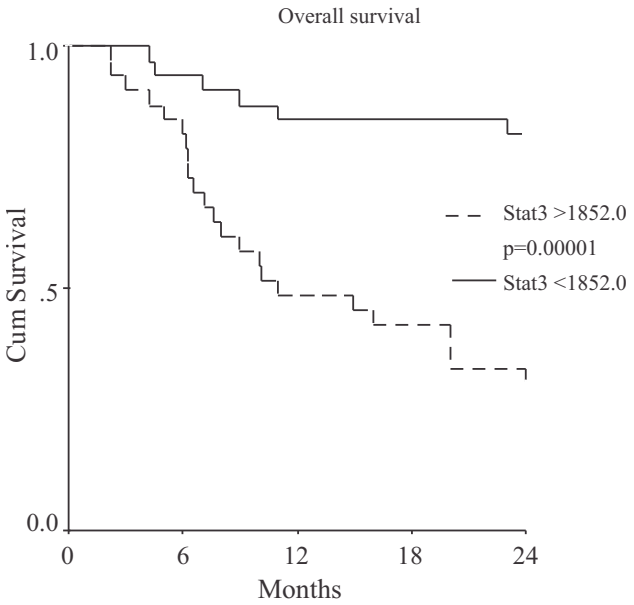

Figure 3: Kaplan-Meier survival curve for OS indicated that patients with Stat3 mRNA expression ⩾ 1852.0 in ANM had significantly inferior OS compared to patients with Stat3 mRNA <1852

histopathological parameters and disease outcome, Stat3 mRNA expression in tumors showed a significant correlation with tumor size only and was unrelated to survival. To our knowledge there are no reports that have investigated the relevance of Stat3 mRNA in terms of survival. In OSCC, Nagpal et al[8] have reported higher Stat3 mRNA expression in T1 and T2 classification (early stages), moderate in T3 and T4 (late stages) and absence of expression in normal epithelium. This once again emphasized that Stat3 activation at the transcriptional level is an early event and involved in tumor progression. On the contrary, in colorectal carcinoma, high Stat3 mRNA expression correlated with nodal metastasis and low tumor differentiation.[12]

The detection of Stat3 mRNA expression in ANM indicated that the tissue adjacent to the tumor though histologically normal is not normal but has molecular alterations thereby reinforcing the "field cancerization" concept described by Slaughter.[20] It also suggested Stat3 pathway to be one mechanism whereby the exposed mucosa becomes "condemned" and indicated that Stat3 mRNA may signal an early alteration before phenotypic transformation in oral carcinogenesis. Alternately, the presence of Stat3 mRNA expression in ANM may be explained by the action of paracrine or autocrine factors secreted by tumors. Grandis et al[21]demonstrated that constitutive Stat3 activation is associated with EGFR autocrine stimulation in human squamous cell carcinoma. Besides this, Sriuranpong et al[22] have demonstrated that HNSCC cells with or without active EGFR, use an autocrine/paracrine Stat3 activation mechanism mediated through the gp130 family of cytokine receptors. The findings of Stat3 mRNA in ANM corroborate with the evidence of Stat3 activation/protein expression in epithelium adjacent to tumor reported by Grandis et al[21] in head and neck cancer and by Klosek et al[23] in OSCC. In multivariate analysis, Stat3 mRNA expression in ANM was a significant predictor of RFS at step 1 and OS at step 2 after tumor stage with a relative hazard ratio of 4.81 and 4.78, respectively. To our knowledge, this innovative finding of the prognostic value of Stat3 mRNA in ANM has never been evaluated. Our data for the first time indicate that Stat3 mRNA in ANM is an important prognosticator of recurrence in OSCC.

Conclusion

In conclusion, the present data demonstrate a strong association between Stat3 activation at

Table-6: Univariate and multivariate survival analysis for overall survival (N=66)

Variables	Wald	HR	95% CI		p value
			lower	upper	
Univariate analysis					
Tumor size	12.42	3.81	1.81	8.04	**0.0001**
Nodal status	10.84	3.44	1.65	7.19	**0.001**
Tumor stage	17.23	6.80	2.75	16.84	**0.0001**
Histological grade	0.94	1.46	0.67	3.14	0.331
Keratin	0.50	2.05	0.28	15.13	0.478
Growth pattern	0.02	1.09	0.38	3.14	0.867
Lymphatic permeation	13.50	4.07	1.92	8.60	**0.0001**
Vascular permeation	5.38	2.91	1.18	7.21	**0.020**
Stat3 mRNA in primary tumors	3.23	1.99	0.94	4.22	0.072
Stat3 mRNA in ANM*	13.95	5.59	2.26	13.82	**0.0001**
Multivariate analysis					
Step 1 Tumor stage	17.23	6.80	2.75	16.84	**0.0001**
Step 2 Stat3 mRNA in ANM*	11.11	4.78	1.90	12.01	**0.001**

*Adjacent normal mucosa

Table-7: Kaplan-Meier survival analysis for relapse-free and overall survival (N=66)

	Relapse-free survival			Overall survival		
Stat3 mRNA expression	**N**	**Patients relapsed N (%)**	**p value**	**N**	**Patients died N (%)**	**p value**
Tumour	66	35 (53)		66	29 (44)	
<2246.0	34	14 (41)	NS	34	11 (32)	0.065
⩾2246.0	32	21 (66)		32	18 (56)	
ANM*	66	35 (53)		66	29 (44)	
<1852.0	33	09 (27)	**0.00001**	33	06 (18)	**0.00001**
⩾1852.0	33	26 (79)		33	23 (70)	

*Adjacent normal mucosa

transcriptional level in oral carcinogenesis and strengthen our previous study[5] of Stat3 activation is an early event in oral carcinogenesis mediated by tyrosine kinase signaling pathway. Moreover, Stat3 mRNA expression in ANM may be regarded as a marker of early undetectable disease and an important prognostic tool.

References

1. Neville BW, Day TA: Oral cancer and precancerous lesions. CA Cancer J Clin 2002; 52: 195-215
2. Darnell JE Jr: Transcription factors as targets for cancer therapy. Nat Rev Cancer 2002; 2: 740-749
3. Heim MH: The Jak-STAT pathway: cytokine signaling from the receptor to the nucleus. J Recept Signal Transduct Res 1999; 19: 75-120
4. Song JI, Grandis JR: STAT signaling in head and neck cancer. Oncogene 2000; 19: 2489-2495
5. Shah NG, Trivedi TI, Tankshali RA et al: Molecular alterations in oral carcinogenesis. Int J Biol Markers 2007; 22: 132-143
6. Shah NG, Trivedi TI, Tankshali RA et al: Stat3 expression in oral squamous cell carcinoma: Association with clinicopathological parameters and survival. Int J Biol Markers 2006; 21: 175-183
7. Shah NG and Trivedi TI: Early stage oral squamous cell carcinoma: use of signal transducer and activator of transcription 3 as a risk factor for poor diagnosis. Book Chapter; Kean University, Union, NJ 07083, USA 2009
8. Nagpal JK, Mishra R, Das BR: Activation of Stat-3 as one of the early events in tobacco chewing - mediated oral carcinogenesis. Cancer 2002; 94: 2393-2400
9. Arany I, Chen SH, Megyesi JK et al: Differentiation-dependent expression of signal transducers and activators of transcription (STATs) might modify responses to growth factors in the cancers of the head and neck. Cancer Letters 2003; 199: 83-89
10. Yin Z, Zhang Y, Li Yu et al: Prognostic significance of Stat3 expression and its correlation with chemoresistance of non small cell lung cancer cells. Acta Histochemia 2012; 144: 151-158
11. Ikuta K, Takemura K, Kihara M, et al. Overexpression of constitutive signal transducer and activator of transcription 3 mRNA in cisplatin-resistant human non-small cell lung cancer cells. Oncol Reports 2005; 13: 217-222
12. Zhang H, Wang S, Zhang YC, Ye YJ, Cui ZR, Fang WG: Correlation between Stat3 signal transduction pathway and expression of cyclooxygenase-2 in colorectal cancer cells. Zhonghua Yi Xue Za Zhi 2005; 85: 2899-2904
13. UICC. TNM classification of malignant tumors. UICC Technical Report Series 1980; 5: 2
14. Broders AC: Microscopic grading of cancer. Surgical Clinics of North America 1941; 21: 947-962
15. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. Anal Biochem 1987; 162: 156-159
16. Shao H, Quintero AJ, Tweardy DJ: Identification and characterization of *cis* elements in the STAT3 gene regulating STAT3α and STAT3β messenger RNA splicing. Blood 2001; 98: 3853-3856
17. Chakraborty A, White SM, Schaefer TS, Ball ED, Dyer KF, Tweardy DJ: Granulocyte colony-stimulating factor activation of Stat3α and Stat3β in immature normal and leukemic human myeloid cells. Blood 1996; 88: 2442-2449
18. Biethahn S, Alves F, Wilde S, Hiddemann W, Spiekermann K: Expression of granulocyte colony-stimulating factor- and granulocyte-

macrophage colony-stimulating factor-associated signal transduction proteins of the JAK/STAT pathway in normal granulopoiesis and in blast cells of acute myelogenous leukemia. Exp Hematol. 1999; 27: 885-894
19. Lassmann S, Schuster I, Walch A et al: STAT3 mRNA and protein expression in colorectal cancer: effects on STAT3-inducible targets linked to cell survival and proliferation. J Clin Pathol 2007; 60: 173-179
20. Slaughter DP, Southwick HW, Smejkal W: Field-cancerization in oral stratified squamous epithelium: clinical implications of multicentric origin. Cancer (Phila.) 1953; 6: 963-968
21. Grandis JR, Drenning SD, Zeng Q et al: Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis in vivo. Proc Natl Acad Sci USA 2000; 97: 4227-4232
22. Sriuranpong V, Park JI, Amornphimoltham P, Patel V, Nelkin BD, Gutkind JS: Epidermal growth factor receptor-independent constitutive activation of STAT3 in head and neck squamous cell carcinoma is mediated by the autocrine /paracrine stimulation of the interleukin 6/gp 130 cytokine system. Cancer Res 2003; 63: 2948-2956
23. Klosek SK, Nakashiro KI, Hara S, Li C, Shintani S, Hamakava H: Constitutive activation of Stat3 correlates with increased expression of the c-Met/HGF receptor in oral squamous cell carcinoma. Oncol Reports 2004; 12: 293-296

In-silico Screening of Natural Compounds for Lactate Dehydrogenase-A Inhibition in Leukemia

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Summary

Hypoxia and oncogenic mutations drive glycolysis, with the pyruvate to lactate conversion, being promoted by increased expression of lactate dehydrogenase (LDH) and inactivation of pyruvate dehydrogenase. Lactate produced by hypoxic tumor cells may indeed diffuse and be taken up by oxygenated tumor cells. Its inhibition favours the switch from lactate-fuelled respiration to glycolysis and consecutively kills hypoxic tumor cells from glucose starvation. LDH has significant role in growth and maintenance of cancer cells. Modulation or inhibition of LDH by natural compounds may be a better molecular targeted therapy for leukemias. The current study evaluated LDH-A isoform as potential drug target in leukemias using natural compounds as ligands. Several online and offline tools and databases were used for procuring the structure, preparing the protein & ligand for molecular docking studies and ADMET evaluation etc. Further molecular dynamics simulation was carried out after molecular docking of best docked molecules. Result of our *in-silico* study suggest that curcumin is a best fit ligand against LDH-A isoform which can modulate its activity and therefore may come out to be a potential lead molecule for the treatment of leukemia. However, this requires extensive QSAR studies followed by in-vitro assays to assess its cytotoxicity.

Introduction

Current molecular oncology studies are significantly expanding the knowledge of cellular mechanisms at the basis of transformation of a normal cell into a tumor cell. A new approach to therapeutic strategy is emerging, based on the peculiar metabolism of the cancer cell. Cancer cells differ from healthy cells due to a plethora of molecular changes, many of which may be mechanistically linked to metabolic reprogramming.[1] Specifically, glycolysis has long been considered the main source of energy for the cancer cell. This particular metabolic status was defined as the 'Warburg effect' by Otto Warburg.[2,3] Cancer cells tend to produce ATP mainly by 'aerobic glycolysis,' a metabolic shift characterized by high glucose uptake and increased production of lactate. This strongly induced aerobic glycolysis of cancer is an epiphenomenon that results from a more complex metabolic rearrangement. Some glycolytic enzymes, strongly induced in the cancer cell (i.e. hexokinase II, lactate dehydrogenase A, glucose-6-phosphate isomerase) may possess different biological functions, acting as both facilitators and gatekeepers of malignancy.[4]

Since 1970s 'lactate revolution' has occurred. At present in the midst of a lactate shuttle era the lactate paradigm is shifted. It now appears that increased lactate production and concentration as a result of anoxia or dysoxia are often the exception rather than the rule.[5] Classification of LDH in mammals there are three monomers of LDH found known as M (also called A) and H (also called B), plus a type X (also called C) found only in sperm. The LDH molecule is a tetramer composed of four polypeptide chains. LDHA is located on chromosome 11p15.4 (Mendelian Inheritance in Man (OMIM) 150000) LDHB is located on chromosome 12p12.2-p12.1 (Mendelian Inheritance in Man (OMIM) 150100) and third is LDHC (Mendelian Inheritance in Man (OMIM) 150150) located on the chromosome 11p15.5-p15.3. There are five different LDH isoenzymes based on the proportion of M and H chains existing in the LDH tetrameric structure. Both M and H monomers weigh about 35,000 Daltons (all isoenzymes MW: 140 kDa). LDH1 is composed of four H-subunits and LDH5 of four M-subunits. As the number of the M- over H-chains increases, the LDH isoenzyme becomes more efficient in catalysing the conversion of pyruvate to lactate (LDH5) whereas an increase of H- over M-chains (LDH1) favours the conversion of lactate back to pyruvate.[6]

From a pharmacological point of view understanding the real role of glycolysis as playmaker in cancer-cell metabolism in general could have significant implications not only for diagnosis and prognosis but for the implementation of more selective cancer pharmacotherapy.[4] Several glycolytic inhibitors are currently in preclinical and clinical development. Their clinical value as anticancer

agents, above all in terms of therapeutic index, strictly depends on a careful re-evaluation of the pathophysiological role of the unique metabolism of

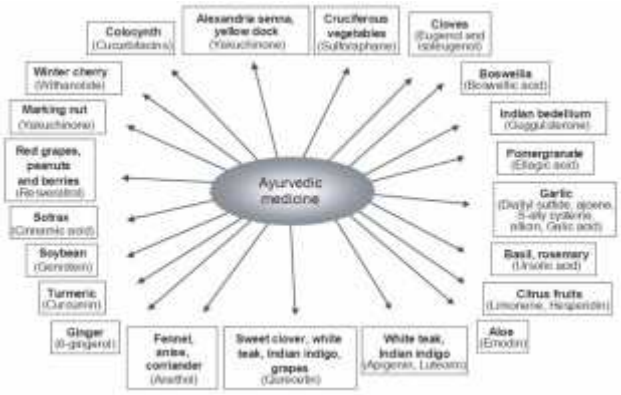

Figure 1: Active components from Ayurvedic medicine used to treat different types of cancer.[12]

cancer cells in general and of Warburg effect in particular.[7]

In ayurvedic medicine, agents derived from plants can be used not only to prevent cancer but also to treat cancer. Of the 121 prescription drugs in use today for cancer treatment, 90 are derived from plants. Almost 74% of these, including taxol, were discovered by investigating a folklore claim.[8,9] Hartwell has collected data on about 3000 plants,[10] those of which possess anticancer properties and subsequently been used as potent anticancer drugs.[11] These dietary agents are believed to suppress the transformative, hyper-proliferative and inflammatory processes that initiate carcinogenesis. Because these chemopreventive agents are derived from natural sources, they are considered pharmacologically safe[12] by checking their ADMET (absorption, distribution, metabolism, elimination, toxicity) properties. Therefore, the present study was carried out to identify possible natural ligand which can be used to modulate LDH activity in leukemic cells. To achieve this task *in-silico*, the following steps were performed:

- Screening of natural ligands to identify potential lead molecule to predict its binding efficiency to substrate and/or cofactor binding sites on LDH
- Pharmacophore study and designing derivatives of best docked ligand
- Molecular Dynamics(MD) simulation and RMSD (Root Mean Square Deviation) of the docked structures for prediction of conformational change due to ligand binding

Materials and Methods

Materials: The following online and offline servers/ tools/databases/software were used for the current study:

Online Servers/Tools/Database used were:

1. NCBI: http://www.ncbi.nlm.nih.gov
2. RCSB Database: http://www.rcsb.org/pdb/ (Protein Data Bank - PDB)
3. MolSoft: http://www.molsoft.com/Offline Tools/ Software used were:

1. Biosuite Software (Version-2.0)
2. Molegro Virtual Docker (MVD)
3. ACD/ChemSketch

Methods: The following steps were performed as a part of pre-docking process**:**

- Downloading the structures of LDH-A from PDB (PDB ID:1i10),
- Searching for natural compounds with anticancer properties from published reports
- Creation of a database of the 104 natural compounds,
- Structural analysis of LDH, protein preparation using Biosuite (removal of chains, hetero atoms and natural ligands from the molecule),
- Binding sites of substrate and co-factor was done by extracting the binding site residues in respective binding sites using 3 Å neighbourhood. Docking studies: Docking of native ligands (NAD/H; Pyruvate/Lactate) was carried out on the respective binding sites of the LDH-A molecule. *In-silico* screening of 104 natural compounds as ligand was performed against LDH-A as receptor as mentioned below:
- Docking with the library of natural ligands with both binding sites (substrate and co-factor) of LDH,
- Ranking of ligands based on their binding free energy score to find out the best interacting compounds,
- Prediction of drug likeness (ADMET) of the hit compounds using MolSoft to obtain the best drug like molecule from top lead compounds,
- Creating possible derivatives of the lead compounds using its pharmacophore property,
- Docking of these derivatives with same binding sites again to assess improvement in binding efficiency due to derivatization. Post docking processing of receptor–ligand complex: The docked receptor ligand complex were then subjected to molecular dynamics simulation using default parameters (1nano sec, 300K) to assess the local changes in and around binding site.
- Superimposition of native structure on MD simulated structure with ligands to calculate their RMSD values. (RMSD >2 suggestive of significant conformational change).

Results and Analysis

The amino acid residues in the NAD binding site and in the lactate binding site found out by the offline tool is summarized in the following Table-1

The natural substrate and co-factors were docked in all possible combinations to their respective binding sites. The binding free energy obtained after docking were noted as shown in Table-2.

Table-1: Substrate and Co-Factor Binding Sites on Chain-A of LDH-A

PDB ID LDH-A	Co-factor Binding Site [NAD(H)]	Substrate Binding Site [Lactate /Pyruvate/Oxamate]
1i10	28-Gly, 95-Ala, 96-Gly, 97-Ala, 98-Arg, 100-Gln, 111-Arg	170-Arg, 185-His, 187-Trp,269-Val,268-Arg,270-His

Docking 104 natural ligands with LDH-A: The Docking of 104 natural ligands on chain-A of LDH-A in is done in 4-different situations as mentioned below:

A. Docking at substrate binding site (when all the binding sites are empty)
B. Docking at cofactor binding site (when all the binding sites are empty)
C. Docking at substrate binding site (with pre-occupied NADH)
D. Docking at cofactor binding site (with pre-occupied Pyruvate)

Table-2: Docking Result of Substrate and Co-Factor with Chain-A LDH-A

Receptor	Co-factor site	Substrate site	Ligand	Energy (Kcal/mol)
LDH A 1i10	Empty	Empty	Lactate	-10.2411
	Empty	Empty	Pyruvate	-7.2985
	Empty	Empty	NAD	-12.3025
	Empty	Empty	NADH	-14.6049
	NAD	Empty	Lactate	-12.1418
	NADH	Empty	Pyruvate	-9.5321
	Empty	Lactate	NAD	-19.1063
	Empty	Pyruvate	NADH	-20.3341

Table-3 indicates Withanolide, Curcumin and Ginkgetin as best fitting ligands in all (A,B,C,D) binding situations based on their binding free energy. Curcumin ranked first with lowest energy level showing a favourable binding.

Table-4 depicts top ten natural ligands ranked according to their binding free energy in increasing order. Curcumin ranked first among the list with lowest binding free energy showing a favourable binding and acceptable molecular mass to qualify as drug like compound.

Table-3: Top Ligand in each Situation Based on their Binding Free Energy

Situation	Ligand	Energy (in Kcal/mol)
A	Withanolide	-21.1823
B	Curcumin	-28.1417
C	Ginkgetin	-21.9790
D	Curcumin	-29.4372

Figure 2 represents a screenshot of molecular docking study between LDH-A with Curcumin and its 2D interaction map with interacting amino acids (Met40, Lys41, Arg170, Asp257, Glu260, Lys264, Arg268, Val269, His270).

Docking of various Curcumin derivatives with of LDH-A: Eleven possible derivatives were derived from the native Curcumin molecule. Docking study was carried out using all these derivatives against same binding site taking native Curcumin as control. The docking scores of various Curcumin derivatives are described in Table-5

Furthermore, as shown in Table-5, the binding free energy of 11 major derivatives of Curcumin with all 4 possible situations was also noted. It was observed that Allyl Curcumin came out to be the best derivatives among all. However, it showed comparable binding energy level as compared to native Curcumin.

MolSoft online server was used to calculate the drug likeness of identified key natural compounds.

Result of MD simulation shows significant change in the conformation at both the binding sites due to Curcumin binding suggestive of its inhibitory effect on LDH-A.

Discussion

Glycolysis is the central metabolic pathway of cancer cells as they proliferate by deriving their excess energy needs from this enhanced process.[3] Tumor cells bearing this metabolic change are uniquely sensitive to inhibition of glycolysis. The possibility of selectively killing cancer cells by deranging their peculiar bioenergetics seems to be an interesting approach. This approach can be used alone or in combination with other therapeutic systems.[4]

LDH-A is an attractive target for cancer therapy because its expression is largely relegated to skeletal muscle. It is not present in red cells, in which glycolysis followed by fermentation is an obligatory process for energy generation.[6] Moreover, it is well known that humans with LDH-A deficiency only show myoglobinuria under intense anaerobic exercise and individuals with complete lack of LDH-A or LDH-B subunit have been documented with no apparent increase in hemolysis. Therefore, it is likely that potential LDH-A inhibitors might show relatively modest systemic toxicity.

Inhibitors of LDHs are under development to suppress the tumor burden, growth rate, and cellular proliferation. Many molecules are in preclinical and clinical phase trials, acting as LDH inhibitors. These molecules, often acting as competitive inhibitors, are capable of reducing tumor burden alone or in association with normal chemotherapy.[4]

The pharmacological actions of these glycolytic enzyme inhibitors, based primarily on ATP

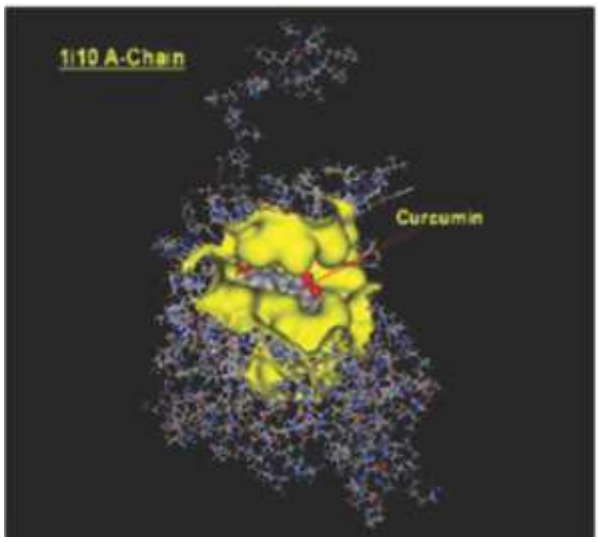

Figure 2(a)

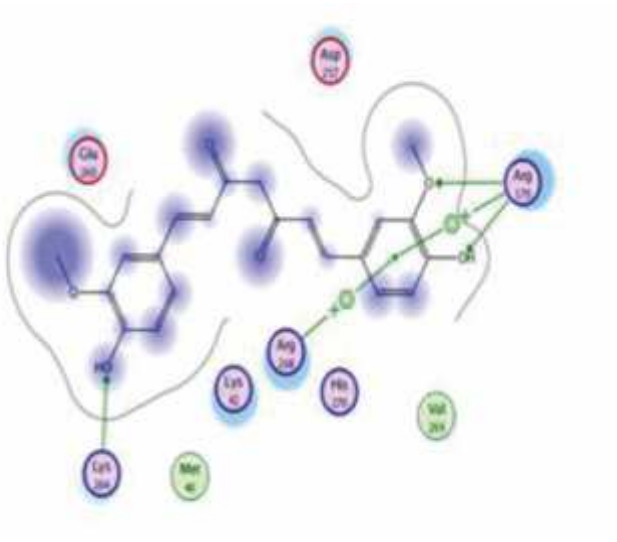

Figure 2(b)

Figure 2: (a) Chain A of LDH-A docked with Curcumin as ligand at substrate binding site. (b) 2D interaction map at the binding site showing Curcumin with interacting amino acids.

Table-4: Docking Result of 10 Natural Ligands with Best Binding Affinity for LDH-A

Sr. No.	Name of the Compound	Energy (Kcal/mol)	Molecular mass
1.	**Curcumin**	**-29.4973**	**368.3**
2.	Cryptoxanthin	-28.3384	552.8
3.	Zeaxanthin	-27.1771	568.8
4.	Capsaicin	-25.7699	305.4
5.	Cicutoxin	-25.3510	258.3
6.	Neohesperidin	-24.2577	610.5
7.	Hesperidin	-24.1344	610.5
8.	Gingerol	-22.1109	294.3
9.	Ginkgetin	-21.9790	566.5
10.	Fangchinoline/ Thalrugosine	-21.4038	608.7

Table-5: Docking Result of 11-Derivatives of Curcumin on LDH –A

Sr. No.	Ligands	On Lactate Site (In Abs of NAD)	On NAD Site (In Abs of Lactate)	On Lactate site (In Presence of NAD)	On NAD site (In Presence of Lactate)
1.	Bisdemethoxy Curcumin	38.9598	-15.6127	9.3495	-16.4391
2.	**Allyl Curcumin**	61.6932	**-27.5131**	**19.8509**	**-29.9491**
3.	Demethoxy Curcumin	62.2513	-16.4490	-12.5375	-15.5355
4.	Curcumin Bisacetate	62.9823	-23.0336	14.1464	-25.7260
5.	Mono-O-(3,3-dimethylallyl)Curcumin	64.8615	-20.4389	-12.9662	-26.8298
6.	Fluoropropyl Curcumin	67.4086	-22.2851	18.1494	-23.3454
7.	Curcumin Dimethyl Ether	69.8265	-22.9642	17.1674	-26.9107
8.	Monodemethoxy Curcumin	74.9683	-16.4207	-13.8398	-22.6942
9.	Curcumin Monoglucoside	82.4046	-15.5382	49.0695	-10.7353
10.	Turmeric Yellow	82.7760	-20.0078	-17.4124	-15.8143
11.	Curcumin Diglucoside	84.1464	-8.4398	14.4562	-15.9606

depletion, could include: i) amelioration of drug selectivity by exploiting the particular glycolysis addiction of cancer cell; ii) inhibition of energetic and anabolic processes; iii) reduction of hypoxia-linked cancer-cell resistance; iv) reduction of ATP-dependent multi-drug resistance; and v) cytotoxic synergism with conventional cancer treatments.[7]

However, in the present study we preferentially took natural compounds as ligand for all possible natural binding sites and tested *in-silico* using molecular docking studies by measuring binding free energy. Curcumin emerged out to be the best scaffold among 104 selected natural compounds in the ligand library.

Conclusion

Among the 104 selected natural compounds

Table-6: RMSD Result of Values of Lactate and Pyruvate with Curcumin

Sr. No.	Receptor	Superpose Ligands after simulation	RMSD (Å)
1	LDH-A (1i10)	Lactate + Curcumin	3.552
2	LDH-A (1i10)	Pyruvate + Curcumin	4.001

Curcumin showed best binding with substrate binding site leading to significant conformational change confirms its modulatory potential. Further ADMET study substantiates its drug likeness property. Therefore, Curcumin can be a suitable scaffold for further derivatization using pharmacophore modelling and QSAR studies.

In spite of few demerits, *in-silico* biology paradigm provides opportunities to both synthetic chemists and biologist to ultimately synthesize a lead compounds with predicted biological activity for the novel target.

Acknowledgement

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References

1. Kroemer G, Pouyssegur J: Tumor Cell Metabolism: Cancer's Achilles' heel. Cancer Cell 2008; 13: 472-482
2. Warburg O: On respiratory impairment in cancer cells. Science 1956; 124: 269-270
3. Warburg O: On the origin of cancer cells. Science 1956; 24; 123: 309-314 Scatena R
4. Bottoni P, Pontoglio A, Mastrototaro L: Glycolytic enzyme inhibitors in cancer treatment. Expert Opinion on Investigational Drugs 2008; 17: 1533-1545 Gladden LB
5. Lactate Metabolism: a new paradigm for the third millennium. J Physiol. 2004; 558: 5-30 Yu Y
6. Deck JA, Hunsaker LA, Deck LM: Selective active site inhibitors of human lactate dehydrogenases A4, B4, and C4. Biochemical Pharmacology 2001; 62: 81–89 Bhardwaj V
7. Rizvi N, Lai MB : Glycolytic enzyme inhibitors affect pancreatic cancer survival by modulating its signaling and energetic. Anticancer Res. 2010; 30:743-749
8. Craig WJ: Phytochemicals: guardians of our health. J. Am. Diet. Assoc. 1997; 97:S199-204
9. Craig WJ: Health-promoting properties of common herbs. Am. J. Clin. Nutr. 1999; 70: 491S-499S
10. Hartwell JL: Plants used against cancer: A survey. Lloydia 1969; 32: 247-296 Balachandran P
11. Govindarajan R: From Cancer—an ayurvedic perspective. Pharmacological Research 2005; 51:19-30
12. Aggarwal BB, Ichikawa H, Garodia P, Weerasinghe P: Traditional Ayurvedic medicine to modern medicine: identification of targets for suppression of inflammation and cancer. Expert Opinion on Therapeutic Targets 2006; 10: 87-118

Paroxysmal Nocturnal Hemoglobinuria (PNH) Evaluation by Multiparametric Flowcytometry

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Summary

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired haemolytic disorder caused by a non-malignant clonal expansion of one or more stem cell lineages. Multiparameter flowcytometry using FLAER is considered as a reliable tool for the diagnosis of PNH. The present study followed same method for PNH evaluation. Twenty-six patients with marrow hypoplasia or aplastic anemia were referred for PNH study. Type II PNH and small PNH clone was observed in 12% (N=3) and in 15% (N=4) of patients, respectively. None of these 7 patients had Type III PNH cells.

Introduction

PNH is considered as a rare chronic disease and the estimated rate is 5-10 times less than that of aplastic anemia. It has been suggested that, like aplastic anemia, PNH may be more frequent in Southeast Asia and in the Far East, with a median survival of about 10.3 years. Morbidity depends on the variable expressions of hemolysis, bone marrow failure, and thrombophilia that define the severity and clinical course of the disease.

PNH is an acquired hematopoietic stem cell disease that is caused by a somatic mutation of the X chromosome-linked phosphatidyl-inositol glycan (PIG-A) gene.[1-3] Since the PIG-A protein is involved in the initial stage of synthesis of the glycosylphosphatidyl-inositol (GPI) anchor; these defects result in partial or absolute deficiency of all GPI linked proteins/glycoproteins in a clone of hematopoietic stem cells. It is characterized by nocturnal hemoglobinuria, chronic hemolytic anemia, thrombosis, pancytopenia and, in some patients, acute or chronic myeloid malignancies. It affects erythroid, granulocytic and megakaryocytic cell lineages. The abnormal cells in PNH have been shown to lack glycosylphosphatidylinositol (GPI)-linked proteins in erythroid, granulocytic, megakaryocytic and, in some instances, lymphoid cells.

A flow cytometric-based assay can detect the presence or absence of these GPI-linked proteins on granulocytes, monocytes, erythrocytes, and/or lymphocytes, thus avoiding the problems associated with red cell-based diagnostic methods (Ham's test) in which recent hemolytic episodes or recent transfusions can give false-negative results. A partial list of known GPI-linked proteins include CD14, CD16, CD24, CD55, CD56, CD58, CD59, C8-binding protein, alkaline phosphatase, acetylcholine esterase, and a variety of high frequency human blood antigens. In addition, FLAER is an Alexa® 488 labeled variant of aerolysin, a unique protein that binds tightly and specifically to GPI-linked proteins. PNH cells do not produce GPI-linked proteins, therefore FLAER will not bind to these cells. Before FLAER, detection of PNH clones by flow cytometry relied on fluorescently labeled antibodies to GPI-linked proteins such as CD59 and CD55. These antibodies do not bind with high affinity, so that small PNH clones are not detected. Also, CD59 and CD55 screen for the absence of a specific protein, rather than loss of the GPI-linked proteins. Since FLAER binds to the GPI-linked proteins, only PNH cells, which lack the GPI-linked proteins, will be negative. FLAER is more sensitive than CD59 in detecting small abnormal granulocyte population to a level of 0.5%. However, FLAER cannot be used to assess PNH clones in erythrocyte lineage, since the latter do not possess surface bound proteolytic enzymes needed to process the proaerolysin and further glycophorin is not GPI-linked protein which binds weakly to proaerolysin.

We, at GCRI, use multiparametric flowcytometry for the diagnosis of PNH including FLAER and follow an international consensus guideline.[4] On RBCs, CD55 and CD59 markers are used. FLAER is used in combination with GPI-linked CD antigens such as CD24, CD16, CD55 on neutrophils and CD14, CD55 on monocytes. The cut-off of >3% abnormal cells found was considered as PNH positive.

Materials and Methods

Patients: In the last two years, 26 patients (male N=16, female=10) with marrow hypoplasia or aplastic anemia were referred to the laboratory from the institute or outside the institute for PNH evaluation. Peripheral blood is preferred for PNH

Table-1: Panel of antibodies used against CD antigens

	On RBCs
Tube 1	Unstained
Tube 2	CD59 FITC (10µl)
	CD55 PE (10µl)
	On WBCs
Tube 1	CD33 PerCPCy5.5 (10µl)
	CD15 APC (5µl)
Tube 2	FLAER FITC (10µl)
	CD24 PE (10µl)
	CD33 PerCPCy5.5 (10µl)
	CD16 PECy7 (5µl)
	CD15 APC (5µl)
Tube 3	FLAER FITC (10µl)
	CD55 PE (10µl)
	CD33 PECy7 (5µl)
	CD14 PerCP (10µl)
	CD15 APC (5µl)

evaluation instead of bone marrow, because bone marrow contains immature precursors that will cause false positive results. The panel of antibodies used on RBCs and WBCs is mentioned in Table-1. With each suspected PNH sample, peripheral blood of healthy donor was analysed simultaneously with the same panel of antibodies for comparison with the normal marker pattern. The FLAER (Alexa 488 proaerolysin variant) was procured from Cedarlane Laboratories Products (Canada) through Bi Biotech India, and antibodies against surface CD antigens and reagents were procured from BD Biosciences (San Jose, CA, USA), and followed manufacturer's instruction.

Flowcytometric analysis on RBCs : Ten micro liter (10µl) of peripheral blood was washed with phosphate buffer saline (PBS), followed by centrifugation at 400g for 5 minutes. One millilitre of PBS was added to the pellet and from that 50µl sample was added into two tubes. The first tube was an unstained control run with omission of antibodies and to the second tube 20µl of CD59 FITC and CD55 PE antibodies were added. After incubation for 15 minutes at room temperature, the sample was washed twice with PBS, followed by centrifugation at 400g for 5 minutes. Then the pellet was resuspended in 500 µl of PBS.

Flowcytometric analysis on WBCs; Hundred

Table-2: Patient characteristics with PNH findings

No.	Age/ Sex	Bone marrow findings	PNH results	PNH clone on RBC	PNH clone on Neutophils	PNH clone on Monocytes
1.	36/F	Aplastic anemia	PNH clone not detected	-	-	-
2.	65/M	Aplastic anemia	PNH clone not detected	-	-	-
3.	13/F	Aplastic anemia	**Small clone detected**	-	+	-
4.	13/M	Aplastic anemia	**Small clone detected**	-	+	-
5.	41/M	Hemolytic anemia	**Type II PNH**	+	+	-
6.	22/F	Hemolytic anemia	PNH clone not detected	-	-	-
7.	21/F	Hemolytic anemia	PNH clone not detected	-	-	-
8.	18/F	Hypocellular marrow	PNH clone not detected	-	-	-
9.	16/F	Hypocellular marrow	PNH clone not detected	-	-	-
10.	19/M	Hypocellular marrow	PNH clone not detected	-	-	-
11.	13/M	Hypocellular marrow	PNH clone not detected	-	-	-
12.	60/M	Marrow hypoplasia	PNH clone not detected	-	-	-
13.	13/M	Marrow hypoplasia	PNH clone not detected	-	-	-
14.	32/F	Marrow hypoplasia	PNH clone not detected	-	-	-
15.	30/M	Marrow Hypoplasia	**Type II PNH**	-	+	+
16.	27/M	Moderate cellular marrow	**Small clone detected**	-	+	+
17.	11/M	Moderate marrow hypoplasia	PNH clone not detected	-	-	-
18.	22/F	Not done	PNH clone not detected	-	-	-
19.	27/M	Not done	PNH clone not detected	-	-	-
20.	18/F	Not done	PNH clone not detected	-	-	-
21.	22/M	Not done	PNH clone not detected	-	-	-
22.	38/M	Not done	PNH clone not detected	-	-	-
23.	21/M	Not done	PNH clone not detected	-	-	-
24.	20/M	Not done	PNH clone not detected	-	-	-
25.	35/M	Not done	**Type II PNH**	+	+	+
26.	6/F	Reactive marrow	**Small clone detected**	-	+	+

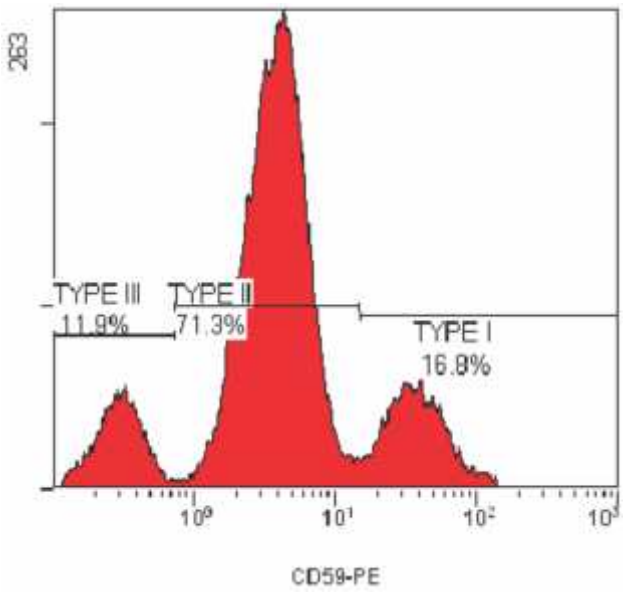

Figure 1: CD59 staining on RBCs showing Type I normal cells, Type II partial deficient PNH cells and Type III complete deficient PNH cells.[4]

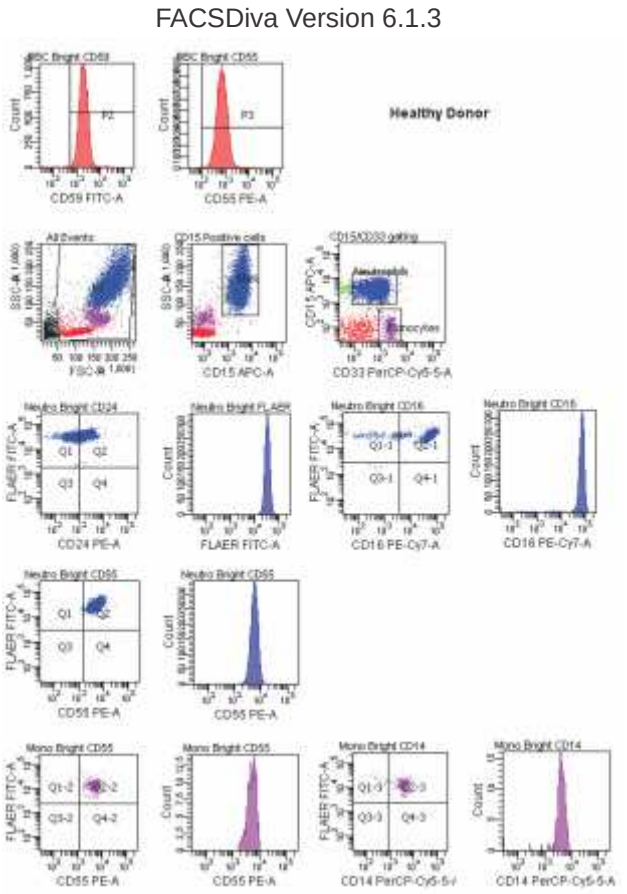

Figure 2: The normal pattern of GPI-linked proteins in a healthy donor.

micro liters (100 µl) of peripheral blood was incubated with 20 µl of respective antibody for 15 minutes as mentioned in Table-1. Then incubated with 2 ml of lysing solution (1:10 in DDW) for 10 minutes

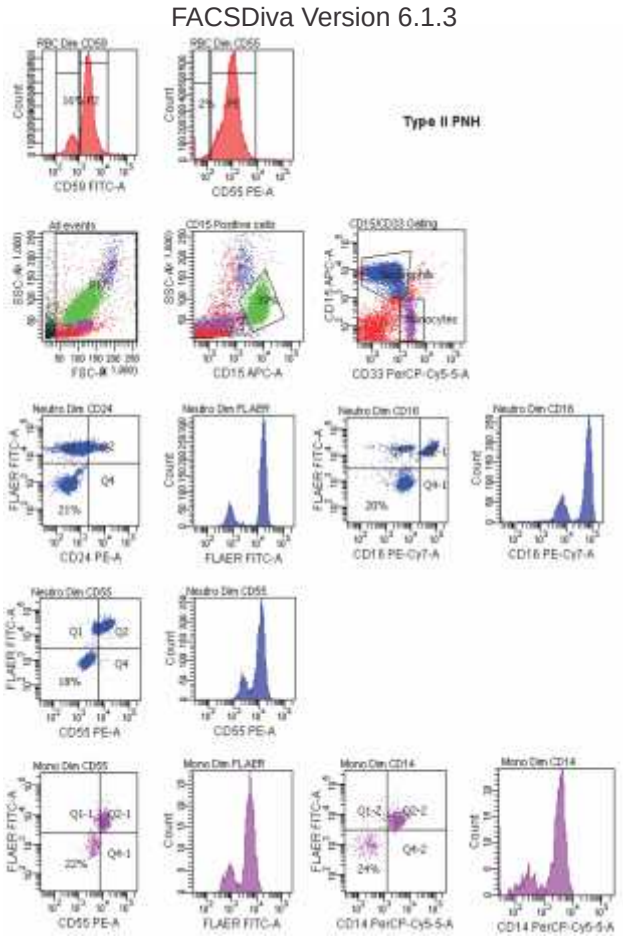

Figure 3: A case of Type II PNH. Partial deficient CD59 cells (16% Type II PNH) seen on RBCs. Dim FLAER vs CD24 (21%), CD16 (20%) and CD55 (18%) cells were seen on neurtrophils. Dim FLAER vs CD14 (24%), and CD55 22%), cells were seen on monocytes.

for RBC lysis, followed by centrifugation at 400 g for 5 minutes. Then pellet was washed twice with PBS and then resuspended in 500 µl of PBS.

Data acquisition and analysis: The samples were acquired in FACS Canto II flowcytometer and analysed in BD Diva software (BD Biosciences). At least 30,000 RBCs and 15,000 neutrophils and monocytes were acquired.

Gating strategy for the analysis of RBCs: RBSs were gated on log forward vs log side scatter plot, and on gated RBCs CD59 and CD55 expression was determined using histogram plot.

Gating strategy for the analysis of neutrophils and monocytes

All nucleated cells were identified on linear forward vs linear side scatter plot and population1 (P1) gate was drawn on all nucleated white blood cells to exclude debris after the red cell lysis step. On a second dot plot CD15 APC vs side scatter, lymphocytes with bright CD15 staining and high side scatter were gated (P2), further on third dot plot CD33

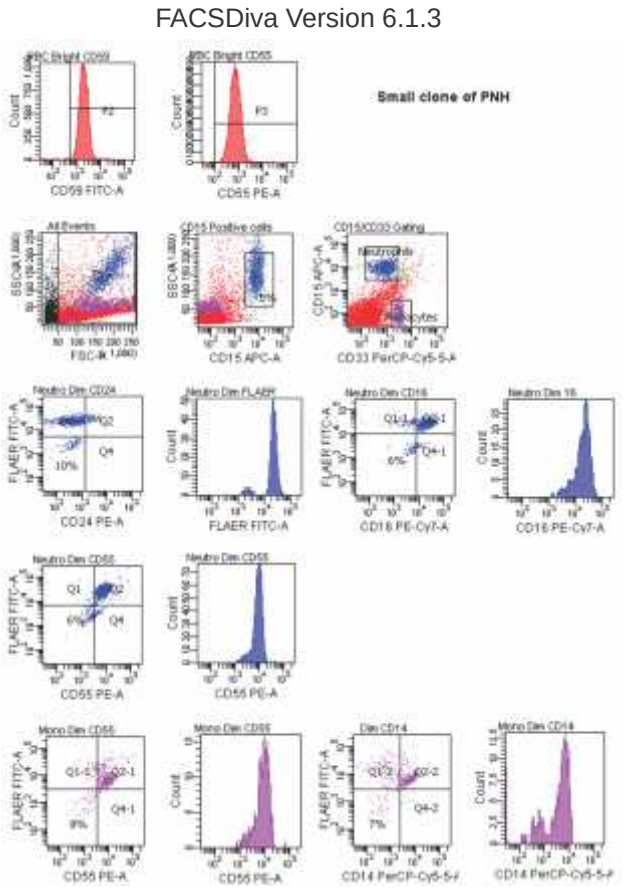

Figure 4: A case of small clone PNH. Normal cells (Type I) were seen on RBCs. Dim FLAER vs CD24 (10%), CD16 (6%) and CD55 (6%) cells were seen on neurtrophils. Dim FLAER vs CD14 (7%), and CD55 (8%), cells were seen on monocytes.

PerCP vs CD15 APC neutrophils (P3) and monocytes (P4) were gated. Then dot plots of FLAER vs CD55, CD24 and CD16, and dot plots of FLAER vs CD55, CD14, CD16 on monocytes along with histograms were made for further quantitative analysis.

Interpretation on RBCS: On RBCs, single colour histograms facilitate comparison of the level of RBC staining with that expected for normal and deficient CD59 or CD55 cells. Type I are normal cells, Type II partial deficient cells and Type III are complete deficient cells for GPI-linked proteins (Figure 1).

Interpretation on neutrophils and monocytes: For large clones it might be possible to have a single GPI-linked marker or FLAER and discriminate positive from negative events using a single parameter histogram, but for the majority of cases it is most desirable to use two different markers, and set quadrant markers or regions that readily identify the negative population of interest (Figure 2-4). Occasionally a population of type II granulocytes may be identified, especially when FLAER is used; in such cases it is important to combine both the type II and type III granulocytes to report the total PNH population.

Observations

The patient characteristics and PNH findings are depicted in Table-2. PNH evaluation was carried out on RBCs using CD55 and CD59 markers. FLAER was used in combination with GPI-linked CD antigens such as CD24, CD16, and CD55 on neutrophils and CD14 and CD55 on monocytes. CD15 and CD33 were used for neutrophils and monocytes gating. Normal marker pattern in healthy donor, Type II PNH cells on RBCs, neutrophils, and monocytes; and small clone of PNH on neutrophils and monocytes were depicted in Figure 2, Figure 3 and Figure 4, respectively.

Of 26 patients, 27% (7/26) of patients had Type II (N=3) or small PNH clone (N=4) and 73% (19/26) of patients had normal Type I cells. Type II PNH cells on all three lineages. RBCs, neutrophils and monocytes, and either of the two lineages were observed in one patient each. Small PNH clone on neutrophils and monocytes, and neutrophils alone was observed in two patients each. None of these 7 patients had Type III PNH cells.

Conclusion

By this multiparametric flow cytometry using FLAER, PNH clone was detected on neutrophils in 7 patients and on monocytes in 4 patients, whereas on RBCs, PNH clone was detected in only 2 patients using CD59 and CD55 alone. Thus, the FLAER assay on WBCs is a more sensitive and robust primary screening assay for detecting PNH clone. PIG-A gene mutation analysis is still limited to research laboratories and, although very specific, is still not diagnostic for PNH.

References

1. Miyata T, Takeda J, Iida Y et al. The cloning of PIG-A, a component in the early step of GPI-anchor biosynthesis. Science 1993; 259: 1318–1320
2. Takeda J, Miyata T, Kawagoe K et al. Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. Cell 1993; 73: 703–711
3. Bessler M, Mason PJ, Hillmen P et al. Paroxysmal nocturnal hemoglobinuria (PNH) is caused by somatic mutations in the PIG-A gene. EMBO J 1994; 13: 110–117
4. Borowitz MJ, Craig FE, Digiuseppe et al. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. Cytometry B Clin Cytom 2010; 78: 211-230

Prevalence of Hepatitis B Virus, Hepatitis C Virus and HIV Infection in Patients Attending The Gujarat Cancer & Research Institute (GCRI): A Call for Action

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Summary

This study shows awareness regarding the burden of disease related to blood borne viral infections and the need for taking actions to prevent HBV, HIV and HCV transmission. Chronic liver disease related to viral hepatitis has emerged as a public health problem. Many of these infections are acquired in health care settings. Implementation of infection control, injection safety and blood safety are major challenges in hospitals. The objective of this study is to provide prevalence of Hepatitis B, Hepatitis C and Human Immunodeficiency Virus (HIV) in the cancer patients attending The Gujarat cancer & Research Institute (GCRI). Total of 26267 blood samples were received in laboratory between January to December 2011 and 26267 serum samples were tested for HbsAg, 25912 tested for HIV and 12421 tested for HCV by Enzyme immunoassay Kits (Biomerieux Inc.) using Fully automated ELISA DVQ system. HIV reactivity was reported according to National AIDS Control Organization (NACO) guidelines. Of 26267 patients, 4.42% (1114/26267) had HBV, 0.77% (200/25712) patients had HIV and 0.72 % (90/12421) patients had HCV infection. And 5.91% patients had at least one type of infection. Seroprevalence of HbsAg positivity was highest amongst medical oncology group (7.94%) followed by pediatrics patients (5.44%) and HIV positivity was highest amongst medical oncology group (0.88%) followed by gynec oncology group (0.79%), and HCV seroprevalence was highest amongst medical patients (1.07%) followed by pediatrics patients (0.89%). This study demonstrates the seroprevalence of blood borne viral pathogens in cancer patients. The infection is highest in medical oncology group (7.94%). Key strategies to reduce HBV, HCV and also HIV transmission include promotion of injection safety and infection control in the health care setting, ensuring safety of blood and blood products, and reducing the demand of unnecessary blood transfusions. Special studies and enhanced surveillance activities are needed to refine prevention strategies and monitor the impact of prevention activities.

Introduction

Blood borne viral infection, such as Hepatitis B (HBV), Hepatitis C (HCV) and Human Immunodeficiency Virus (HIV) are of special concern to cancer patients. While the incidence of these blood borne pathogens is 0.69% for HBV, 0.42% for HCV and 0.18% for HIV in the general population (blood donor-GCRI), little is known about the current prevalence of the exposure amongst patients and health care workers.[3] Transmission of blood borne infections in the health care setting should be viewed within the overall context of health-care associated infections. These are emerging as a leading cause of infectious disease deaths worldwide and affect both developed and resource poor countries. Each year, over 1.4 million people develop serious infections for an unrelated condition during health care delivery. About 5-10% of hospitalized patients in developed countries acquire health care associated infections at any given time and the risk is 2-20 fold higher in developing countries. WHO has confirmed that this is a growing challenge to quality of health care in India.[1] During the past two decades, health care delivery systems in the country have experienced rapid introduction of new technologies, including provision of complex services and increasing numbers of staff performing invasive procedures for the patients either for diagnosis of diseases or treatment. The introduction of new technologies and advances in provision of services like endoscopic biopsies, laparoscopic surgeries, bone marrow biopsies, stem cell therapy, often occur without significant development of appropriate safeguards to prevent HBV, HCV and HIV infections.[1] Therefore, monitoring of blood borne viral infection

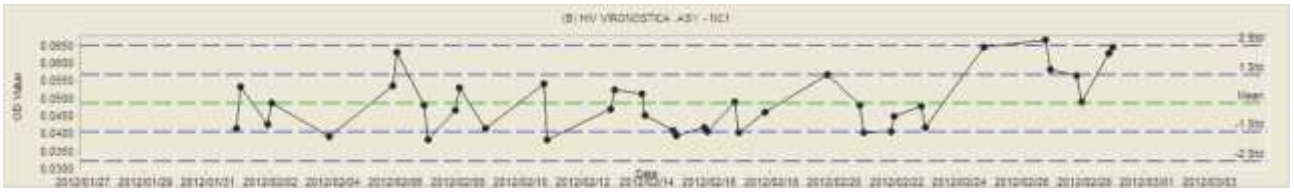

Chart I: L-J chart for HIV-NC

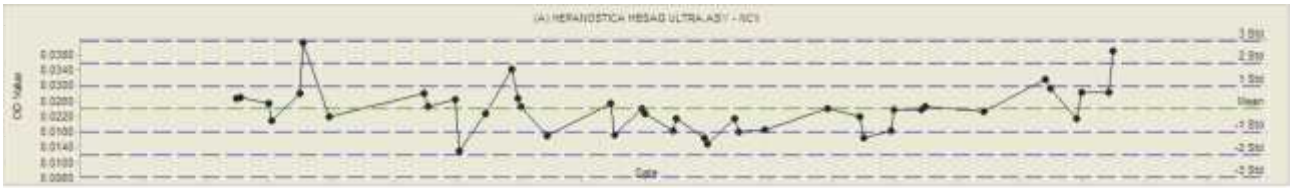

Chart II: L-J chart for HbsAg-NC

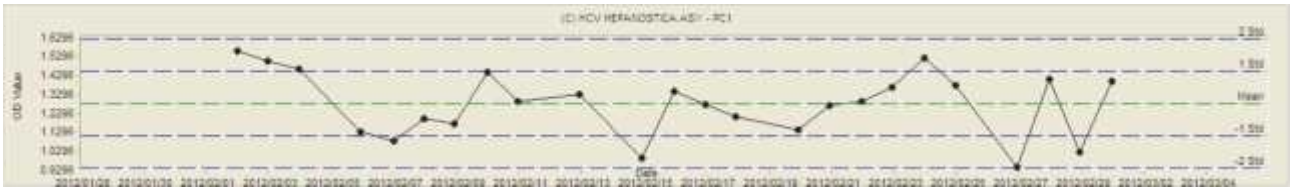

Chart III: L-J chart for HCV-PC

we found that the prevalence of HBV is 1.1 % .

Therefore we intended to have an awareness regarding the prevalence of these blood borne viral infections in GCRI and the need for taking appropriate actions to prevent HBV, HIV and HCV transmission.

Materials and Methods

This was a retrospective study carried out from January to December 2011, to know the prevalence of blood borne viral agents in cancer patients getting admitted to different oncology units like medical, surgery, gynecology etc. for various treatment and diagnostic invasive procedures. The clinical history of patients was recorded. Between this period a total of 26267 blood samples were received for screening of HbsAg, 25912 samples for HIV antigen and antibody and 12421 for anti-HCV from different units of the hospital. All samples were tested by Enzyme Linked Immuno Sorbent Assay (ELISA) using kits procured from Biomerieux, France on fully automated ELISA system (DA VINCI QUATTRO, Biomerieux, France). HIV, HBV and HCV infection were tested by using 4th generation ELISA kits, Virinostika HIV Uni-form II Ag/Ab kit for HIV Ag and Ab detection, Hepanostika HbsAg Ultra kit for HbsAg detection and Hepanostika HCV ultra kit for detection of anti-HCV.

All positive samples were tested twice to confirm the result according to the set guidelines, and HIV-positive samples were further confirmed by rapid immunochromatography using TRI-DOT kit (J.Mitra, India) and Enzyme linked Fluorescence assay (ELFA) using HIV-duo kit procured from Biomerieux on fully automated ELFA system (MINI-VIDAS, Biomerieux, France) according to NACO guidelines.

pathogens is expressed as the number of cases with a positive test result divided by the total number of cases tested. Comparisons of prevalence rates by patient's characteristics were performed using χ^2 testing. For all statistical analysis, a P value of less than 0.05 was considered significant. Statistical analysis was performed using SPSS (SPSS Inc, Chicago, IL version 13) and Epi-Info (version 3) statistical software.

Results

Between January to December 2011, out of total 64400 blood samples, blood borne viral infections was 5.91%. HBV infection was 4.42%, HIV was 0.77% and HCV was 0.72% in cancer patients in GCRI (Chart-IV). Table-1 shows the prevalence of infections in different units and departments of hospitals.

According to Chart-V, the prevalence of HBV infection was 43% in medical, 36% in surgical, 9% in paediatric and 6 % in gynec oncology group. Chart-VI shows prevalence of HIV infection and it was 49% in surgical, 25% in medical, 11% in gynec and 4 % in paediatric oncology group. Chart-VII shows prevalence of HCV infection which was 42% in surgical, 35% in medical and 11% in paediatric oncology group. Seroprevalence of blood borne pathogens was found to be highly significant (P=0.0001) as compared to seroprevalence of blood donor of GCRI.

Discussion and Conclusion

The prevalence of viral markers in patient population was higher (5.91 %) than that when compared with the blood donors (0.43 %) in our hospital. Prevalence of HBV infection is highest

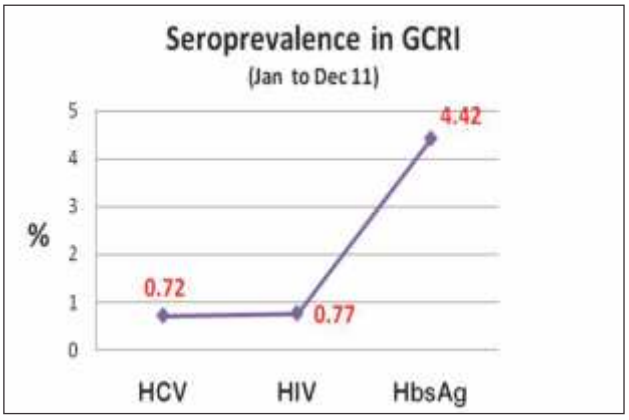

Chart IV: Seroprevalence in GCRI

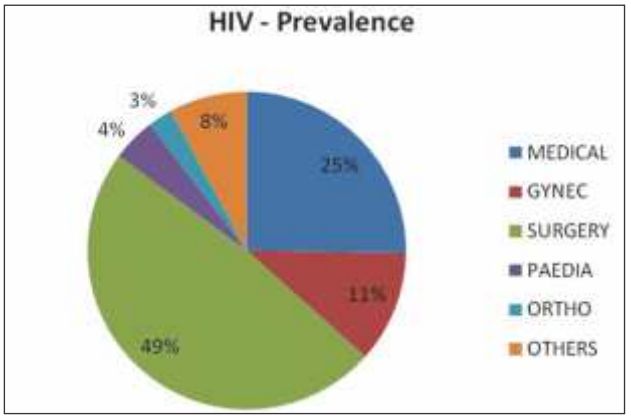

Chart VI : HIV Prevalence – Jan to Dec 2011

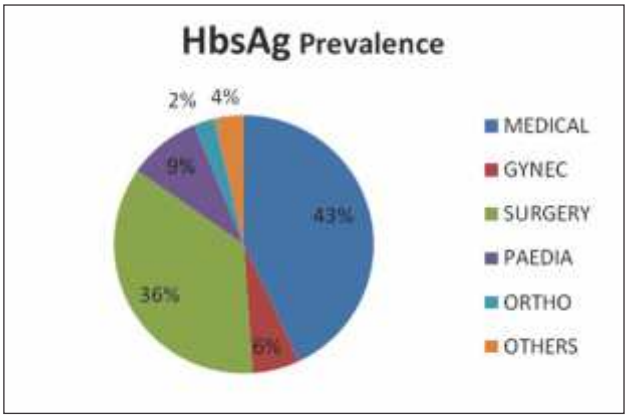

Chart V : HbsAg Prevalence – Jan to Dec 2011

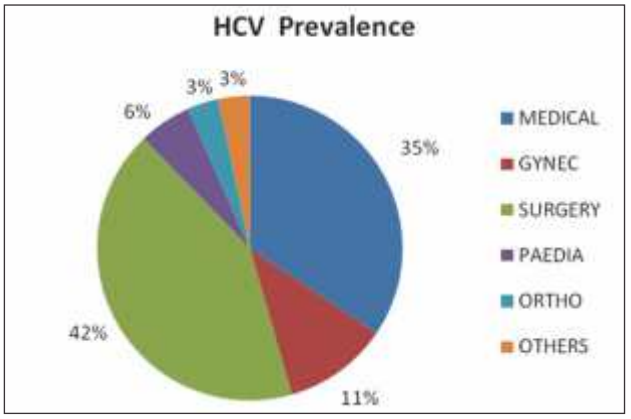

Chart VII : HCV Prevalence – Jan to Dec 2011

amongst the three, where as prevalence of HIV and HCV was lower when compared with other studies.[2,4,5] Midori Kato-Maeda et al showed that the prevalence of Hepatitis B virus was 6.9%, HCV was 7.8% and HIV was 3.3% in patients from emergency room of the Instituto Nacional de Ciencias Medicas y Nutricion in Mexico city which was higher as compared to our study.[3] A great proportion of the patients were unaware of their infection status which demonstrates that the clinical history is not enough to recognize those patients who may have had the blood borne viral infections.[3]

WHO estimates approximately 4.3 million persons are infected with HBV and 800 000 persons are infected with HCV each year. Most of these infections are acquired in the health care settings. It is estimated that approximately 17 million people have chronic HCV infection. Based on current treatment guidelines for hepatitis C, the cost to treat 50% of eligible patients is estimated to be >5625 billion rupees. The cost of treating patients with chronic HBV or HCV infection far outweighs the cost of implementing prevention programmes.

Key strategies to reduce HBV and HCV transmission include [1]

1. Promotion of injection safety and infection control in the health care setting recommending AD syringes
2. Ensuring safety of blood and blood products by routine screening
3. Reducing the demand for unnecessary transmission
4. The epidemiology of HBV and HCV infection is not well characterized in many countries. Special studies and enhanced surveillance activities are needed to refine prevention strategies and monitor the impact of prevention activities.
5. Cleaning and disinfections of endoscopes, gastroscopes and laproscopes by Orthothaldehyde must be followed as per the guidelines and practiced on a regular basis.

WHO Recommendations [1]

1. Expand hepatitis B vaccination programmes to include: provision of a birth dose of vaccine to all infants within the first 24 hours of life; vaccination of all persons with occupational exposure to blood and infectious body fluids; and vaccination of other high-risk populations, including injecting drug users.
2. Promote infection control through adoption of national guidelines and an accreditation process to monitor compliance.
3. Conduct necessary studies/surveillance activities to better understand the epidemiology of hepatitis

Table-1: Seroprevalence of blood borne virus infection in different oncology units of GCRI

Oncology Units	HbsAg			HIV			HCV		
	Total	Positive	%	Total	Positive	%	Total	Positive	%
Medical									
I	2271	165	7.27	2199	19	0.86	1324	10	0.76
II	2100	167	7.95	2020	14	0.69	524	8	1.53
III	1633	147	9.00	1545	18	1.17	1036	13	1.25
Total	**6004**	**479**	**8.07**	**5764**	**51**	**0.88**	**2884**	**31**	**1.07**
Gynecological									
I	1168	19	1.63	1168	7	0.60	170	2	1.18
II	934	26	2.78	933	8	0.86	667	4	0.6
III	820	21	2.56	809	8	0.99	302	4	1.32
Total	**2922**	**66**	**2.25**	**2910**	**23**	**0.79**	**1139**	**10**	**0.87**
Surgical									
I	3348	82	2.45	3346	18	0.54	304	3	0.99
II	2691	85	3.16	2704	16	0.59	670	8	1.19
III	2168	56	2.58	2156	16	0.74	1981	14	0.71
IV	1756	59	3.36	1742	7	0.40	1275	3	0.24
V	2203	71	3.22	2203	34	1.54	1770	7	0.40
VI	1316	45	3.42	1300	7	0.54	719	3	0.42
Total	**13482**	**398**	**2.95**	**13451**	**98**	**0.72**	**6719**	**38**	**0.56**
Paediatric									
I	527	35	6.64	503	3	0.60	176	2	1.14
II	782	28	3.58	764	5	0.65	139	1	0.72
III	622	38	6.11	594	1	0.17	244	2	0.82
Total	**1931**	**101**	**5.23**	**1861**	**9**	**0.48**	**559**	**5**	**0.89**
Orthopedic									
I	260	15	5.77	253	5	1.98	207	2	0.97
II	228	14	6.14	228	0	0.00	192	1	0.52
Total	**488**	**29**	**5.94**	**481**	**5**	**1.03**	**399**	**3**	**0.75**
Radiotherapy	671	15	2.24	672	6	0.89	160	1	0.63
Urology	63	3	4.76	65	0	0.00	11	0	0.00
Plastic Surgery	13	0	0	13	0	0.00	2	0	0.00
Neurology	563	16	2.84	563	6	1.07	509	2	0.39
TOTAL	**26267**	**1114**	**4.42**	**25912**	**200**	**0.77**	**12421**	**90**	**0.72**

C in selected countries.

4. Comprehensive training programs for HCPs (Health Care Practitioners) are fundamental and important tools in preventing the transmission of blood borne pathogens. HCPs who perform procedures that put them at risk for exposure to potentially infected blood or other body fluids should be educated about the risks of blood borne pathogen transmission. The training should include general information about blood borne pathogens, mechanisms of transmission, methods to prevent exposure to blood and other potentially contaminated fluids, and ways to implement those methods during various procedures. Preventing patient-to-HCP transmission as well as HCP-to-patient transmission should be included in the training program.

References

1. World health organization, Regional Committee for the Eastern Mediterranean, Fifty sixth, and August 2009: The Growing threats of hepatitis B and C in Eastern Mediterranean Region: a call for action.
2. Weiss E, Makary MA, Wang T, Syin D, Pronovost PJ, Chang D, Cornwell EE: Prevalence of Blood-Borne pathogens in an urban, university based general surgical practice. Annals of Surgery 2005; 241: 803-809
3. Midori KM, Ponce-de-Leon S, Sifuentes-Osornio

J: Blood borne viral infections in patients attending an emergency room in Mexico city Estimate of seroconversion probability in healthcare workers after an occupational exposure. Infection Control and Hospital Epidemiology 2000; 21: 600-602
4. Soreide K: Seroprevalence of blood borne viruses in Scandinavian trauma victims. Scandianavian Journal of Surgery 2007; 96: 88
5. Dessie A, Abera B, Wale F: Seroprevalence of major blood-borne infections among blood donors at Felege Hiwot referral hospital, northwest Ethiopia. Transfusion Science 1997; 18: 167-179
6. Simmons JH: Development, application and quality control of serology assays used for diagnostic monitoring of laboratory nonhuman primates. National Academy of Sciences 2011; 49: 157-170
7. Ananthanarayan and Paniker's Textbook of Microbiology, 8th Edition 2009 :536-546

Experience with Hickman Catheter and Implantable Ports at GCRI

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Summary

A retrospective study of Hickman and implantable ports for a period of one year was conducted and there were a wide variety of indications for Central Venous Catheter (CVC) in this study. Implantable ports had least complications and hence they had longer durability as compared to Hickman catheter.

Introduction

The development of increasingly complex treatment regimens for patients with malignancies have led to a greater reliance on a variety of intravenous delivery systems. Patients who have cancer usually require repeated venous punctures for treatment monitoring, administration of chemotherapy or blood transfusions. Therefore, central venous catheters and implantable port systems have substantially facilitated the problem of vascular access. However, CVCs have inevitable problems such as insertion-related and long-term complications despite providing benefits to patients. The incidence of CVC-related complications is relatively high based on data from a study commissioned by the Food and Drug Administration in the 1990s, which showed a 10-25% complication rate and 10% morbidity.[1] Insertion- related complications have dramatically decreased since the use of ultra sonogram (USG) guidance, although one report described negative effects of USG.[2] Major complications related to CVCs include infection and thrombosis.[3,4] Various management strategies have been recommended to prevent and treat infection and thrombosis,[5] and CVC outcomes have improved considerably. Along with improvements in insertion technique and CVC management, improvements have been made in catheter design and material, which have contributed in reducing CVC related complications. A central venous catheter currently represents the most frequently adopted intravenous line for patients undergoing infusional chemotherapy or high-dose chemotherapy. Therefore, this study was conducted to study function and complications of Hickman catheter and implantable ports.

Materials and Methods

This is a retrospective study of continuous function and complication rates of 103 CVCs consecutively placed in children (N=79) and adults (N=34) at our institution over a period of one year (1st January to 31st December 2010). Total of 80 Hickman and 23 ports were studied. The age groups ranged from 1 month infant to 62 year old adult. Average time between admissions to Hickman catheter was 15-20 days where as for ports it was 7 days. Wide variety of indications such as hematological malignancies (25 Acute Leukemia and 15 Lymphomas), solid tumors such as Primitive Neuro Ectodermal Tumor (PNET) and soft tissue sarcomas (N=18), other solid tumors (N=29), retinoblastoma (N=9) and non-malignant conditions (Aplastic anemia and Thalassemia Major N=7) were included (Figure 1).

Results

Compared with implantable ports, Hickman catheters were associated with a significantly higher rate of bloodstream infections (major complication), shorter time to first infection, shorter duration of catheterization and higher rate of removal because of mechanical complications. Gram-positive bacterial infections were more prevalent in both Hickman and port, two had exit site infection, one had hemorrhagic pleural effusion and one patient of hematopoietic stem cell transplantation had fungal infection. Four out of fifteen complicated Hickman's were salvaged. Overall, when catheter failures (removal) for infection, obstruction or dislodgement were considered, ports had a significantly longer failurefree duration (median 200 days) of use than did Hickman catheter (118 days). Both infectious and non-infectious complications in Hickman catheters and ports were 18.75% vs 8.7% respectively. Among these complications, 8 patients (7.7%) had infectious complications, 3 patients (2.91%) each had

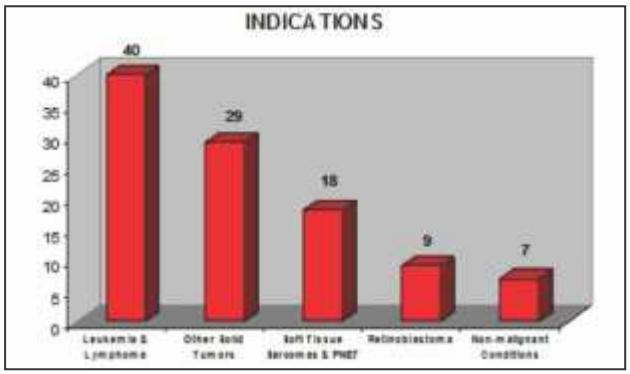

Figure 1: Various indications of Hickman catheter and implantable ports

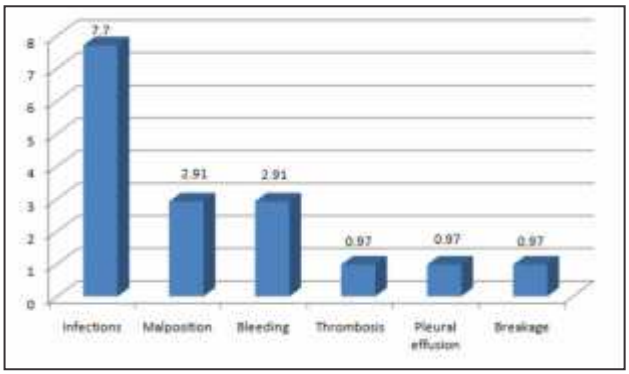

Figure 2: Various complications of Hickman catheter and implantable ports

malposition and bleeding and one patient (1%) each had thrombosis, pleural effusion and breakage of catheter (Figure 2).

Discussion

CVCs have a very important role throughout the management of an oncology patient. The insertion of a CVC may be the initial therapeutic step in some patients who have cancer (poor venous access and prolonged chemotherapy). Catheter-related infection occurred in 7.7% of patients in this study, and was observed more in Hickman catheters than in ports. This particular complication is less compared to Korean study where in it was 12.8%.[6] Possible explanation for fewer incidences of infectious complications was that in our study we did not include PICCs (peripherally inserted central catheter). The incidence of thrombosis was 1% in our study, which was relatively low despite not using prophylactic anticoagulation. Hyun et al have reported 8 incidences of thrombosis among 179 episodes of catheterization.[6] Median duration of port life was 200 days in the present study which was comparable to the study by Hyun et al, which had a median duration of port life of 269 days and a longer catheter life span with ports.[6] However, a prospective study comprising of much larger number of patients would be an ideal study.

Conclusion

This study shows that even in limited resource settings Hickman catheter has an acceptable role with acceptable complications but for long term use implantable ports are superior.

References

1. Scott WL: Central venous catheters. An overview of Food and Drug Administration activities. Surg Oncol Clin N Am 1995; 4: 377-393
2. Mansfield PF, Hohn DC, Fornage BD, Gregurich MA, Ota DM: Complications and failures of subclavian-vein catheterization. N Engl J Med 1994; 331: 1735-1738
3. Yildizeli B, Lacin T, Batirel HF, Yuksel M: Complications and management of long-term central venous access catheters and ports. J Vasc Access 2004; 5: 174-178
4. Cortelezzia A, Fracchiolla NS, Maisonneuve P et al: Central venous catheter-related complications in patients with hematological malignancies: a retrospective analysis of risk factors and prophylactic measures. Leuk Lymphoma 2003; 44: 1495-1501
5. McGee DC, Gould MK: Preventing complications of central venous catheterization. N Engl J Med 2003; 348: 1123-1133
6. Kim HJ, Yun J, Kim HJ et al: Safety and Effectiveness of Central Venous Catheterization in Patients with Cancer: Prospective Observational Study. Korean Med Sci 2010; 25: 1748-1753

An Indian Experience of Bfm 93 Protocol in Pediatric Acute Myeloid Leukemia (AML)

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Summary

We report a series of twenty-seven newly diagnosed paediatric Acute Myeloid Leukemia (AML) patients treated with BFM 93 protocol. Overall 84.61% of the patients achieved complete response (CR), mortality was 11.11% and 3.84% of patients had persistent disease.

Introduction

Pediatric AML patients have poor prognosis and outcome as compared to pediatric Acute Lymphoblastic Leukemia patients.[1] BFM 93 protocol has shown 5-year survival, event-free survival (EFS), and disease-free survival rates of 60%, 51%, and 62% respectively, in paediatric AML after introduction of high-dose cytarabine and mitoxantrone (HAM) in high risk patients.[2] We report our experience with this high intensity protocols (BFM 93) in our patients who are likely to be genetically, physically and nutritionally different from western population..

Materials and Methods

Study of clinical and hematological profile, cytogenetic abnormalities and induction outcome with BFM 93 protocol. Newly diagnosed AML patients, up to 14 years, between May 2008 to December 2009 were treated with BFM 93 protocol. Induction was given with three drugs (Ara-C 100 mg/m^2 days 1 and 2, followed by 12 hourly on days 3 to 8; daunorubicin 30 mg/m^2 12 hourly on days 3 to 5; and Etoposide 150 mg/m^2 on days 6 to 8, and HAM intensification block (high-dose Ara-C 3 g/m^2 every 12 hours for 3 days and mitoxantrone 10 mg/m^2 days 4 and 5) was given in high risk patients (M0,M5,M6,M7 morphology and greater than 5% blasts in bone marrow post 15 day of induction) followed by consolidation, intensification and maintenance chemotherapy according to BFM 93 protocol.[2]

Results and Analysis

Total 27 newly diagnosed AML (19 male and 8 female) patients, up to 14 years of age were treated with BFM 93 protocol, between May 2008 and December 2009. Median age of presentation was 7 years. Common symptoms were fever (96%), weakness (37%) and bleeding (33%). Common findings were pallor (77%), lymphadenopathy (44%) and infections (37%). Anemia was seen in 25 patients (92.60%) - median haemoglobin (Hb) level of 6.5 gm/dl, 25 patients (92.60%) had abnormal total leucocyte count (TLC) with a median of 37,400/mm^3, 26 patients (96.29%) had thrombocytopenia - median PC of 22,000/mm^3. (Table-1)

Table-1: Major symptoms at presentation (n = 27)

Symptoms	No of patients	Percent
Fever	26	96.29
Weakness	10	37.03
Bleeding episodes	9	33.33
Bodyache	5	18.51
Cough	5	18.51
Proptosis	2	7.40

Morphology was reported in 24 patients, 21 had favourable morphology (5- M1, 15- M2, 1-M4E0) & 3 had unfavourable morphology (M5, M7, M0, 1 each). Cytogenetics was reported in 17 patients, 10 had good risk, (7- t(8;21) and 3- inv16) and 7 patients had high risk cytogenetics (2-hypodiploidy, 1-hyperdiploidy, 1 +8 , 1 complex karyotype and 2- normal karyotype). (Table-2)

The median number of days required for recovery of counts was 20 with a range of 14 to 50 days. Neutropenia occurred in all 27 patients while 25 (95.59%) were febrile during neutropenic period. Other common complications were diarrhea in 15, pneumonia in 11 and enterocolitis in 3 patients. Blood culture was positive in 7 out 25 patients who had febrile neutropenia, Staph. aureus was most common isolate grown in 4 out of 7 patients (57.14%). Stool culture was positive in 8 out of 13 patients who had diarrhea & E .coli was most common isolate in 6 out of 8 (75.0%) stool samples. (Table-3)

Induction outcome: Twenty-two patients

Table-2: Cytogenetics and morphology at presentation

Cytogenetics	No of patients N=17	Percent of patients	Morphological subtypes
t(8;21)	4	22.20	M2 = 3
t(8;21),delY	3	16.66	M2 = 2 M1 = 1
t(16;16)/inv(16)	1	5.55	M2 = 1
inv(16),+22	1	5.55	M2 = 1
inv(16),+22,+8	1	5.55	M4 = 1
+8	1	5.55	M2 = 1
hypodiploidy	2	11.10	M2 = 1
hyperdiploidy	1	5.55	M7 = 1
normal karyotype	2	11.10	M1 = 1 M2 = 1
complex	1	5.55	M5 = 1

Table-3: Major complication during induction chemotherapy

Complication	No of patients	Percent of patients	Mortality
Febrile neutropenia	25	95.59	
Loose motion	15	55.55	
Pneumonitis	11	40.74	1
Entrocolitis	3	11.11	2
Thrombophlebitis	5	18.51	
Bleeding	7	25.92	
Hickman site inf.	3	11.11	
Mucositis	3	11.11	
Neurological complication	2	7.40	

(84.61%) achieved remission with BFM 93 Protocol induction. Total 5 patients (3 having persistent disease and 2 with unfavorable morphology) received HAM chemotherapy and 1 patient had persistent disease. (Table-4)

Table-4: Morphological subtypes and induction outcome (n=24)

Subtypes	No of patients	Remission	Death
Mo	1	0	
M1	5	5	
M2	15	12	2
M4	1	1	
M5	1	1	
M7	1		1
Total	**24**	**22**	**1**

Induction mortality was 3 (11.11%) after 8+3+3 chemotherapy, 1 patient died due to pneumonia and 2 due to enterocolitis. There was no mortality with HAM.

Two out of 6 patients (33.33%) who had TLC>1 lac at presentation did not achieve a complete response (CR) with 8+3+3 chemotherapy. All three patients who died during induction had TLC< 20000 at presentation.

Fourteen out of 15 patients (93.33%) who attained absolute neutrophil count (ANC) 0 within 5 days were found to be in CR on subsequent marrow examination. On the other hand, 3 patients out of 4 (75%) who had persistent disease after 8+3+3 chemotherapy attained ANC 0 after 5th post chemotherapy day. So, attaining an ANC 0 within first 5 days after chemotherapy could be used as a surrogate marker for PCT day15 bone marrow examination as it is easier and less traumatic to perform.

We evaluated cytogenetic at presentation with induction outcome. Nine patients out of 10 (90%) with good risk cytogenetic achieved CR after 8+3+3 chemotherapy. Out of 7 patients with intermediate and high risk cytogenetic, 4 patients (57.14%) achieved remission after 8+3+3 chemotherapy. (Table-5)

Discussion

Pediatric AML patients have poor prognosis and outcome as compared to pediatric Acute Lymphoblastic Leukemia patients.[1] The BFM 93 protocol is one of the most commonly used and highly efficient protocol for newly diagnosed pediatric AML patients at present. The BFM 93 study has reported a CR rate of 82.2% with a mortality of 7.45 and 10.4%

Table-5: Cytogenetic at presentation and induction outcome

Cytogenetics	No. of patients N=17	Induction outcome			
		Remission after 8+3+3	Remission after HAM	Progressive disease	Death
Good Cytogenetics					
t(8;21)	4	3	1	0	0
t(8;21),del Y	3	3	0	0	0
t(16;16)/inv(16)	1	1	0	0	0
inv(16),+22	1	1	0	0	0
inv(16),+22,+8	1	1	0	0	0
Bad Cytogenetics					
+8	1	1	0	0	0
hypodiploidy	2	1	0	1	0
hyperdiploidy	1	0	0	0	1
normal karyotype	2	1	1	0	0
complex	1	1	0	0	0

patients had persistent disease. The CR rates of 84.61% in our study are similar to BFM study but the mortality rate of 11.11% is higher than that seen in BFM study. The possible reason for less mortality in BFM studies may be due to the improved supportive care and better experience of BFM group with these protocols as mortality reported with earlier protocols i.e. BFM 78, 83 and 87 were 13%, 12% and 9% respectively, which are almost similar to mortality seen in our study.[3]

In our study 15 patients out of 24 (62.5%) attained ANC 0 within 5 days of completion of chemotherapy. Fourteen out of these15 patients (93.33%) who attained ANC 0 within 5 days were found to be in CR on subsequent marrow examination. On the other hand, 3 patients out of 4 (75%) who had persistent disease after 8+3+3 chemotherapy attained ANC 0 after 5th post chemotherapy day.

So, attaining an ANC 0 within first 5 days after chemotherapy could be used as a surrogate marker for PCT day 15 bone marrow examinations as it is easier and less traumatic to perform and all patients may not be suitable for bone marrow examination due to serious infection and poor general condition. Moreover patients who achieve ANC 0 after 5 days can be selectively subjected to bone marrow examination on PCT day 15 for risk stratification. This observation is derived from small number of patients and needs to be tested in a larger study.

Cytogenetics was not used in BFM 93 protocol for risk stratification of paediatric AML patients. We evaluated cytogenetic at presentation with induction outcome. Nine patients out of 10 (90%) with good risk cytogenetic achieved CR after 8+3+3 chemotherapy. Out of 7 patients with intermediate and high risk cytogenetic, 4 patients (57.14%) achieved remission after 8+3+3 chemotherapy.

Considering cause of death in the BFM 93 study[2], 81.81% of the patients died due to pneumonia where as 9.09% patients died due to enterocolitis and septicemia each. In present study enterocolitis was the underlying cause of death in 66.66% patients and pneumonia was responsible in 33.33% patients. Ten patients out of 11 (90.90%) who had radiologically diagnosed pneumonia recovered in our study where as 2 patients out of 3 (66.66%), who had enterocolitis, died.

BFM93 protocol was found to be effective and safe in our population and should be considered in all paediatric AML patients who can afford the chemotherapy and supportive care.

Conclusion

The results were comparable to original BFM 93 with manageable toxicity in our set of population. With the help of better experience, monitoring, support care in form of antibiotics and blood component and financial support (as given by Gujarat State School Health Program in most of our patients), we can definitely achieve comparable results as good as western data with such high intensity and efficient protocol in our set of pediatric AML patients .

References

1. Lt. Col.H P Singh, Col. Ranga Rao, Col Velu Nair, Lt Col A J Veniyoor: Indian Journal Of Medical and Paediatric Oncology. 2005; 26: 10-14
2. Creutzig U, Ritter J, Zimmermann M et al: Improved Treatment Results in High-Risk Pediatric AML Patients After Intensification With High-Dose Cytarabine and Mitoxantrone: Results of Study Acute Myeloid

Leukemia–Berlin-Frankfurt-Münster 93 Journal of Clinical Oncology, 2001; 19: 2705-2713
3. Creutzig U, Zimmermann M, Lehrnbecher T: Less Toxicity by Optimizing Chemotherapy, but Not by Addition of Granulocyte Colony-Stimulating Factor in Children and Adolescents With Acute Myeloid Leukemia: Results of AML-BFM 98 Journal of Clinical Oncology 2006; 24: 4499-4506

Diagnostic Dilemma in Gynecologic Oncology: Role of PET-CT Scan

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Summary

In the last two decades, the invention of positron emission tomography (PET), a non invasive method, using the radionuclide-labeled analogue of Glucose,[18]Fluro-deoxyglucose (FDG), which is one of the major source of energy in cancer cells, makes possible for us to detect regional metabolism in metabolically active tumor foci more accurately than with that of morphologic imaging techniques. We are presenting anecdotal cases to study the role of PET-CT in the management of different gynecological malignancies in the patients who have registered for treatment in Unit III, Department of Gynecologic Oncology at Gujarat Cancer & Research Institute (GCRI) from June 2011 to February 2012. Integrated PET-CT imaging offers high diagnostic accuracy both at diagnosis and in the evaluation of suspected tumor recurrence and persistent disease in selected cases. In addition, it provides high management impact and superior prognostic stratification compared with conventional techniques in the restaging of a range of malignancies. The potential use of PET-CT appears promising in several decision making steps in the management of patients with gynecological malignancies. It is possible that the addition of PET to the oncologist's imaging armamentarium may ultimately improve both outcomes and costs by altering management strategies in primary and recurrent settings.

Introduction

Computed tomography scan (CT-scan) and magnetic resonance imaging (MRI) are anatomic, high resolution imaging techniques that are commonly used to guide the management of patients with gynecologic cancers. Despite their widespread use, concerns remain that use of these conventional imaging techniques may result in false negatives due to their inability to resolve small volumes (diameter<1 cm) of disease and false positives due to their inability to distinguish between viable tumor masses and masses consisting of necrotic or scar tissue. In the last two decades, the invention of positron emission tomography (PET), a non invasive method, which can establish the metabolic or functional parameters of tissue, may aid in these distinctions. Instead of using anatomical deviations to identify areas of abnormality, PET uses positron-emitting radioactive tracer that accumulates in abnormal tissue. The most commonly used radioisotope tracer is [18]Fluro-deoxyglucose (FDG), a glucose analog which is preferentially taken up by and retained within malignant cells. Depending on the area or organ under study, baseline glucose metabolism may be low, further establishing the difference between normal background tissue and tumor. As PET alone lacks in its ability to give precise anatomical information, combined PET-CT integrating morphologic data of CT-scan and functional data of PET have gained wide clinical acceptance. Thus, compared to structural imaging techniques, FDG-PET has the potential to be a more accurate technique for diagnosis, staging and treatment decisions in oncology.[1-4]

Materials and Methods

Patients: A retrospective analysis was conducted in anecdotal cases to study the role of PET-CT in the management of different gynecological malignancies in the patients who have registered for treatment in Unit III, Department of Gynecologic Oncology at GCRI from June 2011 to February 2012. Of these five patients, clinical information, hematological and radiological investigations, PET-CT studies and histopathological reports were considered.

Imaging techniques : Protocol: PET-CT was performed with BGO plus, Full ring PET-CT (GE Discovery 600) using radio isotope [18]Fluro-deoxyglucose-370 MBq and 60 minutes uptake period. Study mode was PET-3D mode and CT 120 Kv AutomA. Extent of study is from vertex to upper mid of thigh. The standardized uptake values (SUV) values mentioned are in gm/ml. All subjects were studied in fasting condition (>6h) and only patients with blood glucose levels less than 140mg/dl were injected with ([18]F) FDG. Diluted oral contrast and water is given with I.V. contrast for non diagnostic CT-scan protocol. In addition, all patients were orally

hydrated during the (^{18}F) FDG uptake period and were asked to empty their bladder before positioning on the tomograph bed. Emission imaging started 60 min. after intravenous bolus injection of (^{18}F) FDG.

Image analysis: CT-scan and PET images were evaluated separately and as fused images in consensus by the same nuclear physician and radiologist. The peak SUVs were measured for each lesion with FDG accumulation. The SUVmax is a unique and noninvasive marker for studying the biochemical and metastatic changes in cancer tissues. SUVmax value has been reported to be correlated with tumor proliferation, tumor grade and expression of glucose transporters, all of which are biomarkers for various types of malignant tumors. If SUV was over 3 gm/ml, it was determined as positive.[5-7]

Results

Summary of indication for PET-CT is as follows:

Case 1

Mrs. A, a 34 year old lady diagnosed as a case of cervical carcinoma stage Ib_1 was subjected to radical abdominal hysterectomy with radical pelvic lymph node dissection on 5th April 2010. On Histopathological examination (HPE) of her surgical specimen, she was found to be an intermediate risk, node negative. Adjuvant radical radiotherapy (RT) was given. She was advised 3 monthly follow up for first 2 years. Fifteen months after her surgery, clinical examination and Pap test were negative but ultrasonography (USG) was suggestive of possibility of a nodal mass along right iliac vessels. Repeat USG was performed after 1 month. The lesion persisted therefore we performed USG guided fine needle aspiration cytology (FNAC) from the solid area. Pathology report was no evidence of malignancy. However USG after 1 month showed the persistent nodal mass of same size. CT-scan revealed thick walled, same sized peripherally enhancing hypodense lesion along right iliac vessels, possibility of nodal mass. There was no regression in the size of lesion. In view of persistent nodal mass, decision for PET-CT scan was taken which showed non hypermetabolic cystic mass lesion of size 34x33 mm involving right external iliac region (SUV max-Initial-2.1, delayed-2.6). In view of young age, persistent nodal mass, negative FNAC and PET-CT scan findings suggesting no evidence of disease elsewhere in body except nodal mass, decision for surgery was taken. Extraperitoneal right pelvic nodal mass removal was done on 26th September 2011. HPE revealed no evidence of malignancy. Patient is on follow up and disease free till date.

Case 2

Mrs. B, a 22 year old female was referred to GCRI on 25th May 2011 after left salpingo oophorectomy for dysgerminoma. She was unstaged but was given 1 cycle of chemotherapy (CT) - Paclitaxel and Carboplatin. Clinical examination was normal, tumor markers for germ cell tumor (GCT) were normal but. USG was suggestive of 56x24x35 mm sized multiloculated ovarian cyst on right side. Medical oncologist referred the case for reexploration to decide the nature of right ovarian mass. Since reexploration and ovarian biopsy can lead to interference in future pregnancy, PET-CT was planned. Comments were hypermetabolic nodule in left adnexa, non FDG avid right ovarian cyst and absence of FDG avid lesion in rest of the body scanned. On basis of these findings, reexploration was deferred and patient received 2 more cycles of CT. At present she is on regular follow up with no evidence of disease.

Case 3

Mrs. C, a 36 year old female patient, $G_3P_0A_3L_0$, was referred with HPE report of choriocarcinoma with preevacuation β hCG report of 1,93,000 IU/L on 8th June 2011. After investigations and metastatic work up, her final diagnosis was Gestational Trophoblastic Neoplasia, FIGO stage III: 8 (high risk with lung metastasis). She was given 4 cycles of etoposide, methotrexate, actinomycine, cyclophosphamide and vincristine (EMA CO). Third cycle of CT was delayed for 1 week due to neutropenia. As there was rise in β hCG level, patient was given EMA EP (cisplatin) after metastatic work up. After 2 cycles of EMA EP i.e. 12 weeks after starting CT, β hCG titre started increasing. It was 30 IU/L after 7th cycle of CT which increased to 238 IU/L after 8th cycle. In view of still rising β hCG in a nulliparous patient decision for hysterectomy/change of chemotherapy was to be taken and so PET-CT scan was planned. Findings were moderate FDG enhancing (SUV max-5.60) hypodense to cystic lesion 58x32 mm in endometrial cavity with peripheral soft calcification showing loss of endomyometrial interface and heterogeneous myometrial enhancement (Figure 1-2) suggestive of residual lesion. There was no other abnormal FDG uptake seen elsewhere in the body. In view of rising β hCG titre, history of life threatening complication of chemotherapy (neutropaenia) and findings of PET-CT scan suggesting active lesion only in endometrial cavity, hysterectomy was planned after informed consent. Exploratory laparotomy followed by total abdominal hysterectomy and right salpingectomy as there was hydrosalpinx was done on 25th November 2011. Intra operative findings were 5x4 cm firm smooth growth found perforating the serosa of uterus.

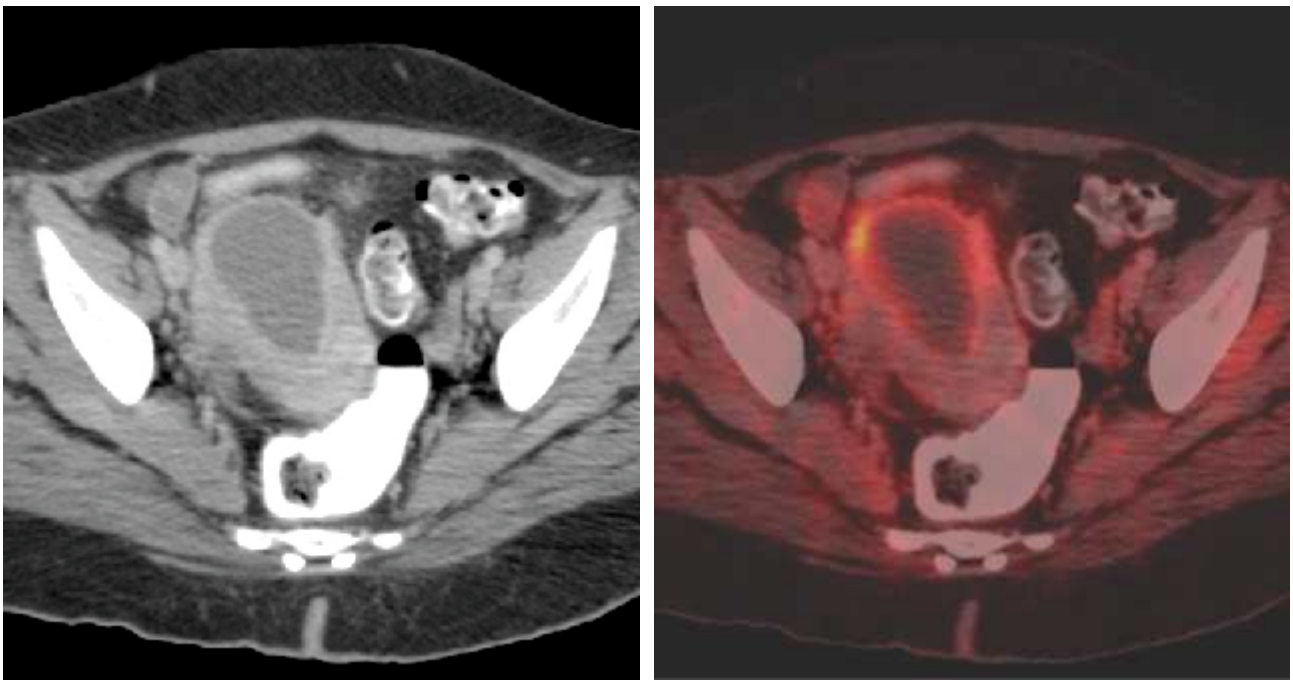

Figure 1-2 : FDG avid hypermetabolic lesion involving the endometrial cavity favour persistant active diseases

HPE confirmed choriocarcinoma of uterus, perforating the serosa and showing viable cells. Post operative period was uneventful. Patient is in remission and on close follow up.

Case 4

Mrs. D, a 48 year old multiparous female patient was referred to GCRI in December 2011 after subtotal hysterectomy with right oophorectomy + cystectomy for low grade fibrosarcoma on immunohistochemical examination (IHC). Her clinical examination was normal. As such there are no tumor markers for sarcoma. Her CT-scan thorax and CA 125 were normal but CT-scan abdomen & pelvis findings showed an inhomogenously enhancing soft tissue lesion in right adnexa suggestive of residual lesion more likely than post operative changes. As she was unstaged patient, decision for PET-CT was taken to rule out any metastatic lesion. It revealed nonmetabolic right adnexal mildly enhancing lesion suggestive of post operative changes, absence of FDG avid disease in operative bed or elsewhere in the body scanned. Completion of surgery followed by bilateral salpingo-oophorectomy + bilateral pelvic lymph node dissection + infracolic omentectomy with nil residual disease was done. HPE report revealed no evidence of malignancy. After reviewing the literature. She was advised adjuvant paclitaxel + carboplatin for 4-6 cycles.

Case 5

Mrs. E, a 32 year old multiparous female patient was referred after unstaged surgery (right ovarian mass removal + total abdominal hysterectomy + omental sampling) with HPE report of serous cystadenocarcinoma of right ovary on June 2010. She received 7 cycles of cisplatin + cyclophosphamide. She was advised 3 monthly regular follow up with clinical examination, USG and CA 125 levels for first two years. Twelve months after completion of CT, her clinical examination was normal but CA 125 titre was raised to 49.3 U/L and USG revealed 21x11 mm sized node in mid aortocaval region. PET-CT scan was advised to determine extent of metastatic disease. It showed solitary hypermetabolic (SUV max-9.9) 17x12 mm lymph node involving aorotcaval region at the level of renal hilum (Figure 3-4) and no evidence of disease elsewhere in the body. Based on these findings exploratory laparotomy followed by paraaortic lymphnode dissection with total omentectomy was done in which 20x15 mm paraaortic lymph node above renal vein was removed. HPE showed metastatic papillary adenocarcinoma in paraaortic lymphnode. By removing recurrent lesion the chances of disease free survival will improve for the patient.

Discussion

Our institute is a tertiary referral cancer centre. It is not unusual to have suboptimally operated, unstaged patient referred to us for further management. Thus patients pose a therapeutic dilemma. Despite continuing advances in surgical and non-surgical therapeutic strategies, cancer recurrence and distant metastasis after initial treatment are often a major problem for women with gynecologic cancers.

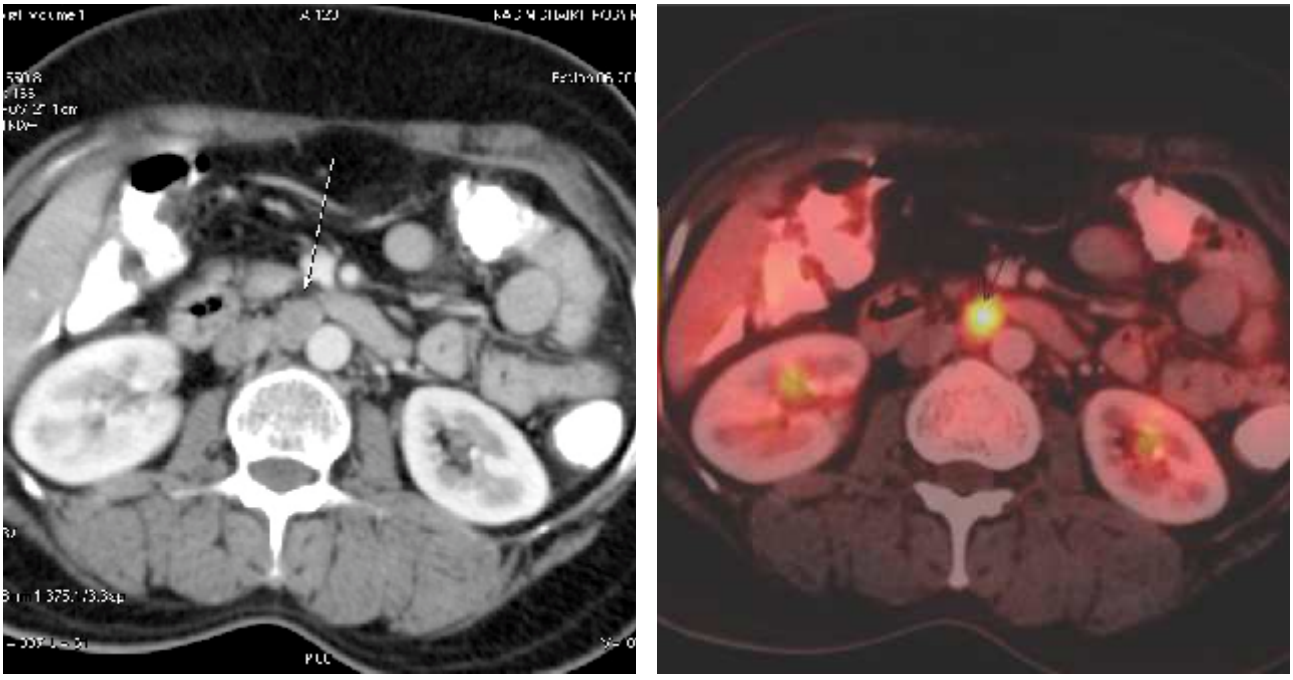

Figure 3-4 Small size hypermetabolic paraaortic lymphnode, suggest disease spread in otherwise normal looking lymphnode or sized based criteria

The primary value for PET in cervical cancer is in diagnosis of extrapelvic disease in initial staging and in detection of recurrence. The PET has little role as a primary modality in the evaluation of primary ovarian masses. Although PET is accurate in the diagnosis of ovarian cancer recurrence, the sensitivity of PET depends upon the level of clinical suspicion. PET sensitivity is 90% and specificity is 86% if there is clinical suspicion of recurrence.[8] The role of PET-CT has been promising though limited studies are available on their accuracies.

MRI or CT-scan has suboptimal accuracy because small or normal-sized lymph node metastases may be missed, whereas an enlarged reactive lymph node may be diagnosed as a false-positive result. It is crucial and important to distinguish malignancy from benign processes in previously treated areas.[5] Patient of carcinoma cervix (Mrs. A) required PET-CT scan for this purpose as a nodal mass was found in the area where previously lymphnode dissection was done. PET is more accurate than MRI for pelvic nodal metastases with sensitivity of 79% and specificity of 99%.[8]

PET-CT scan has been shown effective in the identification of malignant tissue in different primary and metastatic tumor types.[5] It can detect lesions otherwise missed or misinterpreted on conventional morphological imaging studies including CT-scan as we have reported with patient of dysgerminoma of ovary (Mrs. B) and fibrosarcoma of ovary (Mrs. D).[2] In case of dysgerminoma of ovary (Mrs. B) reexploration to decide the nature of ovarian cyst detected on USG was avoided on the base of PET-CT findings. In case of fibrosarcoma of ovary (Mrs. D), completion of surgery was done after findings of PET-CT showing no evidence of disease elsewhere in the body.

It is well established fact that PET-CT can improve the detection of recurrent cervical and ovarian cancer, which has two potential means of benefit: improved survival and reduced morbidity. Hence by means of PET-CT if local recurrence can be detected earlier and curative salvage therapy is possible, survival may improve. Our patient of carcinoma ovary (Mrs. E) was scheduled for surgery as recurrence was found only in paraaortic node on PET-CT findings and HPE confirmed the malignancy in paraaortic node. PET-CT defines the extent of metastatic disease which enables the clinician to decided regarding salvageable surgical intervention/ palliative measures.[1]

A more interesting application is interim PET-CT during therapy, in order to identify non –responder patients who could benefit from other therapeutic approaches, thus reducing toxicity and costs. In the management of patients with gestational trophoblastic tumor as in our patient of choriocarcinoma (Mrs. C), correct identification of the presence of site of metastases was crucial. In fact, it has been shown that resection of a viable metastatic lesion, in highly selected patients with drug-resistant disease, may be successful in inducing remission. However, standard imaging techniques including CT-scan may have considerable limitations in differentiating viable tumor tissue from necrotic tissue. Conversely, metabolic imaging, by identifying

regions of viable disease, may provide additional information and better address therapy.[2]

PET-CT scan may be most valuable and useful when there is no other evidence of metastatic disease by other imaging studies, and thus, biopsy confirmation of disease is not possible. However, one must develop a comfort level and confidence with PET that allows one to act based on its results alone and treats what therefore may be early, potentially curable disease as we have done in the case of fibrosarcoma of ovary (Mrs. D) where completion of surgery was done on the basis of PET-CT findings as no tumor markers are reliable for monitoring.[4]

Despite the relatively high sensitivity of FDG-PET, there are several disadvantages of FDG PET. False negatives can occur with lesions smaller than 1.0 cm and with certain tumor types that demonstrate low metabolic activity. Relatively low specificity with false-positive results can be seen due to increased FDG uptake in normal organs, severe inflammatory disorders such as tuberculosis and granulomatous disease and benign processes.[3,7]

Conclusion

It is possible that the addition of PET-CT to the oncologist's imaging armamentarium may ultimately improve both outcomes and costs by altering management strategies in primary and recurrent settings. It should be considered as an adjunct to rather than a replacement for conventional imaging. The whole-body FDG-PET scan is a sensitive post-therapy surveillance modality for detection of recurrent gynecological cancer and aids in deciding treatment plans and, eventually, may have favorable impact on prognosis and survival. It is not only a useful tool for early detection of subtle recurrences, but also allows to individualize optimal treatment plan by selecting patients for curative intent with localized recurrence and avoiding administration of unnecessary treatment to patients with incurable disease. As further research is done, more applications of FDG-PET in evaluation of gynecologic malignancies will become known.

References

1. Havrilesky LJ, Kulasingam SL, Matchar DB, Myers ER: FDG-PET for management of cervical and ovarian cancer. Gynecol Oncol 2005; 97:183-191
2. Sironi S, Picchio M, Mangili G et al: (^{18}F)Fluorodeoxyglucose positron emission tomography as a useful indicator of metastatic gestational trophoblastic tumor: preliminary results in three patients. Gynecol Oncol 2003; 91: 226-230
3. Wolfman DJ, Ascher SM, Hricak H et al: Diagnostic Imaging Techniques in Gynecologic Oncology. Principles and Practice of Gynecologic Oncology 2009; Fifth Edition:197-230
4. Unger JB, Lilien DL, Caldito G et al: The prognostic value of pretreatment 2-(^{18}F)-fluoro-2-deoxy-D-glucose positron emission tomography scan in women with cervical cancer. Int J Gynceol Cancer 2007: 17: 1062-1067
5. Chung HH, Kim SK, Kim TH et al: Clinical impact of FDG-PET imaging in post-therapy surveillance of uterine cervical cancer: Form diagnosis to prognosis. Gynecologic Oncology 2006; 103: 165-171
6. Nakamura K, Hongo A, Kodama J et al: The measurement of SUVmax of the primary tumor is predictive of prognosis for patients with endometrial cancer. Gynecologic Oncology 2011; 123: 82-87
7. Risum S, Hogdall C, Loft A et al: The diagnostic value of PET/CT for primary ovarian cancer-A prospective study. Gynecologic Oncology 2007; 105: 145-149
8. Lin EC, Alavi A: Gynecological tumors. PET and PET/CT A Clinical Guide 2005; First Edition: 137-142

Post Menopausal Bleeding–Patient Profile and their Management at GCRI, Ahmedabad

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Summary

This is a retrospective study of the patients with postmenopausal bleeding (PMB) of uterine origin with respect to their diagnosis, management and adjuvant therapy. The present study was conducted in a total 34 patients with PMB, at the Department of Gynecological Oncology, at our institute, from May 2011 to October 2011. Patients presenting with post-menopausal bleeding of only uterine origin were included and those having cervical and vaginal etiology were excluded. It was observed that out of 34 patients, based on the fractional curettage evaluation, 14 patients had endometrial cancer, 7 had atrophic endometrium, 5 had proliferative endometrium, and 2 each had endometrial polyp and pyometra. On trans abdominal ultrasonography (TAS), atrophic endometritis patients had endometrial thickness (ET) range between 3-6 mm, proliferative endometritis patients had ET range 8-35 mm and those with endometrial cancer had ET range 18-50 mm. In these 34 patients, 2 were diabetic, 12 were hypertensive, 6 were both hypertensive and diabetic and 3 patients had breast cancer who were on Tamoxifen. Based on the diagnosis, out of 14 patients of carcinoma endometrium - staging laparotomy was done in 12 patients, primary chemotherapy was given to 2 patients with advanced carcinoma. Pyometra drainage was done in 2 patients, antibiotics and anti-inflammatory treatment was advised in atrophic endometritis and observation and yearly sonography in rest of the patients. Postmenopausal bleeding is an ominous symptom and should be properly investigated, no matter how minimal or non-persistent. Most patients with carcinoma endometrium present with abnormal peri-menopausal or postmenopausal bleeding, early in the development of the disease, when the tumor is still confined to the uterus. Appropriate diagnosis and timely treatment yields high cure rate.

Introduction

Post menopausal bleeding (PMB) is not an uncommon clinical presentation in today's gynecological practice. Contributory factors are perhaps increasing longevity, obesity and hormone therapy (supervised and unsupervised). Increasing numbers of women seeking help or reassurance for this problem due to increased awareness would also contribute to this increase. PMB occurs in approximately 3 % of post menopausal women. Patients with PMB have 10-15% chance of having endometrial carcinoma and therefore the diagnostic workup is aimed at excluding malignancy.[1]The risk of endometrial cancer at the age of 50 in a woman with PMB is approximately 1 % and rises to 25 % at the age of 80 years. Seventy five percent of women with endometrial cancer are postmenopausal. Hence in the present retrospective study, evaluation of the patients with postmenopausal bleeding of uterine origin was done in relation to their diagnosis, management and adjuvant therapy.

Materials and Methods

Patients: In this retrospective study, 34 patients of PMB were diagnosed and treated at our institute, from May 2011-October 2011 were enrolled. Detailed clinical history, clinical examination, trans abdominal ultrasonography (TAS), fractional curettage, histopathological findings, treatment offered and adjuvant treatments were noted from the case files maintained at the institute. Patient's characteristics are depicted in Table-1.

Exclusion criteria: Patients having PMB of cervical, vaginal or any other etiologies were excluded from this study.

Results

Demographic data: Ages of the patients who presented with PMB ranged between 44 years and 82 years with a median age of 59 years. Of the 34 patients, 11 (32%) patients presented with duration of menopause less than 5 years. Sixty-eight percent of the patients presented with single episode of PMB. In majority of the (56%) patients the duration of bleeding was less than 10 days (Table-1).

Table-1: Patient characteristics

Parameters	N	Percent
Total patients	34	100
Age (years)		
Range	44 - 82	
Median	59	
Type of episode of PMB		
Single	23	68
On-Off	10	32
Duration of PMB		
<10 Days	19	56
10- 30 Days	7	20
>1 Month	8	24
Duration of menopause (years)		
<5	11	32
6-10	6	18
11-15	6	18
16-20	4	12
>20	7	20
Risk factors		
Obesity	18	53
Hypertension (HT)	12	35
Diabetes Mellitus (DM)	2	6
Both (HT + DM)	6	18
Cancer of Breast on Tamoxifen	3	9

Correlation with risk factors: When correlated with the risk factor, it was observed that 53% PMB patients were obese, 35% patients had hypertension and 6% patients had diabetes mellitus. Eighteen percent patients presented with both HT and DM (Table-1). Interestingly, all the 3 breast cancer patients who were on tamoxifen therapy with PMB had benign etiologies.

Correlation with TAS: The correlation of trans abdominal ultrasono-graphy findings with fractional curettage reports in patients with PMB is shown in Table-2. In 13 patients ET was > 10 mm, out of which 6 patients were found to be associated with malignancy. On the other hand, none of the six patients in group having ET<5 mm were associated with malignancy. Seven out of nine patients having mass lesion on TAS were found to be associated with malignancy.

Correlation with histopathological reports: All the 34 patients with PMB were subjected to fractional curettage. Out of which 20 (59%) patients had benign lesions in endometrium while 14 (41%) patients had malignant lesions i.e carcinoma endometrium. Benign lesions in endometrium were further classified as functional disorder and organic disorder as shown in the Table-3. In the subgroup of patients with functional disorder, 7 (20%), 5(15%) and 4 (12%) patients had atrophic endometritis, proliferative endometrium and normal endometrium, respectively. In the subgroup of patient's organic disorder, 2 (6%) patients each had endometrial polyp and pyometra.

Management

Benign disorders: Patients with functional disorders were managed conservatively and subjected to observation with yearly ultrasonography. Patients having pyometra were treated with cervical dilation and drainage. Treatment with cervical drainage or insertion of a Foley catheter was successful in these patients. No significant predictors for persistence were identified. Patient having endometrial polyp were diagnosed on the basis of fractional curettage report which was also a curative mean for these patients.

Malignant disorder: Out of 14 patients with carcinoma, 12 underwent staging laparotomy while 2 with advanced disease were directly subjected to palliative chemotherapy (Table-4). Based on histopathological reports and staging, patients were offered adjuvant treatment after surgery. Post operatively 6 patients of endometroid adenocarcinoma required adjuvant radiotherapy. Two patients required adjuvant chemotherapy, one patient required concurrent chemo-radiation and one patient was given hormonal treatment. Two patients with early cancer were kept on observation.

Table-2: Correlation of trans abdominal ultrasonography findings with fractional curettage reports in patients with PMB

Parameters	N	Percent	Patients having cancer (on fractional curettage)	
			N	Percent
ET				
<5 mm	6	18	0	0
5-10 mm	4	12	0	0
>10 mm	13	38	6	46
Endometrial Collection	2	6	1	50
Mass Lesion	9	26	7	78

Table-3: Histopathological reports in PMB patients

Histopathological Reports	N	Percent
Benign	20	59
(a) Functional Disorder		
Atrophic endometritis	7	20
Proliferative endometrium	5	15
Normal endometrium	4	12
(b) Organic Disorder		
Pyometra	2	6
Endometrial polyp	2	6
Malignant		
Carcinoma endometrium	14	41

Table-4 : Management of endometrial cancer patients

Parameters	N
Endometrial cancer	14
Primary chemotherapy	2
Staging laparotomy	12
Stage	
I	7
II	2
III	2
IV	1
Grade	
I	6
II	3
III	3
Histopathology report	
Endometroid adeno carcinoma	8
Serous papillary adeno carcinoma	3
Mixed mullerian tumor	1

Discussion

Genital tract bleeding in postmenopausal women is a sign of underlying pathologic condition. The incidence of malignancy increases with delay in presentation of this symptom. The peak incidence of malignancy is observed in the age group of 56-65 years. The incidence of postmenopausal bleeding decreases with increasing age, however probability of malignancy as underlying cause increases.[1] We also observed the similar findings.

The Norwich study has reported that women in the endometrial cancer group were significantly more likely to be older, have higher BMI, recurrent episodes of bleeding, diabetes, hypertension, or a previous history of breast cancer.[2] In present study 53% patients had obesity and 68% patients presented with single episode of PMB. Therefore, it can be presuned that diabetes and hypertension is associated with a modestly increased risk for endometrial cancer.[3]

The data relating to endometrial thickness in the presence of carcinoma has been largely reviewed. Karlsson et al. in the Nordic multicenter trial, the largest study evaluating endometrial measurements in postmenopausal women with bleeding, found that in women with endometrial cancer the mean endometrial thickness $\pm$ SD was 21.1 $\pm$ 11.8 mm and no malignant endometrium was thinner than 5 mm.[4] Similarly none amongst 6 patients who had ET <5 mm were found to have malignancy in this study. As the endometrial thickness increases, the probability of finding endometrial pathology in curettage increases.[5]As in this study maximum cancers were detected at ET > 10 mm.

The atrophic endometrium inclusive of senile cystic atrophy was the predominant finding (20%). The exact cause of bleeding from atrophic endometrium is not known. It is postulated to be due to anatomic vascular variations or local abnormal haemostatic mechanism.[1]

The incidence of proliferative endometrium is 15% in this study. The occurrence of proliferative endometrium could be due to fluctuating levels of progesterone from follicular remnants, the effect of which persists even up to 15 years after cessation of menses.

Risk of endometrial cancer increases following tamoxifen therapy for invasive breast cancer; however, net benefit greatly outweighs risk. Endometrial cancers occurring after tamoxifen therapy do not appear to be of a different type with a worse prognosis than are such tumors in non-tamoxifen treated patients.[6] This study showed that all 3 patients who were on tamoxifen were found to have PMB due to benign causes.

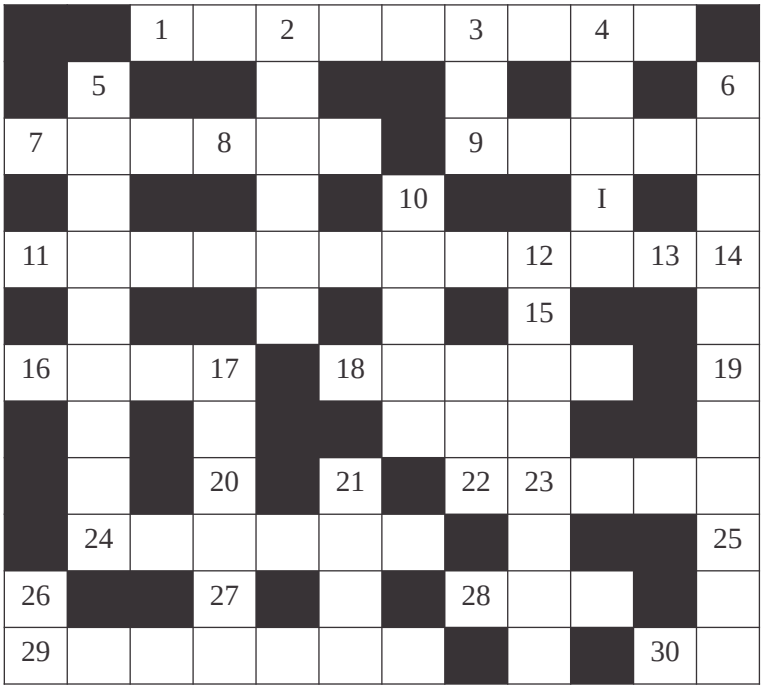
As in this study most of the patients with carcinoma endometrium are detected at an early stage, when the tumor is still confined to the uterus. Appropriate diagnosis and timely treatment yields high cure rate.The duration or amount (staining vs. gross) of bleeding does not make any difference. PMB should always be taken seriously and be properly investigated, no matter how minimal or non-persistent.

References

1. Naik VS, Rege JD, Jashnani KD: Pathology of Genital Tract in Postmenopausal Bleeding. Original/Research, Bombay Hospital Journal 2005; 47
2. Burbos N, Musonda P, Giarenis I, et al: Predicting the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding: the Norwich DEFAB risk assessment tool. British Journal of Cancer 2010; 102: 1201–1206
3. Anderson KE, Anderson E, Mink PJ et al: Diabetes and endometrial cancer in the Iowa women's health study. Cancer Epidemiol

Biomarkers Prev 2001; 10: 611-616
4. Karlsson B, Granberg S, Wikland M et al: Endovaginal scanning of the endometrium compared to cytology and histology in women with postmenopausal bleeding. Gynecol Oncol 1993; 50:173–178
5. Güner H, Tiras MB, Karabacak O et al: Endometrial assessment by vaginal ultrasonography might reduce endometrial sampling in patients with postmenopausal bleeding: a prospective study. ObstetGynaecol 1996; 36: 175-178
6. Fisher B, Costantino JP, Redmond CK et al: Surgical Adjuvant Breast and Bowel Project (NSABP). JNCI J Natl Cancer Inst 1994; 86: 527-537

Crossword Puzzle - I



ACROSS:

1. Central Venous catheter most commonly used in children at GCRI (7)
3. Squamous cell carcinoma of GI Tract (4)
7. Doctor of Podiatric medicine treats ____ (4)
8. According to Rural Cancer Registry of Ahmedabad district, more than half of all cancers in males are _____ (3-abbv)
9. Material used for making gloves (5)
11. Tumour secreting vasoactive peptides (9)
12. Policy for terminally ill patients (3-abbv)
13. Abbreviation of Reference Group (2-abbv)
16. Isotope scan for Neural crest tumors (4-abbv)
18. Dame Cicely Saunders has described cancer pain as _____ pain (5)
22. A heavy chain of immunoglobulins (5)
24. Room No. 27 at GCRI is for _____ therapy (6)
28. Test to check functional capacity of liver (3-abbv)
29. In multiple myeloma FDG-PET is more sensitive for bone lesions than _____bone scan
30. An important end point for cancer clinical trials (2-abbv)

DOWN:

2. Cancer of _____ is frequently associated with obstructive uropathy (6)
3. FLT3 mutation denotes bad prognosis in this haematological malignancy (3-abbv)
4. Muscle specific protein found in Endometrial stromal sarcoma (5)
5. Blood borne viral infection (9)
6. In one lung anesthesia PEEP and CPAP combination improve arterial partial pressure of _____ (6)
10. In the year 2025, major surgeries may be done with their assistance (5)
12. Gene Polymorphism study in oral cancers is done on genomic _____ (3-abbv)
14. TP53 is a tumor suppressor ____ (4)
15. Another word for umbilicus (5)
17. All doctors participating in clinical trials must be trained in ___ (3-abbv)
19. Contiguous spread of tumor occurs in tissue that is ____ the primary (4)
20. Central venous catheter associated with fewer incidence of infection (4)
21. New class of drugs useful in BRCA1 and 2 mutation cancer, inhibits this enzyme (4-abbv)
23. A rapid, automatized diagnosis method for HIV (4-abbv)
25. Mutation of this gene is found in 90% of pancreatic cancers (3-abbv)
26. Major responsibility of clinical trial is on _____ (2-abbv)
27. An integral mode of treating locally advanced carcinoma of cervix (2-abbv)

(Majority of the clues can be found in this issue-so look carefully)

1. Submit your answers to the editorial team for exciting prizes.
2. The first three correct entries will be announced in the next issue of the journal along with the answers.

Cancer Detection and Treatment in 2025

Bhat R

Fellow, Deprtment of Gynecological Oncology

Cancer detection and treatment in 2025 is a topic of interest and awe to every oncologist. It was in 1997 that I first walked gingerly into the medical school. Fifteen years on, the day seems not so far after all. The year 2025 is 13 years away. Long enough time for several governments to change or for the advent of i-pad 15, but being pragmatic, it's not enough time for bringing miracles in the field of oncology. But let's be optimistic in hoping that we might have cancer survivors in 2025, who might lead as good a life, if not a better one viz a viz a diabetic or hypertensive.

In our quest for the future it is apt to keep track of history. It is said that those who fail to read history, history will fail them. Mankind has taken giant steps in the field of science in the past century. We have moved from the 1900s, with an alarmingly high infant mortality rates and life expectancy of not more than 40 years in most countries; to an era where a child born at 28 weeks has more than 80% chance of survival and the life expectancy is soaring at 70-80 years. While attempts to predict the future are likely to be inaccurate, misguided or simply naïve; the world is witnessing a dramatic change on several fronts.

The most important transforming factor probably is the explosion in the field of genetic knowledge. We have been able to map out the entire human genome, giving us an opening to target our drug therapies towards specific defective genes, much like Trastuzumab in the treatment of breast cancer. The revolution in the field of computers has given us cutting edge technology. The world has grown only to become smaller! Today with advanced technology we are able to get a cardiac catheterization done in Ahmedabad and get it reviewed in New York the next day. A Computerized Axial Tomography scan done at Mumbai can be reviewed by radiologists at The Gujarat Cancer & Research Institute (GCRI). It is already a reality that miniature devices are providing information about colonoscopy, without having to undergo one. Wrist watch devices to continually monitor one's vital parameters might become an everyday affair. Telemedicine will allow the remote delivery of medical care to far flung corners of the world. Robotics will allow the performance of guided microsurgeries across the seven seas. Vaccines similar to the cancer cervix vaccines (HPV) are on the horizon. Probably by 2125, if not at 2025, we might be seeing designer babies free of all malignancies!

Finally, nanotechnology, which is already spreading its tentacles everywhere, might be at its zenith in 2025. Scientists are designing micelles of 25-50 nm size, to deliver the right quantity of drug to the right place, sparing the innocent bystanders.

Probably in 2025, my typical day would involve reviewing the lab parameters of my patients sitting at home, using my i-pad; performing a laparoscopic Wertheim, a robotic hemicolectomy guided by an accomplished GCRI oncosurgeon on one of my patients, vaccinating the young girls attending my clinic against carcinoma cervix and of course preparing for my undergraduate classes where in my heart of hearts I know I might soon have to stop teaching about the morbidity of carcinoma cervix and the vagaries of even ovarian malignancies.

Finally, I sign off knowing very well that it might be farfetched to imagine this in 2025. However, as a firm believer of Prof Mel Greaves, who says, “if you are not an optimist, you should not be in medical science”, I hope to see it in my life time!

(The author received first prize in elocution competition held at GCRI on Annual Science Day Celebration, February 2012)

Persistent Arterio-Venous Fistula following Trophoblastic Disease: A Case of Post Vesicular Mole Vaginal Bleeding, managed by Uterine Arterial Embolization

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Summary

Arterio-venous malformations (AVM) of uterus and pelvis are considered to be one of the rarest vascular malformations. They are usually associated with uterine trauma or pregnancy. The patient presents with intractable vaginal bleeding of seemingly unknown etiology. With advances in radiological investigatory tools, more and more such cases are being identified and reported. Many of these cases, which would have either ended up in hysterectomy or develop severe anemia, are now being managed with conservative treatment options such as uterine artery embolization and high doses of progestins. We present a case report about a patient who presented to our department with severe vaginal bleeding during follow up of vesicular mole. In this patient vaginal bleeding was not controlled by high dose of oral progestins and oral contraceptive pills. Successful coiling of bilateral uterine arteries was done. Post-embolization she had normal bleeding during menstrual cycles.

Introduction

Arterio-venous malformations (AVM) of uterus and pelvis are considered to be one of the rarest vascular malformations. They are usually associated with uterine trauma or pregnancy. The patient presents with intractable vaginal bleeding of seemingly unknown etiology.[1] Till quite recently, this entity was rarely ever diagnosed. But with the current advances in radiological investigatory tools, more and more such cases are being identified and reported. Many of these cases, which would have either ended up in hysterectomy or develop severe anemia, are now being managed with conservative treatment options such as uterine artery embolization and high doses of progestins. We discuss here about a patient who presented to our department with severe vaginal bleeding during follow up of vesicular mole.

Case Report

Mrs. X was a 32 year old patient with a single live child born by normal delivery five years back and a previous history of one abortion two years back. She underwent dilatation and evacuation (D&E) twice. The first D&E was done following vesicular mole on 2nd January 2011 and the second was done on 9thApril 2011 for irregular bleeding per vaginum. Histopathology revealed vesicular mole. Both D&E were done at a local hospital. Injection Methotrexate was given for three cycles which concluded on 26th May 2011.After completion of treatment she developed severe menorrhagia for which she was given oral contraceptive pills for 3 months. The patient was admitted with severe bleeding to a private hospital on 2nd September 2011.Her hemoglobin was 4 gm%. She was transfused 3 units of blood and 4 units of fresh frozen plasma. She was started on oral Medroxy progesterone Acetate 10 mg four times daily. She presented to us on 13th October 2011 with complaints of heavy bleeding since 10 days.

Physical examination revealed mild pallor and vital signs showed that the patient was haemodynamically stable. On speculum examination, the cervix was normal. On vaginal examination, the uterus was found to be bulky. Laboratory investigations showed hemoglobin to be 10gm%, β hCG value of 0.63 IU/L. X-ray chest was normal. On ultrasound the uterus appeared enlarged to 92x80x46mm. The endometrial-myometrial interface was not clearly seen. The myometrium showed heterogeneous echo texture with significant high vascularity suggestive of AVM. In view of retaining her fertility and menstrual functions she was counseled for and given an option of uterine artery embolization (UAE).Successful coiling of bilateral uterine arteries was done on 17th November 2011 (Figure 1 and Figure 2). Post embolization she had normal bleeding during menstrual cycles. She is on regular follow up. Her last follow up was on 16th February 2012.

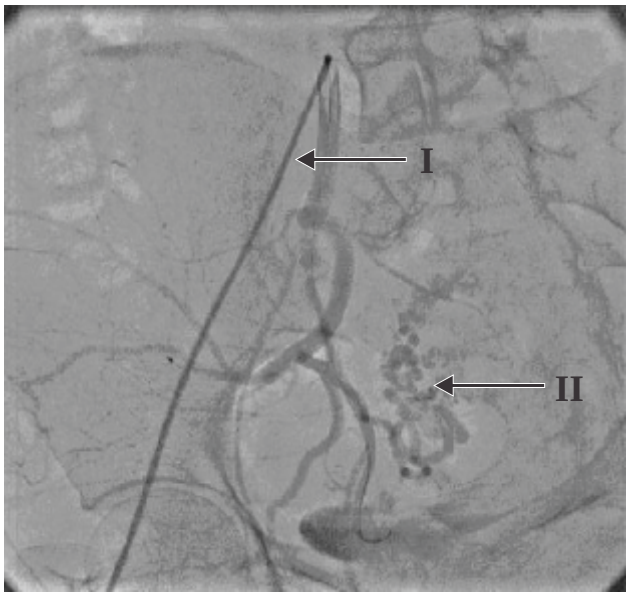

Figure 1: (I) Catheter in right uterine artery
(II) Arterio-venous malformation on right side

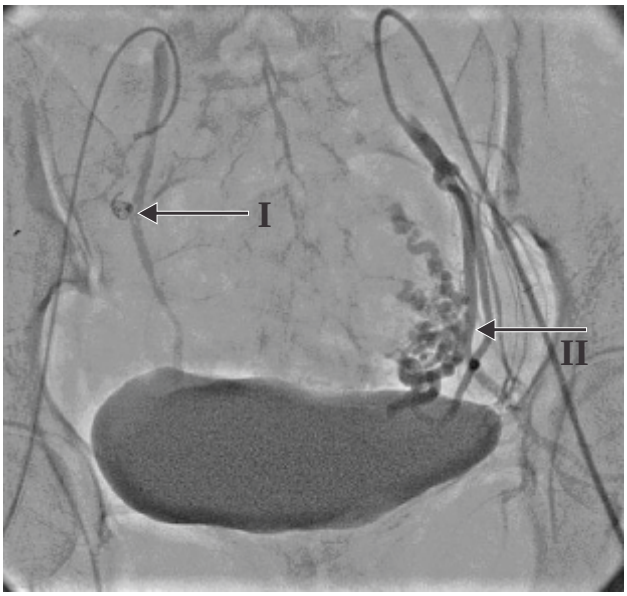

Figure 2: (I) Coil in right uterine artery
(II) Arterio-venous malformation on left side

Discussion

The first case of uterine AVM was reported in 1926 by Dubreuil and Loubat.[2] AVM consists of proliferation of arterial and venous channels with fistula formation and a network of small capillary like channels. In many cases, distinction between the arteries and veins becomes blurred due to secondary intimal thickening in the veins, as a result of increased intraluminal pressure. The lesion has been variably described as cirsoid aneurysm, arterio-venous fistula, arterio-venous aneurysm, pulsating angioma, or cavernous angioma.

AVMs can be congenital or acquired and have been reported in women aged 18 to 72 years. Most congenital uterine AVMs are isolated anomalies but can occur in association with AVM at other sites. Acquired causes[3] of uterine AVM include previous uterine surgeries like curettage, caesarean section[4] or hysterectomy, pelvic trauma,[5] previous pregnancies,[6] gestational trophoblastic disease,[7] exposure to diethylstilbestrol,[8] endometriosis, fibromyoma and endometrial or cervical cancers.

Anywhere in the body AVMs are difficult to manage, mostly because the diagnosis, prognosis, further progress and management are all clinical dilemmas. In cases of uterine AVM the clinical presentation varies from various degrees of menorrhagia to massive life-threatening vaginal bleeding. A strong clinical suspicion is essential for the prompt diagnosis and definitive treatment. Relevant previous history like any history of probable direct trauma to the uterus should be elicited. Clinical and laboratory evaluation has to be done to rule out other more important causes of vaginal bleeding like missed abortion, incomplete abortion, dysfunctional uterine bleeding, hydatidiform mole, endometrial carcinoma, polyp, etc.

In earlier days the diagnosis was usually made based on angiography,[9] during laparotomy or by histopathology. With the advent of newer techniques like colour doppler sonography,[10,11] contrast computed tomography (CT) [12] and magnetic resonance imaging (MRI), the detection of this entity has become easier and therefore nowadays even very small AVMs are being detected.

In present day clinical practice a high index of suspicion, a good patient history and clinical examination, a falling or normal beta hCG value and imaging studies by ultrasound doppler or MR/CT angiography together make the diagnosis almost 100% accurate.[13,14] Customarily, uterine AVM were being treated by artery ligation or hysterectomy. Presently the management depends on factors like the patient's parity status, wish for fertility and the site of AVM. In patients with less severe bleeding long term medical management can be tried. This includes oestrogens, progestins, methyl ergonovine, danazol, 15-methyl prostaglandin F alpha and oral contraceptive pills.[14] Methyl ergonovine maleate induces tetanic myometrial contractions and thus reduces the blood flow to AVM, making it collapse. Intravenous conjugated oestrogen causes the haemorrhaging vessels covered with proliferating endometrium.[14] With the newer addition of uterine artery embolization into the therapeutic armamentarium, mortality from uterine AVM could become a rare entity.

Local complications of UAE include pelvic pain during embolization and in the post embolization period, neurological complications, transient buttock

claudication, puncture site haematoma, abscess formation, vesico-vaginal fistula and endometrial atrophy.

Other management modalities reported include laser coagulation of AVM under hysteroscopic guidance and laparoscopic coagulation of uterine arteries. Hysterectomy is indicated only in those who do not wish to preserve fertility, those with no access to expert medical facility or those in whom UAE fails. Usually bilateral embolization is performed, since unilateral embolization can lead to recurrence of bleed due to collaterals from the opposite uterine artery.

Few pregnancy outcomes after UAE for other pathologies of uterus have been reported, the commonest condition being studied is fibroid uterus. The rate of pregnancy following uterine AVM can probably be increased by super-selective embolization with complete occlusion of the nidus of the malformation, preserving other pelvic branches. But, as of today, no statistically significant study has been reported regarding the pregnancy outcome following uterine AVM embolization.

References

1. Grivell RM, Reid KM, Mellor A: Uterine arteriovenous malformations: a review of the current literature. Obstet Gynecol Surv 2005; 60: 761-767
2. Dubreuil G, Loubat E: Aneurysme crisoide de l'uterus. Ann Anat Pathol 1926; 3: 697-718
3. Ghosh TK: Arteriovenous malformation of the uterus and pelvis.Obstet Gynaecol 1986; 68: 40-43
4. Ho SP, Ong C, Tan BS: A case of uterine artery pseudoaneurysm. Singapore Med J 2002; 43: 202-204
5. Gaylis H, Levine E, Van Dongen LGR, Katz I: Arteriovenous fistulas after gynaecologic operations. Surg Gynecol Obstet 1973; 137: 655-658
6. Junatao L, Fengzhen H, Xuming B: Optimal management of post partum hemorrhage. Chinese Med J 2001; 114: 1280-1282
7. Tacchi D, Loose HW: Successful pregnancy after selective embolisation of post molar vascular malformations. Br J Obstet Gynaecol 1988; 95: 814-817
8. Follen MM, Fox HE, Levine RU: Cervical vascular malformation as a cause of antepartum and intrapartum bleeding in three diethylsilberstol-exposed progeny. Am J Obstet Gynecol 1985; 153: 890-891
9. Fleming H, Ostor AG, Picket H, Fortune DW. Arteriovenous malformation of the uterus. Obstet Gynecol 1989; 73: 209-214
10. Diwan RV, Brennan JN, Selim MA, et al: Sonographic diagnosis of arteriovenous malformation of the uterus and pelvis. J Clin Ultrasound 1983; 11: 295-298
11. Jain KA, Jeffrey RB Jr, Sommer FG: Gynaecologic vascular abnormailties: Diagnosis with doppler ultrasonography. Radiology 1991; 1: 549-551
12. Huang MW, Muradali D, Thurston WA, Burns PN, Wilson SR: Uterine arteriovenous malformations: gray-scale and Doppler US features with MR imaging correlation. Radiology 1998; 206: 115-123
13. Umezu T, Iwase A, Ota T, Suzuki K, Nakagawa A, et al: Three-dimensional CT angiography is useful for diagnosis of postabortion uterine hemorrhage: 3 case reports and review of the literature. J Minim Invasive Gynecol 2010; 17: 246-251
14. Joseph A, Ramalingam D, Rajalakshmi G, Davidson D: A rare case of post-abortal vaginal bleeding, Uterine arterio-venous malformation, managed by Arterial embolization. Pushpagiri Medical Journal 2010; 2: 32-35

Van, Wyk and Grumbach Syndrome or Ovarian Neoplasia? - A Diagnostic Dilemma

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Summary

We report a case of bilateral multicystic ovarian enlargement in a nine year old girl. She was referred to us for surgery as a case of suspected malignant ovarian neoplasia with raised CA-125. She was treated conservatively with thyroid replacement therapy after diagnosing and confirming it as Van, Wyk and Grumbach syndrome.[1,2] This syndrome is characterized by Juvenile hypothyroidism, isosexual precocious puberty, multicystic ovarian mass with delayed bone age.[2,3]

Introduction

The cystic enlargement of ovaries in prepubertal girl leads to diagnostic and treatment dilemma. Differential diagnosis can be simple cyst, polycystic ovaries, Juvenile granulosa cell tumor[4] or cystadenocarcinoma.[5] The association of cystic enlargement with primary hypothyroidism is not widely recognized in medical literature. At present the exact mechanism leading to ovarian cyst formation in primary hypothyroidism remains uncertain. Identification of this Van, Wyk and Grumbach syndrome is important in these girls. It obviates the need for expensive investigation and surgery as they can be treated conservatively with thyroid replacement therapy.[6] Our patient had all the criteria of Van, Wyk and Grumbach syndrome such as juvenile hypothyroidism, isosexual precocious puberty, multicystic ovarian enlargement with delayed bone age.[2,3]

Case Report

A nine year old girl was referred to our institute in May 2011 as a case of bilateral ovarian tumor with raised CA-125 (56 U/ml) and normal β hCG and α feto protein. Ultrasonography (USG) and Computer Tomography Scan (Figure 1) done outside revealed normal size uterus, endometrial thickness of 6 mm and bilateral multicystic ovarian lesions (right ovary 58x44 mm with small hemorrhagic cyst and left ovary 46x 38 mm). Cysts were thick walled, well defined and had regular margins and multiple internal septation. Patient visited the hospital for the complaint of heaviness in lower abdomen since a few days**.** At our institute, on asking leading questions the patient's parents gave history of early menarche with heavy bleeding per vagina for 7 days. They had also noticed stunted growth and weight gain. Her appetite was normal and her scholastic performance continued to be good. There was no history of vomiting or headache, no family history of precocious puberty or hypothyroidism.

On clinical examination she was overweight (25 kilogram) and had stunted growth for her age (height 115cm, less then 5th percentile). She had pallor and dry skin. There was no goiter. Breast enlargement corresponded to stage I-II with no galactorrhoea. She had no axillary and pubic hair. On abdominal examination there was no palpable mass. Her pulse was 68/minute and blood pressure of 90/60mm of Hg. The genitals were well developed for her age.

Laboratory examination showed hemoglobin: 11.5 gm. Due to precocious puberty a hormonal profile was performed. The results were as follows: TSH: >100 mIU/ml, Free T4: < 0.4 ng/dl, prolactin: 78.8 ng/ml, estradiol: 36.4 pg/ml, FSH was normal for her age (8.56 mIU/ml), LH was at lower limit of normal (<0.10 mIU/ml). Hormonal tests confirmed the diagnosis of severe hypothyroidism. Thyroid antibodies were checked to identify the cause of

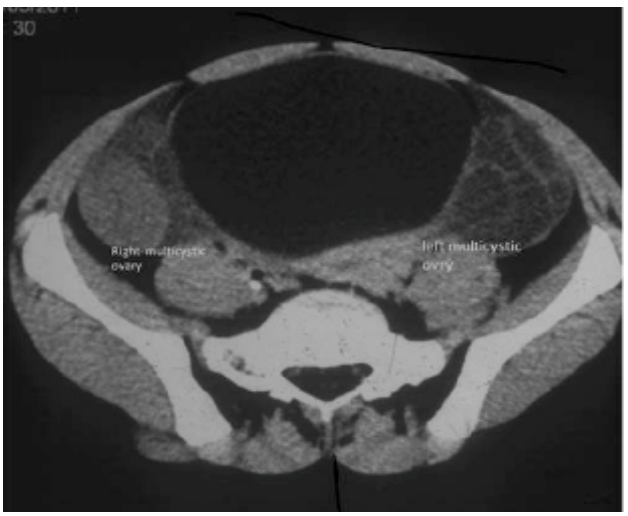

Figure 1: CT scan showing bilateral multicystic ovary with small hemorrhagic cyst on right side

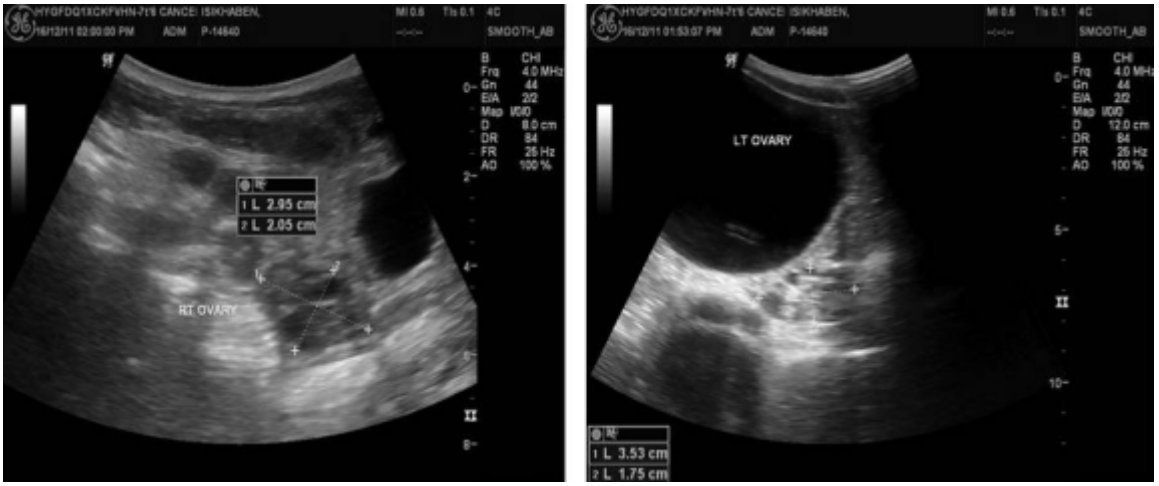

Figure 2 Normal Ovaries after treatment

hypothyroidism. Anti thyroid peroxidase antibodies levels were 14.4 IU/ml (i.e. negative) and her radiological investigation revealed bone age of 7 years. A repeat USG confirmed bilateral multicystic enlarged ovaries with no solid component. USG of neck showed heterogeneous echo texture in both lobes of thyroid gland and small cyst in left lobe of thyroid. MRI brain showed 13x24x11mm lesion in sellar fossa suggestive of pituitary hyperplasia.

An endocrinologist opinion was sought and the literature reviewed. The final diagnosis was precocious puberty and multicystic ovarian enlargement secondary to hypothyroidism i.e. Van, Wyk and Grumbach syndrome.

She was started on thyroid replacement therapy with gradual increase of the dosage. The patient's parents were explained about the condition and need for close and regular follow up with hormonal assay and USG examinations for the size of the ovarian cyst and to confirm the diagnosis. They were asked to return immediately in case of severe abdominal pain or vomiting.

After three months, USG showed regression in the size of cysts and thyroid function showed improvement with normalization of T4 and decrease in TSH level. Six months later there was complete resolution of cysts (right ovary 25x22x31 mm and left ovary 22x25x27mm [Figure 2]) and TSH had reduced to 19.97 mIU/ml. CA-125 had become normal.[7] Her weight had reduced to 20 kg and height had increased to 119 cm. She had no periods since last 5 months.

Discussion

In the present case, precocious puberty and ovarian enlargement suggested an estrogen-secreting ovarian tumor such as juvenile granulosa cell tumor. However, the finding of a delayed bone age with stunted growth in the patient with precocious puberty narrowed the differential diagnosis to long-standing hypothyroidism.[2] In the case of juvenile granulosa cell tumor bone age is advanced with growth spurt. CA-125 can be abnormal even in absence of malignancy as in our case.[6] Another important clue that the cause may be of endocrine origin was bilateral involvement of ovary rather than unilateral.

High circulating levels of TSH along with prepubertal LH levels suggested Van, Wyk and Grumbach syndrome.[7] In girls, the condition usually presents with vaginal bleeding and uncommonly with breast development or galactorrhea. Despite an early stage of puberty, there is lack of pubic hair. The salient diagnostic features include long standing hypothyroidism, high levels of TSH, isosexual precocity with lack of public and axillary hair and delayed bone age along with multicystic ovarian enlargement.[2] The precocious puberty is always isosexual (FSH dominated) and incomplete in patients of Van, Wyk and Grumbach syndrome.[8] Multicystic ovarian disease with hypothyroidism has been previously described in literature. Sella turcica enlargement may be seen at times and it has been attributed to thyrotroph hyperplasia.[9] Thus Van, Wyk and Grumbach syndrome can be diagnosed by the recognition of the salient clinical features, appropriate confirmatory endocrine laboratory tests and without surgical interference.

The exact mechanism of the development of precocious puberty in this syndrome remains speculative. TSH levels are consistently elevated in such patients and the tendency to manifest sexual precocity may be directly related to the severity of TSH elevation.[2] High circulating levels of TSH acting directly on FSH receptors may be the actual mediator of precocity.[8] It is also possible that increased sensitivity of the ovaries to the circulating gonadotropins could result from the hypothyroid state directly or via increased prolactin. However, ovarian enlargement may be secondary to a myxematous infiltration.[10] Our patient also had multicystic ovaries with normal to low gonadotropins suggesting that the

increased sensitivity of ovaries to gonadotropins may be responsible for it. Enlarged pituitary in our patient was probably because of thyrotroph hyperplasia. Although there is little consensus regarding the precise etiopathogenesis of the disorder, the treatment approach is clear. All symptoms subside with thyroxin replacement, the endocrine abnormalities resolve and even the ovarian cysts decrease in size or altogether disappear, as also in the present case during follow-up. There are reports in literature where young girls are operated for large cystic enlargement of ovaries and then subsequently found to have hypothyroidism.[11] These girls will have recurrent development of cystic ovaries even after surgery unless their thyroid deficiency is treated.

Other cystic tumors in young girls are simple functional cyst which regresses by itself and very rarely cystadenocarcinoma[5] a malignant epithelial tumor. This tumor has high level of CA-125 with solid cystic area and it does not present with precocious puberty.

Conclusion

Whenever enlarged multicystic ovaries are found in young girls, the possibility of hypothyroidism should be kept in mind.[12] Correct diagnosis is extremely important as these patients can be successfully treated with hormonal replacement therapy and an unwanted surgical intervention can be avoided.

Reference

1. Durbin KL, Diaz-Montes T, Loveless MB: Van, Wyk and Grumbach Syndrome: an unusual case and review of literature. Journal of Pediatric and Adolescent Gynecology 2011; 24: e93-e96
2. Rastogi A, Bhadasa SK, Bhansali A: An unusual presentation of a usual disorder: Van, Wyk-Grumbach Syndrome. Indian Journal of Endocrinology and Metabolism 2011; 15: 141-143
3. Brown LP, Boswell HP. Crotty EJ, O'Hara SM, Birkemeier KL, Guillerman RP: Van, Wyk and Grumbach Syndrome revisited: imaging and clinical finding in pre–and postpubertal girls. Pedietr Radiol 2008; 38: 538-542
4. Hashemipour M, Moaddab MH, Nazem M, Mahzouni P, Salek M: Granulosa cell tumor in 6 year old girl presented as precocious puberty. J Res Med Sci 2010; 15 94 0: 240-242
5. Blom GP, Torkildsen EM: Ovarian cystadenocarconoma in a 4-year-old girl: Report of a case and review of the literature. Gynecology Oncology 1982; 13: 242-246
6. Hunold A, Alzen G, Stefan A, Blutters-Sawatzki R, Landman E: Ovarian tumor in a 12-year old female with severe hypothyroidism: A case of Van, Wyk and Grumbach Syndrome. Pediatric Blood Cancer 2009; 52: 677- 679
7. Baranowasi E and Hogler W: An unusual presentation of acquired hypothyroidism: the Van, Wyk and Grumbach Syndrome. Eur J Endocrinol 2012; 163: 537-542
8. Ever JL, Rolland R: Primary hypothyroid and ovarian activity evidence for an overlap in the synthesis of pituitary glycoproteins. British J Obstet Gynecol 1981; 88: 195-202
9. Bhansali A, Sreenivasulu PP, Chattopadhyay A, Reddy S, Khandelwal N: Juvenile Primary Hypothyroidism presenting as Acute Abdomen and later with vision loss. Current opinion in Endocrinology, Diabetes and Obesity 2004; 14: 229-232
10. Ansen KA, Tho SP, Hanly M, Moretuzzo RW, Mc Donouogh PG: Massive Ovarian enlargement in primary hypothyroidism. Fertile Sterile 1997; 67: 169-171
11. Sanjeevaiah AR, Sanjay S, Deepak T, Sharda A, Shrikanta SS: Precocious puberty and large multicystic ovaries in young girls with primary hypothyroidism. Endocr Pract 2007; 13: 652-655
12. Gupta SP, Mittal A, Mehta V, Mahendru R: Multicystic ovarian disease secondary to hypothyroidism in a prepubertal girl. Annals of Tropical Medicine and Public Health 2011; 4: 107-109

Choriocarcinoma Emergencies: A Review of Two Cases

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Summary

Gestational trophoblastic disease has long been deemed as "God's first cancer and man's first cure", rightly so with the highly successful management of the disease using chemotherapy. Choriocarcinoma occasionally presents as an emergency, in the form of a hemodynamically unstable patient in shock, usually because of bleeding from primary or metastatic site. We present two such cases requiring emergency surgery.

Introduction

In the second decade of the 21st century the very way in which a gestational trophoblastic neoplasia presents has changed, with decreasing incidence of the more classic and complicated presentations. Choriocarcinoma, the most virulent form in the spectrum of gestational trophoblastic diseases, has been successfully treated with chemotherapy and occasionally surgery in recent times. The use of chemotherapy regimens in the management of gestational trophoblastic disease has reduced the need for surgical intervention once the uterus has been evacuated. Chemotherapy produces high cure-rates while maintaining fertility, allowing women to have further pregnancies.[1] However, we present two cases of choriocarcinoma who presented as emergencies, where surgery was resorted to as a life saving measure.

Case 1

Mrs S. a 35 years old lady, multiparous, presented elsewhere in May 2011 with a history of vaginal bleeding after one and a half months amenorrhoea. A dilation and curettage was done, of which no histopathology report was available. After one month, she again returned with the complaint of vaginal bleeding. Biopsy was taken from a vaginal lesion. She was then referred to us with the diagnosis of metastatic choriocarcinoma and uncontrolled vaginal bleeding.

At presentation, her general condition was poor with severe anemia (Hb-3.5g%), tachycardia, tachyapnoea, normal cardiovascular examination and bleeding from uterus and from the site of vaginal metastases (Figure 1). After initial investigations, the patient was diagnosed to have choriocarcinoma with vaginal and lung metastases. Beta human chorionic gonadotrophin (hCG) level was 3,73,052 IU/L. FIGO 2000 staging revealed a high risk case and she was staged as Stage III:8. It was decided to stabilize the patient and then administer chemotherapy at the earliest. She was started on intravenous tranexamic acid, broad spectrum antibiotics and emergency blood transfusion was given.

However on the second day of admission, before chemotherapy could be initiated, she developed profuse vaginal bleeding. She was immediately shifted to the operating theatre. Bilateral extra-peritoneal internal iliac artery ligation was performed. After a mid-line abdominal incision was given, the anterior sheath of the rectus muscle was exposed and opened below the level of the umbilicus, dissection caudal to the semilunar line of Douglas was performed, and the peritoneal and preperitoneal fat were separated. The peritoneum and its contents were reflected to the right (or left), thus exposing the retroperitoneal structures. Internal iliac arteries on either side were ligated. However excessive bleeding persisted from both the uterus (Figure 2) and vaginal metastasis. Hence, immediately abdominal hysterectomy was performed. Underpinning of the vaginal metastases located just inside the posterior vaginal fourchette was done using 2'0 catgut sutures. Bleeding was then completely controlled. She had an uneventful post operative recovery. Histopathology confirmed the diagnosis of choriocarcinoma. On 13th post operative day, beta hCG was found to be 5,915 IU/L.

As a high risk patient, she was planned for combination chemotherapy. However, because of the poor general condition, she was started on single agent chemotherapy with Actinomycin, from post operative day 13. She demonstrated a log fall with single agent chemotherapy. The medical oncologist hence continued with single agent chemotherapy. She continued to demonstrate more than one log fall in beta hCG in each cycle. Beta hCG normalized after 5

Case I

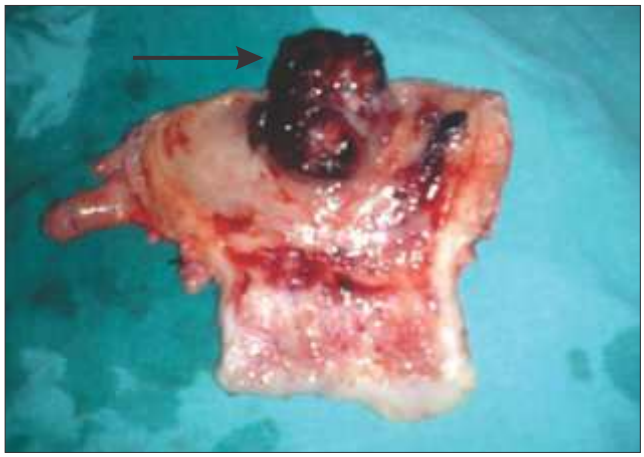

Figure 1: Uterus cut section showing tumor

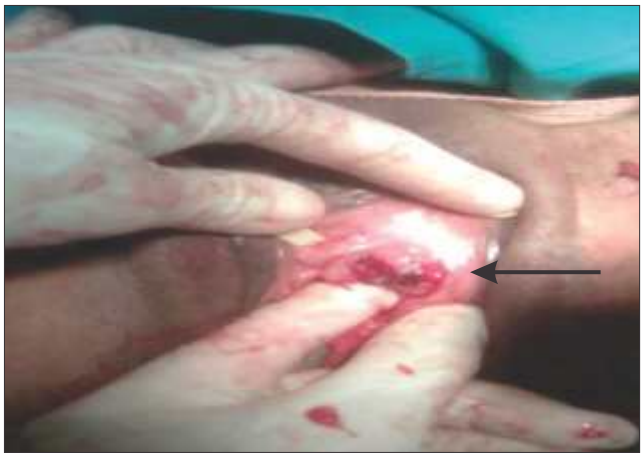

Figure 2: Vaginal metastasis

Case II

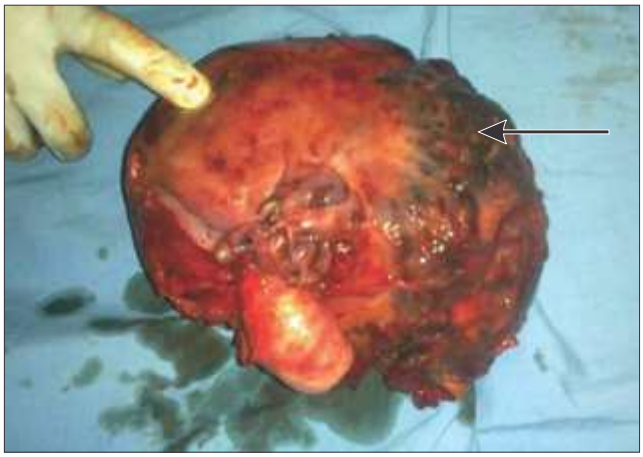

Figure 3: Uterus with the adherent mass

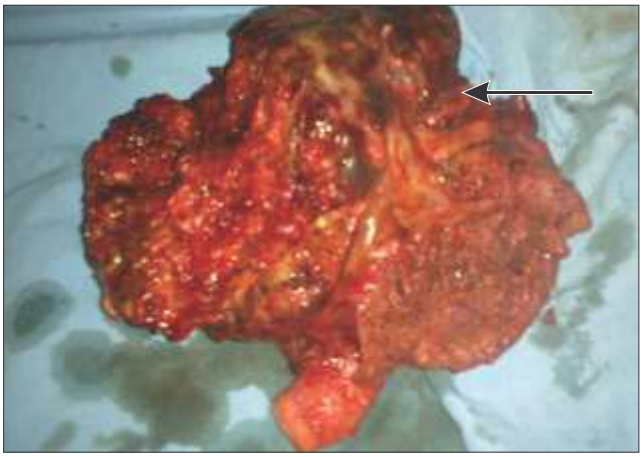

Figure 4: Mass with adherent colon

courses of Actinomycin. She was administered two further courses for consolidation. Presently the patient is on regular follow up and continues to remain in remission.

Case 2

Mrs K. a 40 year old multiparous lady, with the last child birth 5 years back, underwent laparotomy for a presumed uterine fibroid elsewhere. Uncontrolled bleeding was encountered during the surgery. The abdomen was closed and she was immediately shifted to our institute a few hours post operatively.

At presentation, the general condition of the patient was extremely poor. She was in hypotensive shock but was responding to commands. Her abdomen was distended, rigid and tender. There was continuous oozing from the cruciate abdominal incision. The initial investigations confirmed a huge intra peritoneal mass with a large hemoperitoneum. Choriocarcinoma was suspected and a serum beta hCG level was obtained. It was 79,000 IU/L. She was hence diagnosed provisionally as a case of choriocarcinoma in hemodynamic shock.

The patient was immediately taken for exploratory laparotomy. Intra operatively, a massive highly vascular tumor was found arising from the posterior surface of the uterus (Figure 3) and densely adherent to sigmoid colon (Figure 4), bladder and peritoneum. All surfaces were briskly bleeding and there was nearly two liters fresh blood in the peritoneal cavity. The tumor along with the uterus and involved sigmoid colon was removed. Segmental resection of the sigmoid colon was done and a diverting colostomy given. An intra operative bladder injury was repaired. Four large roller packs were used to pack the peritoneal cavity, along with ferroacrylum to control the remaining ooze, a drain was inserted, and the abdomen was closed. The packs were removed uneventfully after 48 hours. The abdominal drain stopped draining after two days. The patient had a stormy immediate post operative period, complicated by pneumonia and septicemia. This was managed conservatively with broad spectrum antibiotics.

Histopathology report confirmed the

diagnosis of choriocarcinoma. According to FIGO 2000 scoring, she was staged as stage IV:7. Due to extensive surgical morbidity, and her poor general condition, on the 12th post operative day she was started on single agent chemotherapy using methotrexate. On the 16th post operative day, the patient went into cardiac arrest during the removal of the central venous line. She was immediately resuscitated using advanced cardio pulmonary resuscitation and placed on mechanical ventilation. She, however, expired on the 18th post operative day due to cardiopulmonary arrest.

Discussion

Gestational trophoblastic neoplasia (GTN) is among the rare human malignancies that can be cured even in the presence of widespread metastases.[2] GTN includes a spectrum of interrelated tumors-including hydatidiform mole, invasive mole, placental-site trophoblastic tumor, and choriocarcinoma. Metastatic GTN occurs in 4% of patients after evacuation of a complete mole and is infrequent after other pregnancies; including normal ones.[3] Chorio-carcinoma has a tendency towards early vascular invasion with widespread dissemination. Pulmonary metastasis are the most common (80%).[4] Vaginal metastasis present in about 30%, are highly vascular and can bleed profusely if biopsied, which was seen in our first case. Hence, attempts at histologic confirmation of the diagnosis should be resisted. Liver, central nervous system, bowel, kidney and spleen are the other sites of distant metastasis.

If choriocarcinoma is suspected at initial presentation, and chemotherapy is promptly instituted, life threatening complications in the form of bleeding can be very effectively prevented, and further highly morbid surgery can be avoided. However, hysterectomy may be incorporated into the initial management of the patient with low-risk metastatic disease resulting in a higher likelihood of single-agent chemotherapy success, fewer courses of chemotherapy, and a shorter duration of treatment.[5,6] Patients with molar pregnancies may suffer from excessive vaginal bleeding; occasionally life threatening either at the time of presentation or after uterine evacuation and then hysterectomy becomes a reasonable option. Patients may occasionally present with severe intra-peritoneal hemorrhage as a result of a penetrative invasive mole leading to uterine perforation. Emergency laparotomy and hysterectomy may be life saving in such situations[1], as was adopted in our second case.

Conservative approaches to persistent vaginal bleeding should be considered in women who are desirous of future fertility and keen to avoid hysterectomy. Selective embolisation of the major pelvic blood supply to tmour can be performed by interventional radiology. Lim et al from Charing Cross hospital has reported the use of arterial embolisation in a series of 14 cases.[7] Selective ligation of the internal iliac artery has been recommended in the management of severe post partum hemorrhage. This technique should be considered in patients with heavy bleeding with persistent Gestational Trophoblastic Disease (GTD) or post molar vascular malformation[8] and there are case reports where this has been successful. Unfortunately few gynaecologists are experienced at this technique.

Unilateral or bilateral hypogastric artery ligation can be life-saving in patients with massive pelvic hemorrhage as was attempted in our first case. Although surgeons may be reluctant to perform bilateral hypogastric artery ligation for fear of injury to the pelvic viscera, there is no evidence that this is the case or that there is any significant impairment of function of the pelvic viscera. If the procedure is performed correctly, there is no morbidity, either short- or long-term.[9]

A mid-line extraperitoneal approach to the aorta, although rare, has been advocated.[10] This was the approach that we adopted in our first case and only on finding that it had failed to stop the bleeding, we proceeded ahead with hysterectomy.

Conclusion

Choriocarcinoma, a highly vascular trophoblastic disease with phenomenal metastatic potential is usually treated using chemotherapy with great success. Early diagnosis and prompt treatment at the right centers goes a long way in the prevention of its complications. However, when it presents with life threatening pelvic hemorrhage, surgery, both radical and conservative can be life saving. Hence it is pertinent on the part of the treating physician to become conversant with the intricacies of the internal iliac artery ligation and difficult hysterectomies and to keep an open mind in employing them when the situation so demands, as has been evident in the aforementioned two cases.

References

1. Tidy J: The role of surgery in the management of gestational trophoblastic disease; Gestational Trophablastic Disease, 2010; Third Edition, Chapter 18; 430-446
2. Goldstein DP, Berkowitz RS: Gestational Trophoblastic Neoplasms; Clinical principles of diagnosis and management, Philadelphia WB Saunders, 1982; 1-301
3. Goldstein DP, Berkowitz RS: Current management of complete and partial molar

pregnancy. J. Reproductive Medicine, 1994; 39: 139-146
4. Berkowitz RS, Goldstein DP: Pathogenesis of gestational trophoblastic neoplasia. Pathology Annuals 1981; 11: 391
5. Hammond CB, Weed JC Jr, Currie JL: The role of operation in the current therapy of gestational trophoblastic disease. American. Journal of. Obstetrics and. Gynecology, 1980; 136: 844-856
6. Soper JT, Clarke-Pearson DL, Berchuck A et al: Five day methotrexate for women with metastatic gestational trophoblastic disease. Gynecologic. Oncology.1994; 54: 76-79
7. Lim AK, Agarwal R, Seckl MJ, Newlands ES, Barrett NK, Mitchell AW: Embolization of bleeding residual uterine. Results with the EMA/CO regimen in high risk GTD, 1979 to 1989. British Journal Obstetric and Gynaecology, 2002; 98: 550-557
8. Bell AM, Hemmingway AP, Neal FE, Millar DR: Arteriovenous malformation of the uterus related to trophoblastic disease: a case for surgery or embolisation. Journal of Interventional Radiology. 1989; 4: 118-121
9. Clark SL, Phelan JP, Yeh SY et al: Hypogastric artery ligation for obstetric hemorrhage. Obstetrics and Gynaecology 1985; 66: 353-356
10. Shumacker HB Jr: Midline extraperitoneal exposure of the abdominal aorta and iliac arteries. Surgery in Gynecology and Obstetrics 1972; 135: 791–792

Bilateral Optic Neuritis following Chemotherapy in a known Case of Leukemia: A Case Report

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Summary

Optic neuritis is one of the important causes of acute loss of vision. It can be caused by various clinical conditions with multiple sclerosis (MS) contributing to the maximum number of cases and less common causes like chemotherapy induced toxicity. Clinical suspicion and imaging modality like ultrasonography (USG) and magnetic resonance imaging (MRI) have revolutionized imaging of it and helped us to come to conclusion. We present a case of a 19 year old male of T-cell Acute lymphoblastic leukemia (ALL) presented with painful acute loss of vision in right eye. Patient also had complain of blurred vision and painful movement of eyeball on left side at the time of presentation. The symptoms remained stable in right eye, however worsening of visual acuity was seen in left eye.

USG of both eye-balls and MRI of brain and orbit were perfomed which suggested findings of bilateral optic neuritis. Patient was put on intra venous (IV) methyleprednisolone immediately. Optic neuritis should be diagnosed at the earliest to find out cause of it, to start appropriate treatment and to prevent recurrent attacks of it.

Introduction

Optic neuritis is an acute inflammatory condition of optic nerve.[1] There are various conditions that can lead to optic neuritis, of which the most common is demyelinating conditions like multiple sclerosis (MS).[1] Other conditions leading to optic neuritis are infectious and parainfectious conditions, inflammation secondary to chemotherapy leading to toxic amblyopia and idiopathic.[1] Diagnosis of optic neuritis is essential. The causes need to be evaluated to direct appropriate treatment in early stage of disease when less damage is done to optic nerve.[1]

Magnetic resonance imaging (MRI) has emerging role in diagnosing optic neuritis specially T2weighted images (T2w) and post contrast fat saturated sequences.[2] Associated advantage of MRI is to evaluate brain for any anatomical mass lesion, aneurysm or infection.[2] Ultrasonography (USG) has limited role in diagnosing optic neuritis, however there are features to support the diagnosis like edematous changes of optic nerve, edema and elevation at optic disc etc.[3]

Case Report

A 19 year old male, known case of T-cell lymphoblastic leukemia was treated with 4 cycles of chemotherapy - vincrsitine, hydrodaunorubisine, cyclophosphamide and prednisolone (CHOP), came to our hospital with complaint of sudden loss of vision in right eye. He had blurring of vision on right side since 5 days associated with painful eye ball movement which progressed to near complete loss of vision on the day of presentation. He also complained of blurring of vision in left eye since 3 days with progressive decrease in vision. Left eye ptosis developed simultaneously with restriction of medial, superior and inferior gaze. Lateral gaze in left eye was preserved. Pain was severe and exaggerated on eyeball movements in left eye. He also had associated right facial nerve palsy.

Patient had no history of trauma, watering, photophobia or redness in eye. On examination eye lid was normal, conjunctiva was white and cornea was clear. On indirect ophthalmoscopy, oedema in optic disc region and surronding macular region was noted. Patient was advised for MRI examination. MRI was performed on 0.4 Tesla MRI machine. T1weighted (T1w) and T2w in axial, coronal and sagittal planes of orbit were taken. Images post IV gadolinium enhancement were taken. MRI showed tortuous dilated right optic nerve with optic disc elevation. The nerve appeared isointense on T1w images (Figure 1a); hyperintense on T2w images (Figure 1b); and isointense on FLAIR images. It showed moderate homogenous enhancement on post contrast study with maximum enhancement in the retro-bulbar portion and the optic disc (Figure 1c). Left optic nerve also showed altered signal intensity with moderate homogenous post contrast. But the diameter of left optic nerve was normal. The altered signal intensity and post contrast enhancement extended to the optic chiasma suggestive of bilateral optic neuritis.

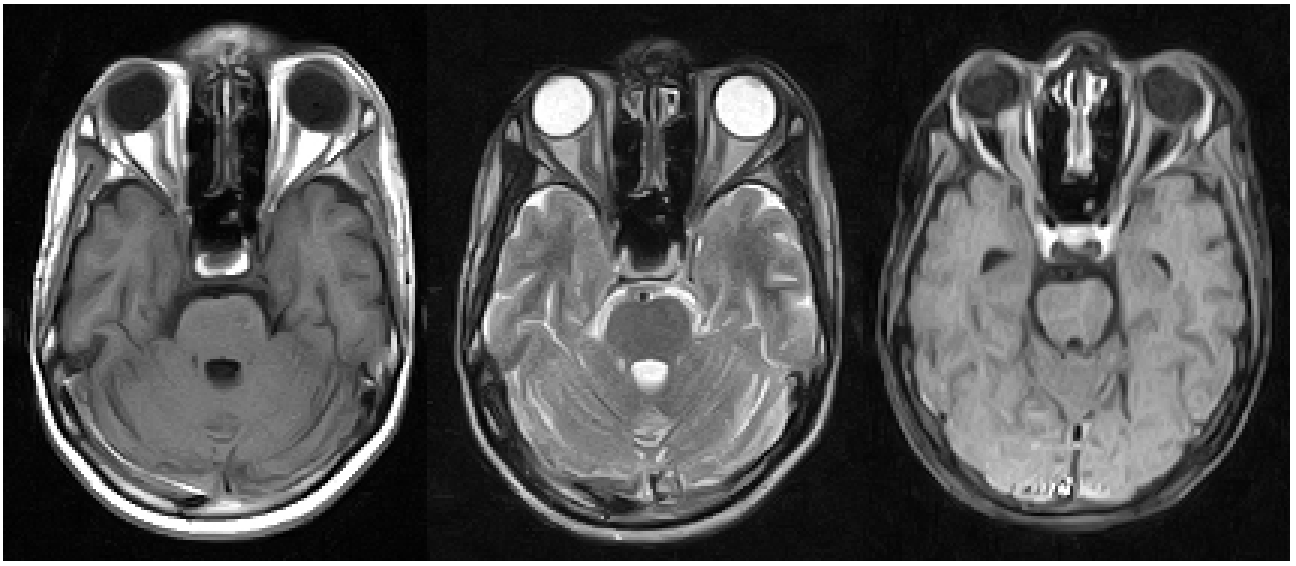

Figure 1: (a) T1w MRI image showing hypointense both optic nerves with thickened and tortuous right optic nerve. (b) T2w MRI image showing hyperintense optic nerves more on right side as compared to left side suggestive of edematous changes. (c) Post contrast fat suppressed T1w showing moderate to intense enhancement of bilateral optic nerves more on right side

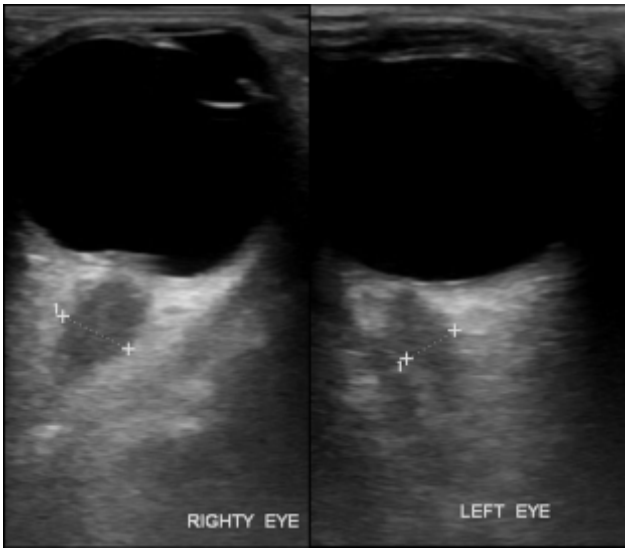

Figure 2: Ultrasound image of both eyes shows thickened and tortuous optic nerves and elevation of right optic disc

High resolution ultrasound of orbit was performed which showed elevation of right optic disc with surrounding disc oedema. Transverse diameter of right optic nerve was more then that of left optic nerve (Figure 2). Other laboratory investigations ruled out infiltration of brain by lymphoma. Patient was diagnosed as bilateral optic neuritis, secondary to toxic side effect of vincristine chemotherapy. In such clinical settings the likely possibility of optic neuritis could be due to vincristine – vinca alkaloids which is a known neurotoxin. Patient was immediately treated with temporary omission of chemotherapy and IV methylprednisolone for 3 days followed by oral prednisolone for 11 days.

Discussion

Optic neuritis is a classic neuro-ophthalmic disease.[1] Etiology of optic neuritis includes demyelinating disease, multiple sclerosis (most common cause), infectious and para-infectious conoditions (viral infections like mumps; measles; chicken-pox; whooping cough; glandular fever), following immunization, secondary to chemical agents-toxic amblyopias, as in cancer chemotherapy i.e. vincristine and hereditory conditions like leber's disease.[1]

Pathologically optic neuritis is primarily immuno-logical inflammation of optic nerve which damages myelin sheath but preserves axons.[4] According to involvement of portion of optic nerve, optic neuritis can be known as either papillitis, neuroretinitis or retro-bulbar neuritis.[1]

The pathogenesis of the peripheral neuropathy induced by vincristine is poorly understood, but interference of vinca-alkaloids with microtubule assembly suggests microtubule changes.[5] In vincristine-exposed axons, there is a shift to shorter length microtubules and the mean measured length of microtubules is significantly shorter.[5] On cross-sections study, the vincristine exposed axons show a decrease in the number of microtubules per square micrometer of axonal area suggestive of loss of portion(s) of each microtubule and support the possibility that microtubules consist of both stable and labile segments.[5] The microtubule changes are associated with malorientation of microtubules and neurofilaments, accompanied by free vesicle accumulation and fragmentation of the smooth endoplasmic reticulum.[5]

Incidence of vincristine induced neurotoxicity is less in leukemia (14%) as compared to lymphomas (61%).[6] Factors affecting neurotoxicity are dose of vincristine (higher dose-cumulative dose 12 mg), age (younger patient), incidence of carcinomatous neuropathy in the various types of malignancies treated with vincristine (i.e leukemic infiltration), the incidence of liver infiltration (uptake and excretion of drug decides its blood level) and effects of other drugs given in combination with vincristine (CHOP combination is particularly prone for neurotoxicity).[7,8] There are some studies showing off-therapy worsening of symptoms (24%) and signs (30%) unexpectedly.[7]

Patients predominantly complain of unilateral and rarely bilateral painful, partial or complete acute loss of vision. The condition is associated with pain on eye movements.[4] Progression to complete loss of vision over a few days to 2 weeks is usually noted with associated decreased color vision and contrast sensitivity.[1,4] Neuro-ophthalmic examination supportive of optic neuritis are relative afferent papillary defect (RAPD) and optic disk edema.[1] At least partial recovery of vision is the norm in 75% to 90% of cases.[1] But progression to monocular blindness is possible specially in recurrent optic neuritis cases due to primary optic atrophy.[1]

Easily accessible imaging modality, like ultrasound has important role in diagnosing papillitis.[3] On ultrasound there is elevation of optic disc and increase in transverse diameter of optic nerve.[3] In our case, we found similar findings in both optic nerves confirming diagnosis of optic neuritis.

MR has emerging role in diagnosing acute optic neuritis. It shows isointense signal on T1w images, high signal intensity on T2w images and post contrast enhancement.[2] Technique of Gadoversatamide combined with fat suppression (post contrast fat suppression) improves enhancing optic nerve lesions.[2] It may show mild bulge of the optic disc, thickened and tortuous optic nerve with edematous changes within.[2] In our case, both optic nerves showed isointense signal on T1w images, high signal intensity on T2w images and post contrast enhancement with thickened and tortuous right optic nerve. Left optic nerve was normal in diameter. These findings confirmed diagnosis of bilateral optic neuritis more severe on right side than left side.

If patient develops chemotherapy induced neurotoxicity, chemotherapy are immediately stopped and patients are treated with steroids, pyridoxin and pyridostigmine[1,9]. Optic neuritis treatment trial (ONTT) has made some recommendations for use of steroids in optic neuritis.[1] IV steroids followed by oral is indicated if patient develops simultaneous loss of vision in both eyes within hours or days of each others or has continuous slow progressive visual loss.[1] As our patient had developed continuous slow progressive visual loss in both eyes simultaneously, he was treated with steroids for 2 weeks. Steroid reduces inflammation and shorten the period of recovery although do not improve final visual outcome.[1] Patient was also given pyridoxin and pyridostigmine. Pyridoxin-pyridostigmine treatment trial showed insufficient conclusion.[10]

References

1. Khurana AK: Neuroophthalmology. Comprehensive Ophthalmology. Fourth Edition. Edited by: New Age International (P) Limited, Publishers: 2007; 12: 294-296
2. Beck RW, Arrington J, Murtagh FR: Brain MRI in acute optic neuritis: Experience of the Optic Neuritis Study Group. Arch Neurol 1993; 8: 841-846
3. Shrivastava PK: Examination of Orbit. Step by Step Ophthalmology Ultrasound. First Edition. Edited by: Jaypee Brothers Medical Publishers (P) Limited: 2007; 8: 178
4. Beck RW, Trobe JD: Optic Neuritis Study Group: What we have learned from the Optic Neuritis Treatment Trial. Ophthalmology 1995; 102: 1504-1508
5. Sahenk Z, Brady ST, Mendell JR: Studies on pathogenesis of vincristine induced neuropathy. Muscle and Nerve 1987; 10: 80-84
6. Watkins SM, Griffin JP: High incidence of vincristine-induced neuropathy in lymphomas. Br Med J. 1978; 1: 610–612
7. Verstappen CCP, Koeppen S, Heimans JJ et al: Dose related vincristine induced peripheral neuropathy with unexpected off therapy worsening, Neurology 2005; 64: 1076-1077
8. Arzanian MT, Mehdizadeh M, Zamani GR: Vincristine induced neurotoxicity: case study of 75 cases. Iranian Journal of Child Neuroradiology 2009; 3: 1-5
9. Ozyurek H, Turker H, Akbalik M et al: Pyridoxin and pyridostigmine treatment in vincristine neuropathy. Pediatr Hematol Oncol 2007; 24: 447-452
10. Jackson DV, Pope EK, McMahan RA et al: Clinical trial of pyridoxin to reduce vincristine neurotoxicity. Journal of Neurooncology. 1984; 4: 37-41

Aggressive Cervical Adenocarcinoma in a Young Lady: A Case Report

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Summary

Cervical cancer is the commonest cancer in females in India, with squamous cell carcinoma of the cervix being the commonest. The incidence of cervical adenocarcinoma is rising world-wide possibly due to changes in sexual habits and increased transmission of HPV at an early age. It accounts for 15%-25% of cervical cancer cases. Adenocarcinoma of cervix has less than optimal response to therapy, particularly irradiation, leading to disease progression or recurrence.

We present a case of cervical adenocarcinoma, in a very young girl with typical features of radio-resistance and aggressiveness. This case highlights the need for wide-spread HPV vaccination between the age of 9-26 years and instituting early and regular Pap smear screening for all, to bring down the rising cervical adenocarcinoma incidence.

Introduction

Cervical cancer is the commonest cancer in females in India. Adenocarcinoma of cervix accounts for 15-25% of cervical cancer cases with squamous cell carcinoma of the cervix as the commonest (upto 83%). Adenocarcinoma of cervix has a poorer clinical outcome than squamous cell carcinoma.[1] It usually has less than optimal response to therapy, particularly irradiation,[1] leading to disease progression or recurrence.

The clinical case described here showed typical features of radio-resistance and aggressiveness in a very young girl and evolved very similar to that described in the literature and thus calls attention for its similarities.

Case Report

A 20 year old lady, laborer by profession, was referred to our outpatient department (OPD) in March 2007 with complaints of purulent discharge, bleeding per vaginum and fever for past one week. She had attained menarche 5 years ago and had normal, regular menstrual cycles. She was sexually active since 1 year. There was no history of use of oral contraceptives. On examination under anesthesia, approximately 6 cm exophytic growth replacing cervix was seen. The cervical growth extended to upper 1/3rd of vaginal wall, and both parametrium were involved upto lateral pelvic wall. A single one cm hard inguinal node was also palpable on right side. Biopsy reports diagnosed papillary adenocarcinoma of cervix (Figure 1) with positive PAS and Alcian blue stains. Sonography of abdomen and pelvis detected a 6.6 x 3.8 cm lesion in cervix and a 1.3 cm right external iliac node. Fine needle aspiration cytology (FNAC) from the inguinal node was suspicious for metastasis. Thus a diagnosis of cervical cancer stage IVB was made. Curative radiotherapy and concurrent chemotherapy were planned.

She was treated with external radiotherapy (50 Gy) and concurrent 5 cycles of weekly cisplatin (50 mg) infusion without complications till May 2007. There was minimal response to treatment. On examination under anesthesia, whole of cervix was replaced by central hard nodule with involvement of lower 2/3rd of vaginal walls laterally. Both parametrium were involved upto lateral pelvic wall. As intra-cavitary radiotherapy would have been ineffective in treating the residual disease, pelvic boost (10 Gy) was administered instead.

She came regularly to the OPD with complaints of incontinence and/or frequency of urination for which she was treated. She refused examination at these visits. After 1 ¾ years of treatment, she presented with multiple inguinal nodes on the left side. FNAC from these nodes diagnosed metastatic poorly differentiated adenocarcinoma (Figure 2). On ultrasonography there was a 47x67 mm hypoechoic lesion in cervix extending into lower uterus and upper vagina. She was given 4 cycles of cisplatin and methotrexate from February-April 2009. Fifteen days later, she presented with vesico-vaginal fistula. Symptomatic treatment, sitz baths and personal hygiene care were advised. Since May 2010, she is lost to follow-up.

Discussion

The incidence of cervical adenocarcinoma is rising world-wide. Data from the USA have shown a two-fold increase, in women aged less than 35 years in absolute and proportional terms.[2] Our institute also reported increase in incidence of adenocarcinoma of cervix from 6.1% in 1977-1988 to 9.5% in the period 1989-1993.[3] In the past 2 years (2010 and 2011), 209 cases of cancer of uterine cervix were diagnosed at our

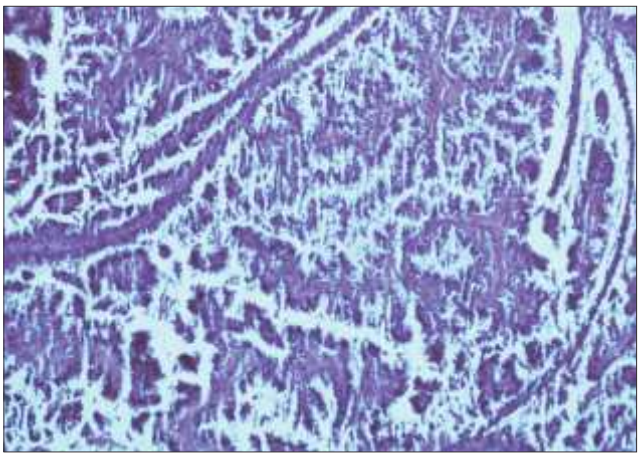

Figure 1: Papilary carcinoma cervix (10x)

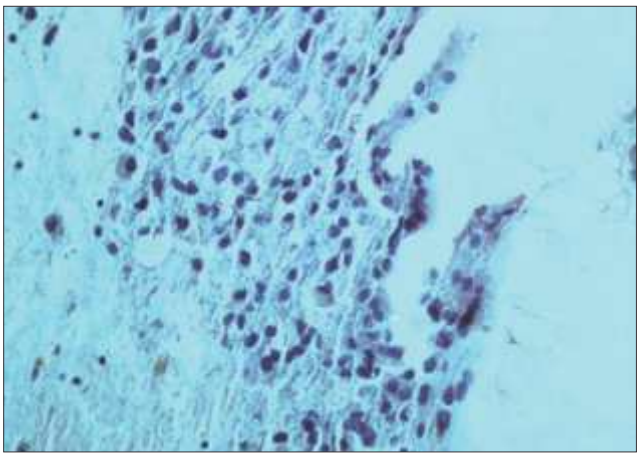

Figure 2: Metastatic papillary carcinoma cervix (40x)

institute. Histopathologically, 186(88.9%) cases of squamous cell carcinoma, 19(9.09%) cases of adenocarcinoma, 2(0.95%) cases of adenosquamous carcinoma and another 2(0.95%) cases of poorly differentiated carcinoma were diagnosed.

An explanation for the rise in incidence of adenocarcinoma of cervix in developed countries is that this is a relative increase as Pap smear screening has brought down the incidence of squamous cell carcinoma of cervix. However, Pap smear screening is less efficient, or even of no benefit, for adenocarcinoma and adenosquamous carcinoma of cervix, or that the risk factors are more pertinent to younger women or both.[4]

The known risk factors for cervical adenocarcinoma are HPV infection, infection by other microbial agents like Chlamydia trachomatis, Herpes Simplex Virus, exo/endogenous immuno-suppression, smoking, dietary deficiencies of Vitamin A or beta carotene etc. The increase in incidence of adenocarcinoma may be possibly due to changes in sexual habits and increased transmission of HPV at an early age.[4] Parazzini et al and many authors consider that risk of cervical adenocarcinoma is inversely related to age at first intercourse and directly to number of sexual partners.[5] Pathologically, this finding is supported by the fact that, during puberty and first pregnancy, the cervix increases in volume in response to hormonal changes. The endo-cervical epithelium everts onto the ecto-cervix (portio vaginalis) exposing it to the acidic pH of the vagina. This provides a stimulus for metaplastic change of the columnar epithelium. The area of the epithelium that has undergone metaplastic change is called the transformation zone. Numerous studies have shown that the immature metaplastic epithelial cells are susceptible to carcinogens and most, if not all, cervical cancers arise here.

The proposed therapy for cervical adenocarcinomas includes radical surgery and radiotherapy. However, the best treatment modality is yet to be established as there is increased radio-resistance and decreased survival with conventional mode of therapy.[5]

In the present case, the patient was young, sexually active at an early age, had poor hygiene and was of low socio-economic class. Poverty and illiteracy were other factors which would have prevented her from accessing health care services earlier. The tumour showed radio-resistance and aggressive nature due to which the disease progressed.

Socially, this disease causes wastage and suffering of human lives during their productive period. This case also reminds us of the need for government programs and help from nongovernmental organizations for wide-spread HPV vaccination between the age of 9-26 years before HPV infection occurs, regular Pap smear screening for all, education regarding reproductive health, especially in teenagers, use of barrier contraceptives and prevention of sexually transmitted diseases to bring down the incidence of cervical cancers.

References

1. Quinn MA: Review Articles - Adenocarcinoma of the cervix. Ann Acad Med Singapore 1998; 27: 662-665
2. Peters RK, Chao A, Mack TM, Thomas D, Bernstein L, Henderson BE: Increased frequency of the uterine cervix in young women in Los Angeles County. J Natl Cancer Inst 1986; 76: 423-428
3. Dave KS, Desai AD, Mankad MH, Kapadia AS: Radical Hysterectomy for adenocarcinoma of the cervix - The Gujarat Cancer & Research Institute Experience. Journal of Obstetrics and Gynecology of India 1996; 46: 146-150
4. Liu S, Semenciw R, Mao Y: Research Letter Cervical cancer: the increasing incidence of adenocarcinoma and adenosquamous carcinoma in younger women. CMAJ 2001; 164: 1151–1152
5. Parazzini F, La Vecchia C, Negri1 E, Fasolil M, Cecchetti G: Risk factors for Adenocarcinoma of the Cervix: A case-Control study Br J Cancer 1988; 57: 201-204

Fertility Preservation in Epithelial Ovarian Cancer: 10 Year Survival without Surgical Completion

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Summary

Early epithelial ovarian cancer (EOC) is optimally managed by surgical staging, total abdominal hysterectomy and bilateral salpingo oophorectomy. However, in the rare event of early stage EOC encountered in a young patient strongly desiring fertility preservation, a therapeutic dilemma arises. Conservation of uterus and the other ovary may be possible for stage 1A ovarian malignancies after fully informed consent regarding the risk of recurrence. In such cases, the patient is explained and should consent for completion of surgery; which is the standard procedure following child bearing.

We present a case, where fertility sparing surgery was done in a patient with stage 1C EOC after a thorough staging, counseling of patient and her husband and informed consent. After childbearing, the patient refused surgical completion. She has been closely followed for ten years and remains disease free.

Introduction

Epithelial ovarian cancers (EOC), a highly malignant group of tumors, are generally seen in advanced stage mainly in postmenopausal women. It is estimated that only 3 - 17% of EOCs occur in women aged $\leqslant$ 40 years.[1] It is optimally managed by surgical staging, total abdominal hysterectomy and bilateral salpingo oophorectomy.[2,3] However, when encountered in young patients in early stage, where fertility preservation is desired, it gives rise to therapeutic dilemma regarding optimal management. In younger patients, who wish to retain their childbearing potential, conservation of uterus and the other ovary may be possible for stage 1A ovarian malignancies.[4] However, there is a risk that women undergoing such a procedure may have higher chances of recurrence and completion of surgery is the standard procedure following child bearing.

Here, we present a case, where fertility sparing surgery was done in a patient with stage 1C EOC after a thorough staging, counseling and informed consent. After childbearing, the patient refused surgical completion and has been closely followed up for ten years.

Case Report

In June 2002, a 25 year old lady was referred to our centre from a private hospital. The patient had undergone laparotomy and excision of a 15cmx15cm left ovarian cystic mass with attached fallopian tube, elsewhere, in April 2002. Tumor was removed without any spillage in peritoneal cavity. Histopathological examination of the mass documented moderately differentiated intestinal type of mucinous cystadenocarcinoma, with capsular invasion. The patient was then referred to our centre.

The patient was of average build and moderately nourished. Clinical examination did not reveal any mass or lymphadenopathy. The histology slides were reviewed and the previous findings were confirmed. Serum cancer antigen 125 (CA-125) and carcinoembryonic antigen (CEA) levels were 5.64 U/ml (normal range up to 35 U/ml) and 3.16 ng/ml (normal Range: up to 3 ng/ml). Ultasonography (USG) showed no evidence of mass. Right ovary was normal and there was no evidence of mass in left adenexal region. DNA ploidy analysis showed diploid tumor.

As the patient had a one and half year old daughter, she was advised completion of surgery. However, she and her family members insisted on fertility preservation as she was desirous of more children. Informed consent of patient and her relatives for surgery was taken. They were explained increased risk of tumor recurrence with fertility preserving surgery compared to women who had complete surgery.

In July 2002, she underwent staging laparotomy with left infundibulopelvic ligament removal, bilateral pelvic lymph node dissection, infracolic omentectomy and appendicectomy. Careful inspection of large and small intestine, liver, undersurface of diaphragm and peritoneal surfaces was done and no disease was found. Wedge biopsy from right ovary was sent for frozen section which was negative for malignancy. Peritoneal fluid cytology report was negative. Histopathology report

of omentum, lymph nodes and appendix showed no evidence of malignancy. Hence, final stage was 1C mucinous cystadeno-carcinoma.

Her post operative course was uneventful. Six cycles of adjuvant chemotherapy with carboplatin were given till December 2002. Repeat USG was done which showed no evidence of mass. Patient was followed up with close observation. She regularly visited the hospital till 2004 and was in good clinical condition and disease free. She was then lost to follow up for four years.

Patient again visited the hospital in 2008. She had a vaginal delivery in 2005, was asymptomatic and disease free after investigation. She was advised completion of surgery but refused even after explaining the risk of recurrence in view of EOC. Hence, she has been kept on close follow up till date and there is no evidence of recurrence on clinical examination, USG and serum CA-125.

Discussion

Standard treatment of early stage EOC is surgical staging, total abdominal hysterectomy and bilateral salpingo oophorectomy. However, early EOC in young reproductive age group females constitutes a therapeutic dilemma. According to the 2007 guidelines of the American College of Obstetrics and Gynecology (ACOG), fertility-sparing surgery for reproductive-age patients with invasive EOC is recommended for highly or moderately differentiated stage IA disease with non-clear-cell histology.[4] Completion of surgery must be done after childbearing.

Fertility preserving surgery has been traditionally adopted for early stage malignant ovarian germ cell tumors and in ovarian sex cord – stromal tumors as they are mostly unilateral, with excellent reproductive outcomes without compromising oncologic safety.[5] For invasive EOC, data are much more constricted and limited to retrospective, non randomised series referring mainly to patients with low-grade stage IA tumors with favorable histology, while data regarding higher stages of the disease or unfavorable constellation of histological characteristics are rather conflicting.[6,7]

Mucinous EOC, as in our case are unilateral in 90% cases. Standard therapy for stage 1C mucinous ovarian cancer, as in our case is surgical staging, total abdominal hysterectomy and bilateral salpingo oophorectomy and appendicectomy followed by chemotherapy with 80% patients having 5 years disease free survival.[2] However, our patient was insistent on fertility preservation. Hence we proceeded with fertility preservation surgery, also buoyed by the fact that there was no disease elsewhere and contralateral ovary was normal. This was followed by six cycles of chemotherapy. Subsequently, on completion of her family, six years later, she was advised completion of surgery, which she steadfastly refused. Hence, she has been kept on close follow up and is disease free for ten years to date. During fertility preserving surgery, current recommendation is close inspection of contralateral ovary. Biopsy of other ovary should be taken only if suspicious area is seen as ovarian biopsy may induce adhesions and subsequent infertility. In our case, we had taken wedge biopsy of the other normal looking ovary, which is not recommended now.

Kajiyama et al. concluded that progression and overall survival of the patients with stage 1C EOC (surface involvement/positive cytology) was significantly poorer than those of patients with stage 1A after fertility preserving surgery.[8] In another study by Morice et al, 7 of 25 ovarian cancer patients treated conservatively recurred in average period of 15 months after surgery. Five of these seven patients had a recurrence in contralateral ovary. In addition, three of nineteen patients with FIGO stage 1A disease and all patients with stage 1C experienced recurrence.[9]

However, Columbo et al have reported more favorable data supporting the role of conservative fertility sparing surgery in young women with apparent stage 1 ovarian cancer. The authors had performed conservative staging surgery on 56 women and radical surgeries on 36 women younger than 40 years with stage 1 EOC from 1982 to 1992. Three of 36 patients with stage 1A disease who were treated conservatively recurred and 2 of 23 stage 1A patients treated with radical surgery recurred. None of twenty stage 1B-1C conservatively treated patients recurred.

When collectively evaluating most published results so far, mean relapse rates of fertility sparing surgery in EOC are estimated to be around 10%. When these results were closely examined, it was found that they mostly belong to subgroup 1C or grade 3 tumors.[8] Stage 1C is a high risk epithelial ovarian tumor, and is associated with high risk of recurrence in contra lateral ovary and at extra ovarian sites, if surgery is not completed. Thus, in patients in whom we have done fertility preserving surgery, completion surgery must be done after completion of family to improve overall survival of patient.[2] Our patient however refused completion surgery.

According to two randomized control trials (International Collaborative Ovarian Neoplasm-1 trial (ICON-1) and Adjuvant Chemotherapy In Ovarian Neoplasm trial (ACTION) adjuvant chemotherapy can improve progression free and overall survival in patients with stage 1 grade 3, stage 1C and stage 2 disease.[11] Chemotherapy would lead to temporary menstrual disorder, and 65–70% patients would regain normal ovarian function after the

completion of chemotherapy, while the congenital malformation rate would not be raised. In 2006, Maltaris et al studied 282 cases of early stage EOC patients undergoing fertility preserving surgery. Amongst them a total of 113 patients managed to become pregnant, among whom, 87 patients reached term pregnancy.[12] Our case also showed similar result suggesting that standard chemotherapy is unlikely to have an adverse impact on ovarian function, fertility and congenital malformations, despite being given adjuvant chemotherapy.

Although patients of subsequent chemotherapy should try to become pregnant as early as possible during the disease-free-period, pregnancy should not begin until at least 12 months after the end of chemotherapy in order to reduce negative effect of chemotherapy on the oocytes.[12] However, prospective (randomized) studies are unrealistic because indications for conservative management are uncommon, only well-conducted retrospective series could provide a better picture of the results of such treatment.

Conclusion

Fertility preserving surgery in early stage EOCs other than stage 1A tumor is fraught with controversies and therapeutic dilemma. This type of surgery may be attempted in properly staged stage 1 EOCs young patients, if they so desire, only after thorough counseling. Completion of surgery is done at the earliest opportunity.

However, in our case of Stage IC EOC, completion surgery was not carried out after child bearing was completed. The patient continues to be disease free for ten years after initial treatment. Further studies are required to confirm the need of completion surgery after child bearing is completed.

References

1. Greenlee RT, Hill-Harmon MB, Murray T, Thun M: Cancer statistics. CA Cancer J Clin.2001; 51: 15–36
2. DiSaia PJ: Conservative management of the patient with early gynecologic cancer. CA Cancer J Clin. 1989; 39: 135–154
3. Bristow RE, Palis BE, Chi DE, Cliby WA: The National Cancer database report on advanced-stage epithelial ovarian cancer: impact of hospital surgical case volume on overall survival and surgical treatment paradigm. Gynecol Oncol 2010; 118: 262–267
4. American College of Obstetricians and Gynecologists.ACOG Practice Bulletin: management of adnexal masses. Obstetrics and Gynecology 2007; 110: 201–214
5. Abdel-Hady ES, Abdel-Hady HR, Gamal A, El-Shamey M: Fertility sparing surgery for ovarian tumors in children and young adults. Archives of Gynecology and Obstetrics 2012; 285: 469–471
6. Rasool N, Rose PG: Fertility-preserving surgical procedures for patients with gynecologic malignancies. Clinical Obstetrics and Gynecology 2010; 53: 804–814
7. Zanetta G, Chiari S, Rota S et al: Conservative surgery for stage I ovarian carcinoma in women of childbearing age. British Journal of Obstetrics and Gynaecology 1997; 104: 1030–1035
8. Kajiyama H, Shibata K, Suzuki S et al: Fertility-sparing surgery in young women with invasive epithelial ovarian cancer. European Journal of Surgical Oncology 2010; 36: 404–408
9. Morice P, Wicart-Poque F, Rey A et al: Results of conservative treatment in epithelial ovarian carcinoma Cancer 2001; 92: 2412–2418
10. Colombo N, Chiari S, Maggioni C: Contoversial issues in the management of early epithelial ovarian cancer: Conservative surgry and role of adjuvant therapy. Gynecol Oncol 1994; 55: S47-S51
11. Trimbos JB, Parmar M, Vergote I et al: International Collaborative Ovarian Neoplasm trial 1 and Adjuvant Chemotherapy In Ovarian Neoplasm trial: two parallel randomized phase III trials of adjuvant chemotherapy in patients with early-stage ovarian carcinoma. J Natl Cancer Inst 2003; 95: 105–112
12. Maltaris T, Seufert R, Fischl F et al: The effect of cancer treatment on female fertility and strategies for preserving fertility. Eur J Obstet Gynecol Reprod Biol 2007; 130: 148–155

Treating Lymphocele with Oxytetracycline: A Case Report

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Summary

The occurrence of pelvic lymphocele is an important complication following systematic lymphadenectomy for gynecological malignancies. This case highlights the importance of early diagnosis and appropriate treatment for pelvic lymphocele following radical hysterectomy which would otherwise lead to increased morbidity in the patient.

Introduction

A lymphocele has been defined as a lymph-filled collection in the peritoneum devoid of any epithelial lining. Pelvic lymphocele is a well recognized complication of radical gynecological surgery and has also been reported following urologic procedures and renal transplantation. It has been reported in up to 30% of cases of lymphadenectomy performed because of gynecologic or prostate malignancy and in 2-18% of cases of renal transplantation. Occurrence of lymphocele has also been decribed in other areas like mediastinum, axilla, neck, aorta and peripheral vasculature.[1] All lymphoceles were diagnosed on the basis of biochemical and cytological findings in aspirated fluid. Small and sterile lymphoceles are often asymptomatic and spontaneously reabsorbed. Large and infected lymphoceles may cause serious symptoms that necessitate treatment. Treatment options include single or multiple aspirations, external drainage, intraperitoneal marsupialization or use of sclerosing agents. Therefore this case highlights the importance of early diagnosis and appropriate treatment for pelvic lymphocele following radical hysterectomy which would otherwise lead to increased morbidity in the patient.

Case Report

Mrs X, 59 years old, fairly nourished, post menopausal, multiparaous, last delivery 25 years back with no comorbidity was admitted in the department of gynecologic oncology of our institute on May 18th 2011 with the complaints of bleeding per vaginum and anorexia since 15 days.

On per speculum examination, there was 4×5cms exophytic growth. All fornices and bilateral parametrium were free. Her pre-operative investigations were within normal limits. Cervical biopsy revealed squamous cell carcinoma.

Radical abdominal hysterectomy with bilateral pelvic lymph node dissection was done on May 23rd 2011 under epidural and general anesthesia. Final histology revealed squamous cell carcinoma of cervix, moderately differentiated type. Tumor infiltrated more than 3/4th thickness of cervical wall. Maximum tumor diameter was 4 cms. Bilateral parametrium, vaginal resected margins and lymph nodes were free of malignancy. She received post operative radiotherapy (RT). During her treatment she complained of painful palpable lump in abdomen. Therefore, RT was withheld and further investigations were conducted. Post operative computed tomography (CT) scan was done on June 28th 2011 which showed 64×45 mm well defined cystic lesion in left iliac fossa anterior to left external iliac vessels possibility of lymphocele appeared likely. Ultrasonography (USG) guided aspiration was done under local anesthesia, 75 ml clear yellowish fluid was drained and sent for cytology examination which showed lymphocytes in clear background consistent with the diagnosis of lymphocele. RT was restarted on July 4th 2011. Repeat USG was done on July 22nd 2011 that showed presence of anechoic cystic lesion 57×54mm (80cc) along left iliac vessels. Refilling of lymphocele occurred within 15 days, hence active management was planned.

USG guided drainage was done along with instillation of 30 cc oxytetracycline on July 29th 2011. Repeat USG was done on August 8th 2011 followed by drainage of 150 cc of clear fluid and instillation of 30 cc oxytetracycline. (Figure-1 and 2). USG done on September 17th 2011 revealed 55×40mm organized lymphocele. Repeat USG after three months showed 50% reduction in size with no fluid content inside.

Discussion

The symptoms of lymphocele manifest within 3 weeks of surgery in 80% to 90% of cases. Symptoms are determined by the degree of external

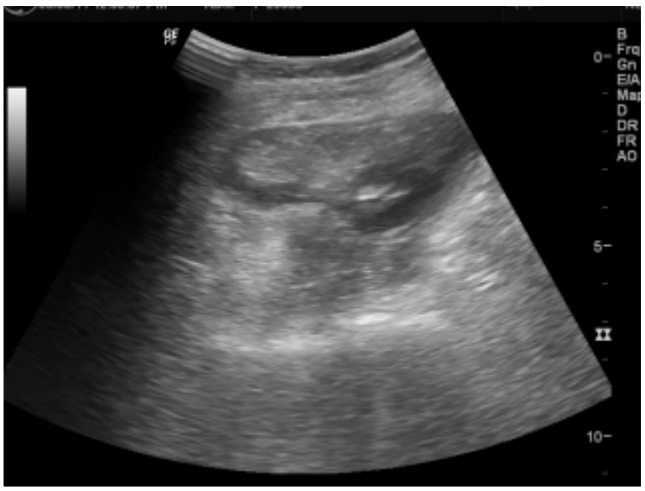

Figure 1: Before Oxytetracycline instillation

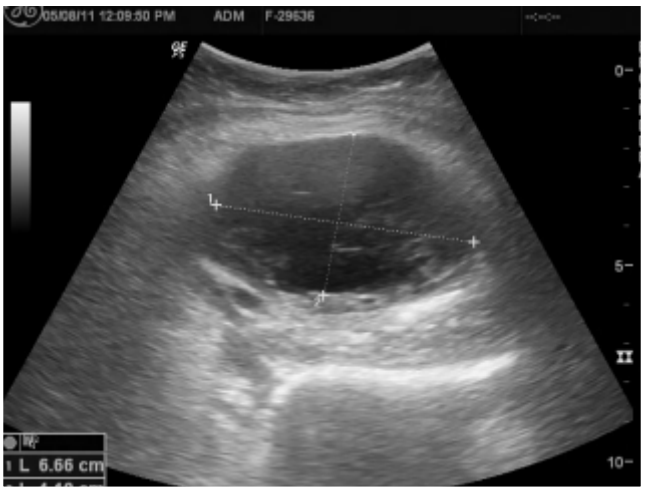

Figure 2: After Oxytetracycline instillation

compression of adjacent ureter, bladder, rectosigmoid, iliac vessels and pelvic nerves. Typical symptoms are lower abdominal pain radiating to leg and back, a sensation of abdominal fullness, urinary frequency, constipation, and edema of vulva and ipsilateral lower extremities.[1] Differential diagnosis includes seroma, haematoma, abscess and urinoma and in late post operative period include ovarian cysts and tumor recurrences. The diagnosis is established by pelvic USG or CT scan.[2]

Conservative management is indicated initially since most of the lymphoceles will be reabsorbed over a period of weeks. For infected lymphocele antibiotics alone are sufficient. Fine needle aspiration (FNA), with CT or USG guidance is undoubtedly helpful where diagnostic uncertainty exits. Due to minor morbidity some regard FNA as the first line treatment.[3] Disadvantages include high recurrence rate and chances of infection. Use of percutaneous catheter drainage is considered superior to FNA.[4]

The surgical management of lymphocele includes both external and internal drainage procedures. External surgical drainage has reported success rates of 80% but post operative care is complex and associated infection rate is 20%. Internal surgical drainage with peritoneal marsupialisation represents definitive therapy; primary success rate of 90% is expected.[1]

A variety of sclerosing agents have been used, gold, sodium tetradecyl sulphate, povidone iodine, tetracycline and alcohol. The possible mechanism of sclerosing agent is local oxidative effects responsible for inciting an inflammatory response in the wall of any fluid containing structure. It also has an anti exudative effect from chelation of proteins. Drawback of this treatment modality is prolonged treatment time, known sensitivity and potential damage to neighbouring structures due to marked local inflammatory response.[5] Application of fibrin sealants was recently published as preventive measures.[6]

Conclusion

Lymphoceles, smaller than 150 ml are treated with single-session sclerotherapy and greater than this are treated by multiple-session sclerotherapy. Percutaneous catheter drainage in combination with sclerosis with Povidone-Iodine has proved to be highly effective in obliterating pelvic lymphoceles. If the lymphocele is nonloculated, sclerosant therapy is the first line therapy. A multiloculated lymphocyst has more chances to recure under sclerotherapy because of the multiple cysts in the lymphocele cavity. The most important issue dealing with lymphocele remains prevention.

References

1. Metcalf KS, Peel KR: Review of Lymphocele. Annala of The Royal College of Surgeons of England 1993; 75: 387-392
2. Yamamoto R, Saitoh Ti, Kusaka T: Prevention of Lymphocyst Formation Following Systemic Lymphadenectomy. Jpn J Clin Oncol 2000; 30: 397-400
3. Jeong YY, Kim YH, Kim YC: Postoperative Pelvic Lymphocele. Radiology1999; 212: 390-394
4. Aronowitz J, Kaplan AL: The Management of Pelvic Lymphocele. Gynecologic oncology 1983; 16 : 292-295
5. Cohan RH, Saeed M, Schwab SJ, Permutt LM: Povidine –Iodine Sclerosis of Pelvic Lymphoceles. Urol Radiol 1988; 10 : 203-206
6. Khoder WY, Trottmann M, Bucher A: International Journal of Urology 2011; 18: 638-643

Summaries of the Published Reports

Department of Community Oncology and Medical Records

(A) Rural Cancer Registry : Ahmedabad District

Year: 2008

Rural Cancer Registry-Ahmedabad District (RCR-AD) has been functioning at Gujarat Cancer & Research Institute (GCRI), Ahmedabad under the network of National Cancer Registry Programme of Indian Council of Medical Research since January 2004. The objectives of this registry are (i) To have a valid estimate of annual incidence of cancer cases in the Ahmedabad district (ii) To study cancer patterns in the male and female population of all ages in Ahmedabad district (iii) To study cancer related mortality through death records from village/nagar panchayat offices.

During year 2008, 804 (males: 485; females: 319) patients with cancer were recorded. The crude cancer incidence rate (CIR) per lac population per year in male was 55.8 and in females 40.8. The corresponding age adjusted rates (AAR) was 74 and 46.6. The truncated incidence rate (TR) among males and females were 142.4 and 109.1 per 1,00,000 persons, respectively. Male/Female ratio was 1.52:1**.**

Majority of cases (12.99%) among males were found in age group of 60-64 years and 14.42% cases among females were found in age group of 45-49 years and also in 50-54 years age group. About 87% (697 cases) of cancers occurred after the age of 35 years, whereas total population above the age of 35 years accounted for about 33% in both sexes thus indicating need for control measures to prevent cancer problem among general population at very beginning of truncated age group.

Pediatric cancers (age 0 -14 years) constituted 3.36% (27 cases) of total cancer load in both sexes with higher percentage of cases among boys (3.51%) than girls (3.13%).

The first five leading sites of cancers among males, cancer of the tongue is the most predominant site of cancer constituting 14.43% of the total cancers followed by cancer of the mouth (12.78%), lung (11.34%), oesophagus (6.39%) and hypopharynx (5.36%). In females, cancer of breast has accounted for 23.82% of the total cancer, followed by cancer of cervix (17.24%), ovary (6.27%), oesophagus and myeloid leukemia (4.39%) and mouth (3.13%).

Tobacco related cancers (TRCs) accounted for more than half (60%) of all cancers in males and 14.11% of all cancers in females. Among the tobacco related cancer sites in males, cancer of tongue was the most common site (24.05%) followed by mouth (21.31%) and lung (18.90%) cancer. These three sites together constituted 64.26% of total TRCs in males. In females, cancer of oesophagus was more common (31.11%) followed by mouth (22.22%) and tongue (15.56%) cancer.

In the year 2008, 136 deaths in males and 80 deaths in female were registered. The crude mortality incidence rate (CMR) per lac population per year in male was 15.7 and in females 10.2. The corresponding age adjusted mortality rates (AAMR) was 21.1 and 11.6. The truncated mortality incidence rate (TMR) among males and females were 40.3 and 27.8 per 1,00,000 persons respectively. Mortality to incidence (M/I) percentage was 26.87% and the cases registered with death certificate only sources (DCOs) accounted for 1.49%.

Year: 2009

During year 2009, 833 (males: 460; females: 373) patients with cancer were recorded. The CIR per lac population per year in male was 52.6 and in females 47.5. The corresponding AAR was 68.9 and 52.9. The TR among males and females were 138.1 and 121.4 per 1, 00,000 persons respectively. Male/Female ratio was 1.23:1**.**

Majority of cases among males (14.78%) and females (15.28%) were found in age group of 60-64 years and 50-54 years respectively. About 85% of cancers occurred after the age of 35 years, whereas total population above the age of 35 years accounted for about 34% in both sexes thus indicating need for control measures to prevent cancer problem among general population at very beginning of truncated age group.

Paediatric cancers (age 0 -14 years) constituted 2.88% (24 cases) of total cancer load in both sexes with lower percentage of cases among boys (2.61%) than girls (3.22%).

The first five leading sites of cancers among males, cancer of the tongue is the most predominant site of cancer constituting 14.57% of the total cancers followed by cancer of the mouth (10.87%), lung (9.57%), hypopharynx (4.78%) and prostate (4.13%). In females, cancer of breast has accounted for 19.57% of the total cancer, followed by cancer of cervix (18.50%), ovary (6.43%), myeloid leukemia (5.36%) and mouth (5.09%) cancer.

TRCs accounted for more than half (55.00%) of all cancers in males and 19.84% of all cancers in females. Among the tobacco related cancer sites in males, cancer of tongue was the most common site (26.48%) followed by cancer of oral cavity (19.76%) and lung (17.39%). These three sites together constituted 63.64% of total TRCs in males. In females, cancer of oral cavity was more common (25.68%) followed by tongue (18.92%) and lung (17.57%).

In the year 2009, 202 deaths in males and 144 deaths in female were registered. The CMR per lac population per year in male was 23.1 and in females 18.3. The corresponding AAMR was 30.7 and 20.9. The TMR among males and females were 63.9 and 42.4 per 1,00,000 persons respectively. M/I percentage was 41.53% and the cases registered with DCOs accounted for 0.36%.

(B) Population Based Cancer Registry: Ahmedabad Urban Agglomeration Area

Year: 2008

Population based cancer registry – Ahmedabad urban agglomeration area was established at GCRI, Ahmedabad under the network of National Cancer Registry Programme of Indian Council of Medical Research in the year 2007 with the main objective of generating reliable data on magnitude and pattern of cancer morbidity and mortality among the residents of Ahmedabad urban agglomerate.

During year 2008, 3850 (males: 2139; females: 1711) patients with cancer were recorded. The CIR per lac population per year in male was 84.8 and in females 77. The corresponding AAR was 115.5 and 89.8. The TR among males and females were 199 and 183.4 per 1,00,000 persons respectively. Male/Female ratio was 1.25:1.

Majority of cases (13%) among males were found in age group of 55-59 years and 13.85% cases among females were found in age group of 50-54 years. About 90% of cancers occurred after the age of 35 years, whereas total population above the age of 35 years accounted for about 35% in both sexes thus indicating need for control measures to prevent cancer problem among general population at very beginning of truncated age group.

Paediatric cancers (age 0-14 years) constituted 83 cases (2.16%) of total cancer load in both sexes with higher percentage of cases among boys (2.52%) than girls (1.69%).

In the year 2008, 591 deaths in males and 379 deaths in female were registered. The CMR per lac population per year in male was 23.4 and in females 17.1. The corresponding AAMR was 32.4 and 20.1. The TMR among males and females were 57.2 and 42 per 1,00,000 persons respectively. M/I percentage was 25.19% and the cases registered with DCOs accounted for 5.71%.

Year: 2009

During year 2009, 4006 (males: 2263; females: 1743) patients with cancer were recorded. The CIR per lac population per year in male was 88.2 and in females 77.2. The corresponding AAR was 119.2 and 89.2. The TR among males and females were 205.8 and 184.8 per 1,00,000 persons respectively. Male/Female ratio was 1.29:1.

Majority of cases among males (12.20%) and females (13.71%) were found in age group of 50-54 years. About 89% of cancers occurred after the age of 35 years, whereas total population above the age of 35 years accounted for about 36% in both sexes thus indicating need for control measures to prevent cancer problem among general population at very beginning of truncated age group.

Paediatric cancers (age 0-14 years) constituted 94 cases (2.35%) of total cancer load in both sexes with higher percentage of cases among boys (2.92%) than girls (1.61%).

In the year 2009, 448 deaths in males and 279 deaths in female were registered. The CMR per lac population per year in male was 17.5 and in females 12.4. The corresponding AAMR was 22.7 and 13.9. The TMR among males and females were 44.1 and 29.6 per 1,00,000 persons respectively. M/I ratio was 18.15% and the cases registered with DCOs accounted for 2.25%.

Summaries of Published Articles

01. Anesthetic Management of an Achondroplastic Dwarf for Transhiatal Oesophegectomy

Sanghavi P, Shah B, Chaudhary N, Patel B
Department of Anaesthesia

Summary

Anesthetic management for an achondroplastic dwarf posted for transhiatal oesophegectomy offers a considerable challenge to anesthesiologist. This report describes successful anesthetic management of achondroplastic dwarf under general anesthesia and epidural analgesia. Since a dwarf tends to have several abnormalities that have anesthetic implications we offer a general approach to preoperative evaluation and suggest guideline for anesthetic management for this patient.

Asian Archives of Anesth & Resuscitation: 2010; 71: 1931-1933

02. Pain Management in a Case of Sensory-Motor Polyneuropathy following Chemotherapy

Sanghavi P, Joshi G, Patel B
Department of Anaesthesia

Summary

Chemotherapy, especially Vincristine, Paclitaxel and Methotrexate are known to cause neuropathy. Intrathecal Methotrexate in treatment of leukemia can produce acute meningitis syndrome in 5-50% of cases. Paclitaxel generates a syndrome of diffuse arthralgia and myalgia in 10-20% of cases. Vincristine, a vinca alkaloid is associated with neuropathic like acute pain syndrome. Here we report a case of sensory-motor polyneuropathy following Intrathecal injection of chemotherapy and its management.

Indian J Pain: 2011; 25: 133-134

03. Effect of Positive End Expiratory Pressure (PEEP) to the Dependent Lung and Effect of Continuous Positive Airway Pressure (CPAP) to the Non-Dependent Lung along with Positive End Expiratory Pressure to the Dependent Lung on Arterial Oxygenation during One Lung Anesthesia

Patel L, Thakkar J, Joshi G, Patel B
Department of Anaesthesia

Summary

This study was carried out to compare the effect of PEEP (8 cm of H_2O) to the dependent lung and combined effect of PEEP (8 cm of H_2O) to the dependent lung along with CPAP (5 cm of H_2O) to nondependent lung on arterial oxygenation. Twenty patients undergoing one lung anesthesia for thoracic surgery were selected for study. ABG was done after fifteen minutes following (a) two lung anesthesia (b) one lung anesthesia (c) after application of PEEP to dependent lung (d) after application of CPAP to nondependent lung along with PEEP to the dependent lung. PaO_2, $PaCO_2$, pH, ventilation perfusion mismatch (V/Q mismatch %) I:E shunt %, buffer bases (HCO_3^-), SpO_2%, $EtCO_2$, ECG changes, heart rate, and blood pressure were monitored. One lung ventilation resulted in fall of PaO_2 from 260.3±19.2mm of Hg (135-318 mm of Hg) to 84.3±4.8 mm of Hg (40-100 mm of Hg). PaO_2 increased during application of PEEP to 96.4±5.6 mm of Hg (72-108 mm of Hg) in 16 patients. Associated fall in shunt% along with rise in PaO_2 was (10-12%). PaO_2 was 74.40±6.68 mm of Hg with PEEP in three patients with rise in shunt % to 10-20%. In one patient there was no change in PaO_2 with PEEP. $PaCO_2$ was reduced from 44±3.2 mm of Hg to 38±6.2 mm of Hg during application of PEEP. All patients subjected to PEEP to dependent lung and CPAP 5 cm of H_2O to non dependent lung showed significant rise of PaO_2 from 84.3±4.4 mm of Hg to 126.3±8.7 mm of Hg (90-158mm of Hg). Fall in shunt% was 18.6±9.8%. No significant changes were observed in $PaCO_2$. Haemodynamic data shows fall in arterial pressure by 20.6±8.9 mm of Hg after application of PEEP which was corrected within 10 min to basal value. $_pH$ and buffer bases showed no significant change with either PEEP or CPAP or both. Application of CPAP to nondependent lung with PEEP to dependent lung is more beneficial than application of PEEP alone to the dependent lung to improve PaO2 during one lung anesthesia.

Asian Archives of Anesth and Resuscitation: 2010; 71(1): 1920-1924

04. A Comparative Study of Ketamine, Diclofenac (Dispersible) and Lignocaine Mouthwash for Pain Relief in Radiation-induced Mucositis

Joshi G, Taneja P, Patel B, Patel B
Department of Anaesthesia

Summary

Patients with moderate to severe mucositis invariably suffer pain and hyperalgesia on chewing food and speaking. Objective of this study was to compare effectiveness of Ketamine, Diclofenac (Dispersible) and Lignocaine viscous in relieving pain in mucositis. Seventy-five adult patients undergoing

radiotherapy for head and neck cancer were enrolled in this randomized, prospective study. They were allocated into three groups of 25 subjects each: Group K, Ketamine 20 mg in 5 ml saline; Group D, Diclofenac (Dispersible tablet) dissolved in 5 ml saline; Group L, Lignocaine viscous 5 ml. Patients were asked to swish and swallow this mixture four times/day. 89.2% patients had mucositis Grade III and 10.7% patients had mucositis Grade IV as per WHO grading for mucositis. Patients' VAS decreased from 7.8 to 4.2 in Group K, 8.2 to 3.8 in Group D and 7.9 to 4.1 in Group L after treatment. Patients in Ketamine group had pain relief within minutes following mouthwash. They were able to chew food after four days, compared to more than 10 days in patients of Group L and Group D. Two patients receiving Ketamine reported giddiness, which was transient. Ketamine, Diclofenac and Lignocaine mouthwash relieves pain of mucositis. Patients treated with Ketamine are able to chew food earlier than those treated with Lignocaine or Diclofenac mouthwash.

Indian Journal of Pain: 2011; 25(01): 29-31

05. Uterine Carcinosarcomas: 8-year Single Center Experience of 25 Cases

Dave K, Chauhan A, Bhansali R, Arora R, Purohit S
Department of Gynecological Oncology

Summary

The aim of this retrospective study was to evaluate the behavior and treatment outcomes of uterine carcinosarcomas in relation to their clinical and pathogenic features and to determine the optimal treatment strategy. Secondary objectives were to identify parameters predictive of survival. The hospital records of all 25 patients of uterine carcinosarcoma operated between 2000 and 2008 in Gujarat cancer research institute, Ahmedabad, were reviewed. Patients who presented with clinical evidence of recurrent disease or those who had incomplete medical records were excluded from our analysis. The status of these patients was updated up to November, 2010. Patients were classified according to the new 2009 FIGO staging system for endometrial carcinoma, to see what difference the assigned stage has on survival with the old treatment strategy. Survival was calculated by Kaplan-Meier method and compared by Log-Rank test. Median survival time was derived with the Brookmeyer 95% confidence interval. For comparison of qualitative data, Chi-Square test and Fisher extract χ^2 were used. Median age of patients was 56 years (range, 36-77 years). Only 36% of patients had stage I at diagnosis and another 36% were stage III. Most of the tumors (56%) were with homologous sarcomatous components and 64% of tumors were high grade (grade 2/3) at diagnosis. Fifty-two percent patients received postoperative adjuvant treatment. Twelve patients had no postoperative treatment: two were lost to follow-up immediately after surgery, four could not receive adjuvant treatment on account of severe medical complications and age factor which could have increased morbidity, and six patients declined treatment. Four of these patients expired within one year of diagnosis, two other within 18 months, and rest were lost to follow-up. The difference in survival of 13 patients who had taken adjuvant treatment was significantly more than the group who had not taken adjuvant therapy (P = 0.025). The overall 3-year disease-free survival of 13 patients who had taken adjuvant therapy was 40%. However, these adjuvant treatment modalities had borderline statistical significance on overall survival of patients (P = 0.075). The only statistically significant predictor of survival in this study was stage of the disease (P = 0.035). This highly aggressive uterine malignancy warrants comprehensive surgical staging to assess tumor dissemination followed by systematic adjuvant therapy in patients with both early and advanced disease. The value of pelvic RT in addition to systemic treatment remains ill-defined. Stage is the significant predictor of survival for the disease. Our results indicate that in this highly aggressive malignancy, further exploration of potential outcome benefits of postoperative treatment, especially chemoradiation, is warranted in larger group of patients after comprehensive surgical staging.

Indian Journal of Medical and Paediatric Oncology: 2011; 32 (3): 162-166

06. Obstructive Uropathy in Cancer Cervix

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Summary

Over 70% patients with cancer cervix present in advanced stage of disease and in many of them, it is difficult to offer the definitive treatment as they present with some urological complications like obstructed uropathy. In this study we evaluated patients with obstructed uropathy with uremia for urinary diversion to facilitate further definitive treatment for cancer cervix. In this study, 24 patients with cancer cervix, whether treated or untreated who had obstructed uropathy were evaluated for type of complication, their management and effect on the primary disease. Eleven patients underwent urinary diversion in form of per-cutaneous nephrostomy and they were followed – up after tumour specific treatment. Among 24 patients, 15 patients (62.5%) had obstructed uropathy with uremia and 9 (37.5%) patients had obstructed uropathy without uremia. In present study, 22 patients were in advanced stage and 2 were in early stage. Eleven patients (45.8%) with

obstructed uropathy were treated by urinary diversion procedure followed by radiotherapy or chemotherapy, curative radiotherapy in 1 patient and palliative radiotherapy in 8 patients and palliative chemotherapy in 2 patients with recurrent disease. Among this, 5 patients came for follow –up for more than 6 months. Urinary diversion procedure in obstructed uropathy in form of per-cutaneous nephrostomy in case of advanced cancer cervix was effective to save renal function until tumour specific treatment could be offered in form of radiotherapy or chemotherapy which helps in prolongation of life in such patients for atleast few months.

Indian Journal of Gynecological Oncology: 2011; 10(1): 25-28

07. Two Case Reports of Radical Abdominal Hysterectomy with Caesarean Section

Purohit S, Dave P, Dave K, Bhansali R, Arora R
Department of Gynecological Oncology

Summary

Invasive cervical cancer associated with pregnancy is relatively unusual, although cervical cancer is the most common malignancy that occurs in pregnancy. This situation is a dilemma for the patients as well as for physicians; effective treatment has to be provided without compromising pregnancy whenever possible, especially because these patients will be deprived of their fertility after surgical procedures. We report 2 cases, a patient with 32 weeks pregnancy and a patient with 36 weeks pregnancy, both underwent LSCS followed by radical hysterectomy at 36 weeks and delivered live healthy babies. Both were advised curative radiotherapy which only one of them received. The other patient had a disease free survival of 6 months and then presented with vault recurrence. Both of them are now lost to follow-up. Carcinoma cervix in pregnancy is a curable disease. When histological diagnosis is confirmed, patient is evaluated individually and a treatment plan is outlined taking into consideration the patient's emotional, religious and ethical beliefs. The well being of both mother and fetus are considered and all options of therapy are discussed with the patient & relatives in detail outlining the associated benefits and risks.

Indian Journal of Gynecological Oncology: 2011; 10(1): 66 - 68

08. Primary Bartholin Gland Adenocarcinoma – Successfully Treated With a Disease Free Survival of more than 8 Years: A Case Report

Dave K, Dave P, Mankad M, Gupta A
Department of Gynecological Oncology

Summary

This is a case of Primary Bartholin Gland Adenocarcinoma successfully treated with radical surgery and radiotherapy with a disease free survival of more than 8 years. Bartholin gland adenocarcinoma is a rare type of vulvar malignancy. Since only limited number of cases have been reported so far, literature review becomes important for management protocols. Case: We report a case of successfully treated Bartholin gland adenocarcinoma. Radical vulvectomy with bilateral groin node dissection was performed. Postoperatively adjuvant radiotherapy was given. Patient has been disease free for the past 8 years and has not developed any recurrence. Bartholin gland adenocarcinoma can be effectively cured by radical surgery and adjuvant radiation.

Indian Journal of Gynecologic Oncology: 2011; 10(1): 52-54

09. Extra Mammary Pagets' Disease of Vulva-A Report of Four Cases

Dave K, Dave P, Mankad M, Gupta A
Department of Gynecological Oncology

Summary

Vulvar Extra Mammary Paget's disease (EMPD) is a rare neoplasm accounting for 2.5% of all vulvar malignancies. Its histogenesis is uncertain. Most commonly it is an intra epidermal lesion. The treatment of choice is primarily surgery. This is often difficult since the margins of the lesion can not be distinguished macroscopically because it extends sub-epithelially beyond the gross lesion. The disease is often multicentric. It has high rate of recurrence which makes optimal management challenging. Cases: We report four patients with EMPD diagnosed between the years 1994 to 1999. Mean age was 57.5 years. Vulvar pruritus was the predominant symptom. All four patients were primarily treated with surgery with adjuvant radiotherapy if required. One patient had underlying apocrine gland adenocarcinoma. EMPD recurred twice in one patient and was treated successfully with wide local excision. By proper individualization of treatment in each case, we have successfully treated this rare neoplasm.

Indian Journal of Gynecologic Oncology: 2011; 10(1): 46-48

10. Bilateral Breast Metastasis from an Adeno-carcinoma of Lung: Case Report

Nasit J, Parikh B, Shah M
Department of Pathology

Summary

Breast metastases from extramammary neoplasms are very rare. The pathologist has a key role in making the diagnosis of metastasis to the breast when histological appearance is similar to primary breast tumor. The clinical history is helpful to make the diagnosis. Metastasis to the breast usually indicates disseminated metastatic disease and poor

prognosis. We report a case of 42-year-old female who developed bilateral breast metastasis 18 months after the diagnosis of primary adenocarcinoma of lung.
National Journal of Medical Research 2011; 1(2): 83-86

11. [131I] Metaiodobenzylguanidine Therapy in Neural Crest Tumors: Varying Outcome in Different Histo-pathologies

Rachh S, Abhyankar S, Basu S
Department of Nuclear Medicine

Summary

To evaluate the response of [131I] metaiodobenzylguanidine ([131I] MIBG) therapy in patients with neuroectodermal tumors and to assess their quality of life using the functional assessment of cancer therapy –general quality-of-life questionnaire for patients who are on follow-up after MIBG therapy. Thirty-two patients diagnosed with various subtypes neuroectodermal tumors and treated with [131I] MIBG were included in this retrospective analysis. Response to therapy was evaluated objectively by comparing pretherapy and posttherapy biochemical markers, radiological investigations, and follow-up MIBG scans. Symptomatic response and quality of life were also evaluated in the follow-up visits. In seven patients with stage III neuroblastoma, an objective response rate was seen in 57% and a symptomatic response rate was seen in 29% of the patients. Among 11 patients with stage IV neuroblastoma, an objective response was observed in 36% and a symptomatic response in 36% of the patients. Among 12 patients with pheochromocytoma and paraganglioma, an objective response was noticed in 8%, but symptomatic improvement and stabilization of disease were seen in 75% of the patients belonging to this category. One patient with medullary carcinoma of the thyroid and one patient with mediastinal carcinoid did not show an objective response but had a stable disease; both patients showed symptomatic improvement. Quality of life has improved in all 11 patients who are still on follow-up. [131I] MIBG therapy can be of significant value in the treatment of patients with chemotherapy-resistant stage III and IV neuroblastomas who demonstrate good tracer uptake in diagnostic scans. MIBG therapy has the potential to stabilize the disease and provide symptomatic improvement in patients with metastatic/recurrent pheochromocytoma/paraganglioma and medullary carcinoma thyroid and carcinoid in which there is evidence of tracer accumulation in the tumor. Both single high dose or multiple fractionated doses are equally effective in improving the quality of life in metastatic/recurrent pheochromocytoma / paraganglioma.

Nuclear Medicine Communications: December 2011; 32(12): 1201–1210

12. Comprehensive FLT3 Analysis in Indian Acute Myeloid Leukaemia

Mehta S, Shukla S, Vora H
Immunohistochemistry and Flowcytometry Division

Summary

Activating mutations of FLT3 are commonly found in AML patients and reported to be associated with poor clinical outcome. We aimed to evaluate the incidence of FLT3 mutations along with FLT3 mRNA and CD135 protein expression in AML patients of western India and their role in prognosis of disease. Analysis for the detection of FLT3 internal tandem duplication (ITD), Tyrosine kinase domain (TKD) point mutations and quantification of mRNA level was carried out in total 174 de novo patients diagnosed with acute myeloid leukemia (AML), myelo-dysplastic syndrome (MDS) and aplastic anemia using PCR and RT-PCR methods. FLT3 protein was quantified by flow cytometry on leukemic blasts. The incidence of FLT3 ITD, FLT3 TKD mutations and CD135 protein expression was found to be 19%, 7% and 62% respectively in AML patients. In MDS, only FLT3 ITD mutation and CD135 protein over expression could be analyzed, incidence of which was 22% and 60%. In aplastic anemia, FLT3 mutations, FLT3 mRNA and protein over expression were not detected. FLT3 mutations as well as FLT3 mRNA and protein overexpression were prominently noted in AML subtypes associated with myelo-monocytic lineage. CD135 protein over expression was significantly associated with reduced disease free survival (DFS) whereas WBC and blasts emerged as poor prognostic factors with respect to DFS and overall survival (OS) respectively in multivariate analysis. Our data suggest that CD135 receptor protein overexpression is a potential prognostic marker, as well as, molecular target for FLT3 inhibitors in AML patients.

Journal of Blood and Lymph 2012, 2:1 http://dx.doi.org/10.4172/2165-7831.1000102 (online publication)

13. Transforming Growth Factor Beta - 2: A Predictive Marker for Breast Cancer

Dave H, Trivedi S, Shah M, Shukla S
Receptor and Growth Factor Laboratory

Summary

Dual role of TGF-ß signaling in breast tumorigenesis as an inhibitor in early stages and promoter in advanced stages has been well established and known as TGF-ß switch. However, the biological mechanisms needs to be explored. Aim of the present study was to look for the usefulness of TGF- ß 2 as a predictive marker for breast cancer and to offer a

better predictability to identify patients likely to benefit from antiTGF- ß strategies. Circulatory as well as transcript levels of TGF- ß 2 were estimated from 118 pretherapeutic breast cancer patients using ELISA and q-PCR with ddCt method. Multifactorial analysis was performed to correlate the results to clinico-pathological prognosticators and Kaplan-Meier survival analysis with a median follow-up of 49 months was also evaluated. Circulating TGF- ß 2 was similar in control and breast cancer patients. TGF- ß 2 was significantly upregulated in advanced tumors compared to early tumors. An inverse correlation was observed between TGF-b2 protein and mRNA; nevertheless both exhibited significant correlations with clinico-pathological prognosticators. Higher expression of TGF- ß 2 mRNA was connected to an early relapse in advanced stage than early stage patients. It is the first report to evaluate circulatory and transcript levels exhibiting TGF-b switch and confirming the utility of TGF- ß 2 as an important predictive marker for breast cancer.

Indian Journal of Experimental Biology: 2011; 49: 879-887

14. Prognostic Utility of Circulating Transforming Growth Factor Beta 1 in Breast Cancer Patients

Dave H, Shah M, Trivedi S, Shukla S
Receptor and Growth Factor Laboratory

Summary

Transforming growth factor betas (TGF-bs) are multifunctional cytokines with a biphasic role in breast tumorigenesis, acting as tumor suppressors at early stages while stimulating tumor progression at later stages (TGF-b switch). Among the 3 human isoforms, TGF-b1 is known to be overexpressed in several tumor types including breast tumors. TGF-b signaling and "crosstalk" in the tumor microenvironment presents a unique challenge and an opportunity to develop novel therapies. We assessed circulating TGF-b1 levels by ELISA in blood samples from 117 previously untreated breast cancer patients in this prospective study to explore the TGF-b switch at the forefront. The levels were correlated with clinicopathological prognosticators like age, menopausal status, nodal status, histological type, histological grade, necrosis, stromal involvement, and survival. Higher mean preoperative serum TGF-b1 was observed in early-stage patients than controls (p=0.05) as revealed by receiver operating characteristic (ROC) analysis. Elevation of TGF-b1 was evident in patients with advanced-stage breast cancer compared with those having early-stage disease (p=0.0001). Prognosticators of an aggressive phenotype were associated with higher TGF-b1 levels, and higher levels thus announced the likelihood of relapse, marking the role of TGF-b1 as a tumor promoter and evidencing the existence of a TGF-β switch. Moreover, higher levels of TGF-b1 shortened the overall survival in breast cancer patients (p=0.010). The results indicate that circulating TGF-b1 may be used as a predictive and prognostic marker in breast carcinoma.

Int J Biol Markers 2011; Oct 20:0. doi: 10.5301/JBM.2011.8736. [Epub ahead of print]

15. A Review on Salivary Genomics and Proteomics Biomarkers in Oral Cancer

Shah F, Begum R, Vajaria B, Patel K, Patel J, Shukla S, Patel P
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Summary

Oral cancer has emerged as an alarming public health problem with increasing incidence and mortality rates all over the world. Therefore, the implementation of newer screening and early detection approaches are of utmost importance which could reduce the morbidity and mortality associated with this disease. Sensitive and specific biomarkers for oral cancer are likely to be most effective for screening, diagnosis, staging and follow-up for this dreaded malignancy. Unlike other deep cancers, oral cancer is located in oral cavity. Hence, the direct contact between saliva and oral cancer lesion makes the measurement of tumor markers in saliva an attractive alternative to serum and tissue testing. The DNA, RNA and protein molecules derived from the living cancer cells can be conveniently obtained from saliva. Thus, salivary biomarkers, a non-invasive alternative to serum and tissue based biomarkers may be an effective modality for early diagnosis, prognostication and monitoring post therapy status. In the current post-genomic era, various technologies provide opportunities for high-throughput approaches to genomics and proteomics; which have been used to evaluate altered expressions of gene and protein targets in saliva of oral cancer patients. The emerging field of salivary biomarkers has great potentials to prove its clinical significance to combat oral cancer. Hence, we have reviewed importance of several salivary genomics and proteomics biomarkers for oral cancer.

Indian Journal of Clinical Biochemistry: 2011; 26 (4): 326 - 334

Summaries of Presentations at Conferences by International Bodies

01. Concurrent Chemoradiaton (CCRT) vs Radiotherapy (RT) Alone in Locally Advanced Carcinoma Cervix: Comparision of Results and Side Effects

Patel B, Dave P, Dave K, Desai A, Mankad M, Chauhan A
Department of Gynecological Oncology

Summary

In India, > 70% of cervical cancer patients are diagnosed in advanced stage. Role of radiotherapy to cure locally advanced cervical cancer is limited by the size & stage of the tumor. CCRT may interact to increase the killing of tumors cells without delaying the course of RT and improves survival. This randomized prospective study is done with aims to evaluate the safety & improvement in the survival of the patients with locally advanced cervical carcinoma. One hundred and thirteen patients with squamous cell carcinoma of the cervix were included from April 2004 to March 2007: 59 in arm A (RT) and 54 in arm B (CCRT with cisplatin 40 mg/m^2 weekly) after examination and investigations at Gujarat Cancer & Research Institute, Ahmedabad. In arm A 49(83.05%) and in arm B 46(85.19%) patients completed the scheduled treatment and evaluated. Twelve (24.49%) & 20(43.48%) patients were stage II arm A and B respectively and rest were stage III. Acute toxicities were transient. Complete response was 87.76% in arm A and 93.48% in arm B. Twenty eight patients (57.14%) in arm A and 33(71.74%) in arm B had > 6 month of follow up (median - 31.25 months for arm A, 37.64 for arm B). Three year disease free survival was 55% for arm A and 82% for arm B. Recurrence was noted in 9(18.37%) patients in arm A and 5(10.87%) in arm B. We concluded that CCRT using weekly cisplatin is well tolerated and superior regimen than RT alone for locally advanced cervical cancer.

International Gynecology Cancer Society (IGCS) Regional Meeting on Gynecologic Cancers, New Delhi, India, April 2-3, 2011 (Oral)

02. Germ Cell Tumour of Ovary in Young Girls - Current Management Strategies

Chawla H, Mankad M, Dave P, Chauhan A, Patel B
Department of Gynecological Oncology

Summary

Germ cell tumors account for 2-3% of all ovarian cancers and usually occur in first two decades. They are also seen in women up to 35 years of age, making fertility sparing treatment of paramount importance. This study was carried out with the objective to review the outcome of treatment of malignant ovarian germ cell tumors in young patients. During last 3 years Unit III of gynec oncology at GCRI, Ahmedabad, and witnessed 119 cases of ovarian mass of which 5 cases were of germ cell tumors (age range 6-18years). Cases: All 5 girls presented with mass and abdominal pain. All were histopathologically reviewed and staged according to FIGO classification (1988). One girl had mass above umbilicus with paraaortic lymph node metastases on CT scan. She was given anterior chemotherapy (BEP - 3 cycles). Unilateral salpingooophorectomy was done in 4(80%) girls and one girl was inoperable as both ovaries and uterus formed a conglomerated mass and could not be removed. A case of dysgerminoma had dysgenetic gonads (46XX, possibility of mosaicism couldn't be ruled out). Reports of Histopathology revealed mixed germ cell tumors in 3(60%), dysgerminoma in one and teratoma in one patient. Post operatively adjuvant chemotherapy (BEP in two, P+E in one) was given in three girls. The mean numbers of cycles of BEP given were 4. The follow up was done by clinical examination, ultrasonography and tumour markers. Management of these patients requires consideration of fertility sparing surgery. These patients should be treated at a specialized centre because of frozen section facility as well as medical oncologist to plan adjuvant therapy.

International Gynecology Cancer Society (IGCS) Regional Meeting on Gynecologic Cancers, New Delhi, India, April 2-3, 2011 (Poster)

03. Vimentin and Epithelial Mesenchymal Transition in Breast Cancer

Patel N, Shukla S, Shah M, Vora H
Division of Immunohistochemistry and Flowcytometry, Cancer Biology

Summary

Epithelial-mesenchymal transition (EMT) is defined by the loss of epithelial characteristics and the acquisition of a mesenchymal phenotype. In carcinoma cells, EMT can be associated with increased aggressiveness, and invasive and metastatic potential. Snail a transcription factor and EMT inducer, act as a transcriptional repressor of epithelial markers E-cadherin and Cytokeratin and inducer of

EMT marker Vimentin. The aim was to identify molecules involved in EMT such as Snail, E-cadherin, Cytokeratin and Vimentin in primary breast carcinoma that may serve as the therapeutic targets for metastasis prevention as EMT can be completely reversed using combination of inhibitors of EMT. Snail, E-cadherin, Cytokeratin and Vimentin expression was determined by immunohistochemical localization in 100 patients with breast cancer. These markers were correlated with conventional clinicopathological parameters and disease-free and overall survival. In breast carcinoma, 58%, 69%, 56% and 22% of patients expressed Snail, Ecadherin, Cytokeratin and Vimentin. A trend of positive correlation was noted of snail with E-cadherin and Cytokeratin alongwith significant positive correlation between E-cadherin and Cytokeratin (r=+0.40, P=0.0001). These markers when correlated with clinicopathologic parameters, a trend of higher incidence of gain of Vimentin expression with loss of E-cadherin and Cytokeratin was noted in patients with age less than 45 years. Loss of E-Cadherin was also seen with advancement of histologic grade. Further, Vimentin over expression correlated with ER and PR negativity, and loss of E-cadherin correlated only with ER negativity. In Kaplan and Meier univariate survival analysis Vimentin expression significantly correlated with reduced overall survival (OS)(Log rank=4.67, df=1, P=0.03) and loss of Cytokeratin and gain of Vimentin showed a trend of reduced disease free survival (DFS). In Cox Forward Step Regression analysis Lymph node status entered at step 1 and Vimentin at step 2 for DFS and OS. Vimentin promotes breast cancer progression by induction of EMT and could be a therapeutic target to prevent EMT.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

04. Comprehensive FLT3 Analysis in Indian Acute Myeloid Leukemia

Mehta S, Shukla S, Vora H
Division of Immunohistochemistry and Flowcytometry, Cancer Biology

Summary

FLT3 is a receptor tyrosine kinase, which play important role in normal hematopoiesis. Activating mutations of FLT3 are commonly found in AML patients and reported to be associated with poor clinical outcome. We aimed to evaluate the incidence of FLT3 mutations along with FLT3 mRNA and CD135 protein expression and their role in prognosis of AML patients. Detection of FLT3 internal tandem duplication (ITD), Tyrosine kinase domain (TKD) point mutations and quantification of mRNA level was carried out in total 174 de novo patients diagnosed as acute myeloid leukemia (AML, N=144), myelodysplastic syndrome (MDS, N=9) and aplastic anemia (N=21) using PCR and RT-PCR methods. The quantitation of FLT3 protein on leukemic blasts was done by flow cytometry. The incidence of FLT3 ITD, FLT3 TKD mutations and CD135 protein expression was found to be 19%, 7% and 62% respectively in 144 de novo AML patients while mRNA was studied in 130 patients and all showed FLT3 mRNA expression. In MDS, only FLT3 ITD mutation and CD135 protein over expression could be analyzed, incidence of which was 22% and 60%. In aplastic anemia, FLT3 mutations, mRNA and protein over expression were not detected. In AML, FLT3 mutations as well as FLT3 mRNA and protein over expression were prominently noted in AML subtypes associated with myelo-monocytic lineage. With clinical and hematological parameters, only FLT3 ITD was significantly associated with blast count $\geqslant$ 61% (P=0.007) and platelet count $<1.5 \times 10^5/\mu l$ (P=0.04). When inter correlation of FLT3 parameters was evaluated, FLT3 TKD mutation (P=0.002) and wild type CD135 protein over expression (P=0.01) was found to be significantly associated with mRNA expression level $\geqslant 16 \times 10^5$. In univariate analysis, a trend of reduced overall survival (OS) with FLT3 ITD positive AML and reduced disease free survival (DFS) with FLT3 TKD mutation positive AML was noted while CD135 protein over expression was significantly associated with reduced DFS with relapse rate of 78% (P=0.04). In multivariate analysis, WBC (P=0.03) and blasts (P=0.001) emerged as poor prognostic factors with respect to DFS and OS respectively. Our data suggest that CD135 receptor protein over expression is a potential prognostic marker as well as molecular target for FLT3 inhibitors in AML patients.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

05. Clinical Significance of Fas Receptor (CD95/APO-1) mRNA in Colorectal Cancer Patients

Gajjar K, Ghosh N, Shah N, Goswami J, Shah S, Shukla S
Division of Molecular Endocrinology, Cancer Biology

Summary

FasR (CD95/APO-1), a cell-surface receptor, activates the apoptosis signaling pathway by binding

to its ligand FasL, resulting in apoptosis. Dysfunction of FasR/FasL system results in abnormal survival and proliferation of tumor cells and may promote malignancies. Further, reports suggested that FasR is widely expressed in normal colonic epithelial cells, colonic adenomas but is downregulated in majority of colorectal adenocarcinomas. However, studies correlating FasR expression with clinical outcome of colorectal cancer patients are lacking. Therefore, the present study evaluated FasR mRNA expression by RT-PCR and its association with clinicopathological parameters and survival in colorectal cancer patients (N=45). FasR mRNA was expressed in 58% (26/45) colorectal tumors and the expression was more frequent in colon cancer patients (77%) than patients with cancer of rectum (46%). When compared to established clinicopathological parameters, FasR mRNA expression was significantly inversely correlated with anatomic site of tumor (r =-0.29, P=0.049) and a trend towards significance was observed with histological grade of tumor (r=-0.28, P=0.061). For survival analysis, 31 colorectal cancer patients were followed for a period of 36 months. Univariate survival analysis revealed that loss or down regulation of FasR mRNA was significantly associated with adverse recurrence free survival (P=0.013) but not with overall survival. Similar significant findings were observed when patients were sub grouped according to anatomic site of the tumor and Dukes stage. Further, in multivariate analysis, FasR mRNA expression remained a significant independent predictor for disease free survival (P=0.004). In conclusion, our results indicated that FasR mRNA expression may be a useful independent predictor of prognosis in patients with colorectal cancer.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

06. Relevance of Interleukin-1 alpha, Interleukin-1 beta, Interleukin-8 and Interferon-alpha in Thyroid Diseases

Kobawala T, Patel K, Parekh R, Patel D, Thakor P, Parekh U, Shah N, Shukla S, Patel G
Division of Molecular Endocrinology, Cancer Biology

Summary

It is very well realized that the development of cancers from inflammation is a process driven by inflammatory cells and mediators, including cytokines. Cytokines are signalling proteins that act as important modulators of tumorigenesis. Cytokines are thought to play a crucial role in autoimmune thyroid diseases, and modulate development and growth of both normal and neoplastic thyroid cells. Therefore, the aim of this study was to explore the occurrence of interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-8 (IL-8) and interferon-alpha (IFN-α) levels in serum of 88 individuals of which 19 were healthy individuals, and 69 were patients with thyroid diseases including goitre (N=21), autoimmune diseases (N=16), and carcinomas (N=32). IL-1β, IL-8 and IFN-α were significantly higher in patients with thyroid disease as compared to healthy individuals while, IL-1α was significantly higher in patients having goitre. Cytokine levels when correlated with clinicopathological parameters of thyroid carcinoma patients showed that IL-1α was significantly inversely correlated with tumor size (r=-0.526, P=0.002) lymphatic permeation (r=-0.385, P=0.029) and differentiation status (r=-0.403, P=0.022). On the other hand, IL-1β significantly correlated with the nodal (r=0.379, P=0.032) and tumor differentiation status (r = 0.629, P< 0.001). IL-8 levels showed significant positive correlation with disease stage (r = 0.431, P=0.014) only while IFN-α levels demonstrated no significant correlation with the studied clinicopathological parameters. Finally it may be concluded that, the studied cytokines have a significant role in thyroid cancer pathogenesis and may represent useful serum biomarkers in patients with thyroid diseases.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

07. Clinical Significance of Prognostic Molecular Markers in Breast Carcinoma

Desai N, Patel P, Shukla S, Shah P, Shah M, Vora H
Division of Immunohistochemistry and Flowcytometry, Cancer Biology

Summary

The purpose of the present study was to investigate clinical significance of prognostic molecular markers in breast carcinoma. For this we study first the expression of apoptosis related proteins such as Bcl2, BAG-1 and p53 in breast tumors and to compare it with established clinicopathologic prognostic factors and disease outcome. Protein expression of Bcl2, BAG-1 and p53 was noted in 57%, 68% and 61% in tumors of breast carcinoma patients (N=165), respectively. Further, a significant decrease in Bcl2 expression was noted with increase in tumor size, disease stage and tumor grade in younger patients. In univariate survival analysis, Bcl2 overexpression significantly associated with better overall survival in lymph node negative patients.

Moreover, in trivariate analysis patients with three marker positivity had reduced overall survival as compared to patients with one or two marker positivity followed by negative subgroup. Thus, Bcl2 negativity and BAG-1 and p53 positivity indicated aggressive phenotypes in breast cancer patients. We have also analysed p53 exons 5 and 7 and Bcl2 gene and protein by IHC in all tumors (N=44). The size of the fragments produced by PCR primers were 290bp for p53 exon 5 and 210bp for p53 exon7 and 390bp for Bcl2 primer. We noted 37% (11/30) of p53 exon 5 expressions, 11% (4/38) of p53 exon 7 expressions and 33% (10/30) of Bcl2 gene expressions. A significant positive correlation was noted between p53 exons 5 and 7 with LN positive subgroup (P=0.02). When all three subgroups were inter correlated, a trend of positive correlation was found between p53 exons 5 and 7 (P=0.06), while no such correlation was noted with Bcl2 gene. p53 exons 5 and 7 expression was found to be associated with hormone dependent breast cancer; also down regulation of p53 protein was noted as compared to incidence of p53 gene expression, which is associated with hormone receptors negativity.

BIT's 4th Annual World Cancer Congress 2011- Breast Cancer Conference, towards personalized breast cancer therapy, Guangzhou, China, November 16-18, 2011 (Oral)

08. Cytogenetic Profile of 2n+Ph+ALL: Doorstep of Challenges and Opportunities for Translational Research Therapy

Patel D, Patel G, Brahmbhatt M, Trivedi P, Shukla S, Patel P

Division of Cell Biology, Cancer Biology

Summary

In ALL (Acute lymphoblastic leukemia), the role of cytogenetics in patient management has largely been centered on the presence of the Philadelphia (Ph) chromosome and hyperdiploidy. The Ph chromosome is observed in 5% of pediatric and 25% of adults ALL cases which is associated with poor outcome. Hyperdiploid karyotype defined by the presence of more than 46 chromosomes is detected in 2% to 9% of adults and in 29% of pediatric patients which is associated with better prognosis and good response to conventional therapy. On the other hand, hyperdiploidy with Ph chromosome accounts for very rare cases of ALL. Such possible crucial cytogenetic events and its clinical significance are a matter of further study. We studied the impact of such events in 77 ALL patients. Cytogenetic analysis of 2n + with Philadelphia positive ALL patients and correlation of overall survival with sole Ph+ve patients and Hyperdiploidy patients. The study included 77 ALL patients; conventional cytogenetic studies were performed in all patients and FISH was performed whenever required. There were 53(68.8 %) males and 24(31.2 %) females. The Ph+ patients were treated with IM (Imatinib mesylate). In terms of chromosomal pattern, 25(32.5 %) patients were with sole t(9;22), 44(57.1 %) were with hyperdiploid and 8(10.4 %) patients showed hyperdiploid with t(9;22). The overall survival was significantly higher in hyperdiploidy group, intermediate for sole t(9;22) and shorter survival for hyperdiploidy with t(9;22). The present data signifies that the t(9;22) is likely to be the primary change and hyperdiploidy is probably a second event, which conferred a shorter survival. This is presumably by alteration in the kinetics of Ph+ neoplastic cells. Thus, a meta analysis of the karyotypic abnormalities may enable risk stratification of Ph+ ALL patients treated with IM and can be a candidate for invention of newer molecular targets.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

09. Roadmap for Developing and Validating Therapeutically Relevant Cytogenetic Classifiers in AML: A GCRI Experience

Vakani M, Brambhatt M, Trivedi P, Patel D, Shukla S, Patel P

Division of Cell Biology, Cancer Biology

Summary

Acute myelogenous leukaemia (AML) is a heterogeneous disease. Cytogenetic findings permit patient risk to be categorised as favourable, intermediate and adverse group with very different cure rates. Cytogenetic risk group strongly predicts response to induction therapy, risk of relapse, and overall survival. The advent of molecular diagnostics has heralded an explosion in new prognostic factors i.e. karyotyping, FISH and M-FISH. The aim of the present study was to evaluate the clinical significance of cytogenetic in AML patients to determine risk stratification and treatment outcome. The aim of the present study was to evaluate the clinical significance of cytogenetic in AML patients to determine the risk stratification and treatment outcome. Cytogenetic study was carried out in 167 untreated AML patients. FISH study was done using different probes and M-FISH was performed as and when required. Based on cytogenetic analysis, AML patients were divided into three cytogenetic risk groups: [1] favourable (33%), [2] Intermediate (62%), [3] Adverse (5%). In favourable risk group, secondary changes observed were mainly loss of sex chromosome. In intermediate risk group, changes were t(10;12), t(5;8), t(3;5) and

t(9;11) whereas in adverse risk group, complex karyotype was observed in 7 patients. Besides recurrent cytogenetic abnormalities, several rare and novel chromosomal abnormalities were also observed. The present study suggests that cytogenetic analysis is the most valuable prognostic factor in AML. Further, molecular cytogenetic study aids in disclosing the hidden abnormalities which is beyond the resolution power of conventional cytogenetic. Thus, characterization of AML by cytogenetic classifiers forms an important basis for selection and designing of targeted therapy for each AML risk group for reducing the risk of relapse and treatment side effects.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

10. SARA – A Critical Downstream Modulator of TGF- Signaling in Human Breast Carcinoma

Patel H, Nanavaty A, Raval A, Dave H, Trivedi S
Receptor and Growth Factor Laboratory, Cancer Biology

Summary

SARA, an important downstream mediator is known to modulate the activity of Smad dependent TGF- signaling. 'TGF- Switch'; conversion of cytokine from tumor inhibitor to promoter is well explored in breast tumorigenesis. Adaptor proteins such as SARA play a crucial role in improving Smad access to TGF- receptors, hence it is envisaged that SARA will make known the role of active TGF- signaling and help in prognostication of breast carcinomas. Gene copy number (CN) of SARA was evaluated in the current study using RQ-PCR from early stage (63) and advanced stage (43) tumors and their adjacent non-neoplastic tissues. This expression was compared with clinicopathological parameters. Moreover, the expression from lymph nodes was assessed from 67 patients. Higher SARA expression was seen in tumor tissues than non-neoplastic tissues ($P<0.01$). Individual comparison in each patient revealed that it was upregulated in 66% tumors than adjacent normal tissue ($P<0.0002$). Higher expression was seen in older age cohort. T2 tumors showed higher SARA transcript compared to T3 tumors ($P<0.01$). SARA expression was higher in early than advanced stage disease. Tumors with absence of lymphatic permeation and vascular permeation exhibited higher SARA than tumors with a positive status ($P<0.02$; $P<0.001$). Malignant lymph nodes (47) showed higher levels than in negative lymph nodes ($P<0.001$). In node negative patients (20), tumoral expression was higher than lymph nodes ($P<0.01$). The active in-vivo signaling is dependent on TGF-1 and TGF-RII; hence their expression was compared with SARA to note its utility as major downstream modulator. Remarkably, SARA was upregulated when TGF-1 and TGF-RII were downregulated (N=52). Moreover, when compared with each molecule independently, SARA was upregulated where TGF-1 was downregulated (N=53) and, downregulation of TGF-RII (N=67) was observed where SARA was upregulated. Overexpression of SARA seen in the growing tumors may announce the shift in the focus of TGF- signaling pathway. Notably, in the absence of the established important players of this signaling cascade, SARA would provide a better understanding of in-vivo signaling in breast carcinoma and may be a useful biomarker.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

11. A Preliminary Assessment of Circulating and Transcript Hepatocyte Growth Factor in Indian Population

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Summary

Hepatocyte Growth Factor (HGF) is well known for its involvement in mammary ductal morphogenesis and spacing. Tumoral microenvironment has been implicated in tumor cell behaviour; a higher expression of HGF is noted in tumor associated fibroblasts. With current focus on personalization of oncology therapies, HGF overexpression may be a potential target for tumor suppression. We evaluated the differential expression of circulating and transcriptional HGF which were compared to several clinical and pathological prognosticators in breast cancer. Methods: Synchronous tumoral and adjacent non-neoplastic tissues were collected after pathological confirmation from 104 newly diagnosed breast cancer patients. HGF copy numbers were analysed by Quantitative Real Time PCR. Serum HGF was estimated from the same set of patients using ELISA and the levels were compared with 58 healthy age matched individuals. Results: ROC curve analysis showed that higher levels of circulatory as well as transcript HGF was observed when compared with controls and adjacent normal tissues respectively ($P<0.013$; $P<0.064$). However, correlations of circulatory and transcript HGF levels showed an inverse albeit non-significant relation. Both serum and mRNA showed increased

expression with increasing age and were higher in postmenopausal patients. In the younger population (>40 years), HGF levels were higher in patients than controls (P<0.01). The same trend was seen in pre- (P<0.02) as well as post-menopausal women (P<0.01). Circulating HGF levels in stage-IV tumors were lower than stage-II and -III tumors (P<0.01). Also, stage-II and -III disease showed higher levels of HGF transcript. Poorly differentiated tumors expressed lower HGF transcript than well or moderately differentiated tumors (P<0.05; P<0.02). Conversely, serum HGF was higher in poor than moderately differentiated tumors (P<0.01). In a subset of 67 patients, HGF transcript was upregulated in malignant lymph nodes than non-malignant lymph nodes (P<0.003). Moreover, higher tumoral transcript was seen in node negative patients whereas inverse was true for node positive disease. Conclusion: Upregulation of HGF at both transcriptional and translational levels stamps its utility as an upcoming biomarker for prognostication as well as for disease monitoring and survival. This may help in segregating patients suited for anti-HGF strategies.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

12. Insulin like Growth Factor Signaling as Upcoming Biomarker in Human Breast Cancer: A Comprehensive Study

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Summary

Increased mitogenic stimulation is the key factor for uncontrolled cell proliferation and differentiation and is known to initiate heterogeneous mammary gland tumors. Assessment of comprehensive signaling is indeed necessary to predict the malignant potential of tumors as well as to predict the likelihood of success for newer therapeutic modalities. Numerous studies have explored IGF axis molecule/s at distinct levels; however collective evaluation from synchronous large cohort of Indian breast cancer patients is yet to be reported. Cumulative circulatory assessment of growth factors (IGF-1, -2) and abundant binding protein (IGFBP-3) from peripheral blood was done from 106 previously untreated breast cancer patients using ELISA and IRMA. Moreover, expression of transcripts of all these molecules alongwith their receptors (IGFR1, -R2) were also determined with qPCR. Multivariate correlations were done with the known clinicopathological prognosticators including survival analysis. Circulating IGF-1 was higher in patients than controls (P<0.02) whereas IGF-1 mRNA was downregulated in 73% tumors than adjacent non-neoplastic tissues. Remarkably, circulatory levels were positively correlated with mRNA (P=0.040). Age (P=0.001), menopausal status (p=0.05) and histologic grade (p=0.05) were inversely correlated with serum IGF-1. IGF-2 mRNA was downregulated in 65% tumors than adjacent normal tissues. Serum IGF-2 was lowest in large tumors (>5 cms) and inversely correlated with tumor size (P<0.01). Histologic grade was negatively correlated with IGF-2 (P=0.014). Serum IGFBP-3 was higher in younger patients than older patients (p<0.05). Upregulation of IGFBP-3 (73%) and IGFR1 (79%) were seen and they were higher in tumors than normal tissues (p<0.001). IGFR2 was upregulated in 84% tumors. Older patients had higher IGFR2 expression than other age groups (P<0.05, P<0.001). Intermolecular comparison revealed that all molecules were linearly correlated at both levels independently except IGFBP-3. Cumulative comparison of IGF axis molecules revealed that serum IGFBP-3 was highest than both ligands (IGF-1, IGF-2) and emerged as the chief player of the signaling cascade in circulation. However, at transcript levels, IGFR1 showed highest expression, emerged as the most active signaling molecule stamping a likelihood of success for anti-IGFR1 agents in breast cancer therapy.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Oral)

13. Implications of Vascular Endothelial Growth Factor Signaling Pathway in different Stages at Diagnosis in Human Breast Carcinoma

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Summary

Vascular Endothelial Growth factor (VEGF), the pivotal mediator of angiogenesis, alongwith it's signalling is well explored in prognostication and therapeutic decisions in breast cancer. However, many anti-VEGF signalling molecules under clinical trials have not been a great success; hence, it is necessary to evaluate in-vivo expression that directly mimic disease progression and help in selecting the potential benefactors likely to respond to anti-angiogenic therapeutic modalities. We assessed circulatory VEGFs (with ELISA) from controls (60), early- (61), advanced- stage (43) and locally advanced breast cancers (LABC) (57) patients. Moreover, gene expression of VEGF and its receptors were also

determined using qPCR from synchronous tissues. Potential prognostic value was determined with multivariate analysis to unearth their utility as predictors of survival. Circulatory VEGF was highest in LABC patients; higher than controls (P<0.001), early- (P<0.01) and advanced-stage (P<0.001) patients. In middle age group, VEGF was higher in patients than controls (P<0.01). Similarly, postmenopausal women showed higher serum VEGF (P<0.02). T2 tumors exhibited highest serum VEGF; higher than T1 (P<0.05) and T4 tumors (P<0.01). VEGF was upregulated in 83.01% tumors and was higher than non-neoplastic tissues (p<0.001). Younger cohort demonstrated lower VEGF transcript than older group (P<0.05). Serum VEGFR-1 was lower in patients than controls (P<0.001) while, VEGFR-2 was similar. Tumoral VEGFR-1 was lower than normal tissues (P<0.08) while no difference for VEGFR-2 mRNA. VEGFR-1 and VEGFR-2 were upregulated in 57.55% and 55.66% patients respectively. Lower VEGFR-1 was observed in perimenopausal (P<0.01) and postmenopausal-patients (p<0.001) than controls. Necrotic tumors showed higher circulating VEGFR-2 (P<0.05). Moreover, VEGFR-2 was higher than VEGFR-1 at circulatory (P<0.001) as well as transcript (P<0.001) level exhibiting a greater signaling by way of VEGFR2 than VEGFR-1. All three molecules were upregulated in 44.33% patients that were stamped as the most suited candidates for anti-VEGF therapies. Patients (23%) who relapsed during study exhibited upregulation of VEGF. Functional VEGF signalling was active in majority of patients, demonstrating high angiogenic potential of tumors with a greater likelihood to metastasize. Alongwith the prognostic utility this study alludes VEGF signalling as surrogate biomarkers in breast cancer and continuous assessment with disease progression would be beneficial.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

14. Incisional Recurrence of Squamous Cell Carcinoma of Cervix Following Simple Hysterectomy

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Summary

Carcinomas of uterine cervix recur most commonly loco regionally after surgery and/ or radiation therapy. The pelvis (parametrium or lymph nodes) and vagina are the most frequent sites of relapse. Distant metastases are rare and usually observed in the lungs, bone, and liver. On the other hand, the common primary sites of patients with skin metastases are the breast, large intestine, lung, and ovary. However, the incidence of incisional skin metastases from carcinoma cervix is extremely rare; incidence ranges from 0.1% to 0.2 %. The present case is unusual because incisional skin metastasis was seen in Squamous cell carcinoma cervix where patient did not receive any adjuvant treatment after simple abdominal hysterectomy and presented to us 2.5 years after surgery with skin and omental metastases. The patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy in December 2006 in private hospital for infiltrating squamous cell carcinoma. She was referred to higher center for radiotherapy but she did not follow the advice. She presented to us with complaint of distension of abdomen. Exploratory laparotomy with removal of metastatic nodule from anterior abdominal wall and infracolic omentectomy was done on September 2009. In view of extensive abdominal disease there was no role of radiotherapy. She was advised 3 cycles of Taxol and Carboplatin. She is on regular follow up. Her last follow up is on 16/01/2011 with normal USG and vault smear.

International Gynecology Cancer Society (IGCS) Regional Meeting on Gynecologic Cancers, New Delhi, India, April 2-3, 2011 (Poster)

15. Polymorphism in *CYP1A1*, *GSTM1* and *GSTT1* Genes as Predictors of Risk and Outcome in Oral Cancer: A Case-Control Study from Gujarat, West India

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Summary

The inter-individual differences in oral cancer risk may be ascribed to the polymorphic variability in the xenobiotic metabolizing enzymes involved in the metabolic activation/detoxification of tobacco related procarcinogens. Therefore, the aim of this study was to investigate CYP1A1, GSTM1, and GSTT1 gene polymorphisms as risk factors in oral cancer and their association with clinicopathologic data. The study included 122 cases of oral carcinoma and 127 controls matched by ethnicity and socioeconomic status. Genomic DNA from heparinised blood was collected. Gene polymorphism for CYP1A1*2A (MspI), GSTM1 and GSTT1genes was assessed using PCR and PCR-RFLP.

GSTM1 and GSTT1 null genotypes showed overrepresentation among cases (43.4% and 23.0%, respectively) as compared to the controls (33.1% and 13.4%, respectively). In contrast, CYP1A1*2A variant genotypes (*1A/*2A and *2A/*2A) were more prevalent in controls than cases (45.7 % and

15.0% vs.36.9% and 13.9%).An increase in risk for oral cancer in patients with GSTM1*0(OR=1.5, 95% CI 0.9-2.6) and GSTT1*0 genotypes (OR=1.9, 95% CI 0.9-3.7) was observed. CYP1A1*2A variant genotypes conferred risk after adjustment of confounding variables like age and tobacco use. CYP1A1*2A genotypes (wild/variant) increased risk of oral cancer together with GSTM1 and GSTT1 deletion genotypes. Moreover, a significant greater risk was observed in patients having concurrent deletion of GSTM1 and GSTT1 (OR= 2.57, 95% CI: 0.96-6.85; P=0.05). The interactions between variant genotypes of CYP1A1*2A, GSTM1 and GSTT1 and tobacco use were statistically significant (OR=7.1, 95% CI: 3.05-16.31, P<0.0001; OR=3.1, 95% CI: 1.17-8.32, P=0.02; OR=4.1, 95% CI: 1.11-15.32, P=0.03).The results also revealed significant interactions between tobacco chewing and oral cancer risk in individuals harbouring variant genotypes of CYP1A1*2A (OR=6.4, 95% CI: 2.72-14.86; P<0.0001), null genotype of GSTM1 (OR=2.89, 95% CI: 1.06-7.85; P=0.03) and GSTT1(OR=5.0, 95% CI: 1.26-19.83; P=0.02). The dose response relationship indicated that the interactions between tobacco chewing and GSTT1*0 resulted in increase in the risk irrespective of the tobacco exposure levels. Furthermore, variants of CYP1A1*2A increased risk at higher life time tobacco exposure levels while GSTM1 showed no such interactions with tobacco exposure levels.

CYP1A1, GSTM1 and GSTT1 showed no association with lymph node involvement. Variant genotypes of CYP1A1*2A and GSTM1 were found to be associated with higher disease stage, however this lacked statistical power. Compared to the referent wild genotype of CYP1A1*2A and GSTM1, the mean survival of individuals with variant genotypes was lower i.e. 38.4 and 40.7 vs 40.5 and 41.7 months, respectively. GSTT1 is an imperative factor in susceptibility to tobacco-induced oral cancer. Further, the gene-gene and gene-environment interactions of CYP1A1*2A, GSTM1 and GSTT1 offer a considerable risk to oral cancer in this population. Our results support the hypothesis that specific genotypes at the CYP1A1*2A, GSTM1 and GSTT1 loci modestly increase the risk of oral cancer. Identification of CYP1A1*2A (MspI), GSTM1 and GSTT1 genotypes as biomarkers in combination is useful as preventive and predictive measure.

4th International Symposia on Translational Cancer Research, Udaipur, India, December 16-19, 2011 (Poster)

16. p53 Polymorphisms and Risk of Oral Cancer: A Case- Control Study

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Summary

Oral cancer constitutes 3-4 % of all malignancies worldwide, however; the Indian subcontinent accounts for one third of the world burden of this malignancy. *p53* gene variants Arg/Pro at codon 72 in exon 4, 16 bp tandem repeat in intron 3 and NciI RFLP in intron 6 have been reported to modulate susceptibility to different types of human neoplasms. Therefore, we undertook this study to evaluate the role of these *p53* SNPs in oral cancer in the population from western region of India. This hospital based case-control study comprised of 79 oral cancer cases and 110 healthy controls. Genomic DNA was extracted from WBC and genotype frequencies at these three *p53* loci were determined by PCR-RFLP method. The statistical significance of the data was studied by computing logistic regression to determine the risk of oral cancer development. Our results suggested that there was a significant difference in the distribution of p53 codon 72 genotypes between cases and controls. The individuals with Arg/Pro genotype at codon 72 of p53 were protected from developing oral cancer (OR=0.46, 95% CI: 0.24 – 0.91). No difference was found in the distribution of intron 3 and Intron 6 genotypes between cases and controls. Combination analysis revealed that Del/Del and Del/Ins genotypes at intron 3 and Arg/Pro genotype at codon 72 provided protection against oral cancer development (OR=0.45, 95% CI: 0.21–0.98; OR=0.42, 95% CI: 0.17–0.99, respectively). Likewise, the combination of heterozygous genotypes at all three loci conferred protective effect against oral cancer development (OR=0.35, 95% CI: 0.14 – 0.84). When subjects were stratified according to tobacco habits; tobacco habituates with combination of *p53* codon 72 Pro/Pro and Arg/Pro genotype showed protection, although marginal against oral cancer development (OR = 0.51, 95% CI : 0.23 – 1.13). Our study provides evidence that the *p53*Arg/Pro genotype at codon 72 in exon 4 is protected from developing oral cancer in this population. Moreover, *p53* codon 72 genotypes might be associated with tobacco associated oral cancer development.

4th International Symposia o Translational Cancer Research, Udaipur, India, December 16-19, 2011 (Poster)

17. Glycoprotein Electrophoretic Patterns have Potential to Monitor Changes Associated with Neoplastic Transformation in Oral Cancer

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Summary

Oral cancer is a major global threat to public health. The alterations in glycoproteins, the important cell surface constituents have long been associated with various malignancies. Therefore, the present investigation explored clinical significance of glycoproteomics approach in patients with oral precancerous conditions (OPC) and oral cancer. The study included 80 oral cancer patients, 50 patients with OPC and 84 controls. Native polyacrylamide gel electrophoresis (PAGE) followed by Schiff's staining was carried out to study the alterations in glycoproteins. The results showed significant elevation (P<0.0001) in 192 kDa, 170 kDa, 116 kDa and 44 kDa glycoproteins in oral cancer patients and in patients with OPC when compared with the controls. Also, the levels were significantly higher (P<0.0001) in oral cancer patients when compared to the patients with OPC. The odds ratio indicated significant higher risk among habituates and especially chewers for oral cancer. The alterations in glycoprotein electrophoretic patterns also showed positive correlation with tobacco habits. The levels of all the glycoprotein bands (192 kDa, 170 kDa, 116kDa and 44 kDa) were found to be higher in patients with habit of tobacco (WHT) than in patients with no habit of tobacco (NHT) and were also higher in WHT controls when compared to NHT controls. Moreover, a 230kDa glycoprotein consistently appeared only in individuals with tobacco habits and an increasing trend was observed from WHT controls to patients with OPC to WHT oral cancer patients. Receiver's operating characteristic (ROC) curve analysis indicated that 192 kDa, 170 kDa, 116 kDa and 44 kDa glycoproteins could significantly discriminate between controls and oral cancer patients, controls and patients with OPC, as well as patients with OPC and oral cancer patients. In conclusion, the results indicated potential utility of glycoprotein alterations in monitoring sequential changes occurring due to tobacco consumption during neoplastic transformation.

4th International Symposia on Translational Cancer Research, Udaipur, India, December 16-19, 2011 (Poster)

18. Altered Glycosylation and its Clinical Significance in Oral Cancer in Current Era of Glycomics

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Division of Biochemistry Research, Cancer Biology

Summary

Glycosylation changes; particularly aberrant sialylation and fucosylation are the characteristic features associated with various stages; initiation, promotion and progression. The present study was focused on evaluation of alterations in terminal sugars, enzymes involved in their metabolism and expressions of sialoproteins and fucoproteins in oral cancer. 100 controls, 184 oral cancer patients and 91 patients with oral precancerous conditions (OPC) were enrolled for the study. Blood samples from all the subjects as well as tissue samples from oral cancer patients were collected. Post-treatment follow-up blood samples (N=100) were also collected from oral cancer patients. Total protein (TP), Total Sialic acid, (TSA) Lipid bound Sialic acid (LSA), fucose, α-L-fucosidase were estimated by spectrophotometric methods, sialidase was estimated by spectrofluorimetric method, α -2,6 and α -2,3 sialoprotein were studied by dot blot, sialyl transferase (SiT) and fucosyl transferase (FucT) expression were studied by RT-PCR and 2-D PAGE was performed to evaluate differential protein expression. The results showed significantly elevated TSA/TP ratio, -2, 6 sialoproteins, -2,6 SiT, fucose, fucose/TP and fucosidase in oral cancer patients and patients with OPC as compared to the controls. Significantly decreased expression of α-(1,3)-fucosyltransferases (Fuc-T III, Fuc-T V and Fuc-VI) was observed in malignant tissues as compared to adjacent normal tissues. Sialyl transferase (ST3 Gal 1) expression was found to be higher in malignant tissues as compared to normal tissues. The 2-D PAGE revealed 60 protein spots showing greater than two fold increase in malignant tissues as compared to adjacent normal tissues. ROC curve revealed that TSA/TP and fucosidase activity could significantly discriminate between controls and oral cancer patients as well as controls and patients with OPC. Serum levels of TSA/TP, fucose, fucose/TP, fucoproteins as well as activity of 2,6 SiT, 2,3 SiT and fucosidases were decreased in responders and remained higher in non-responder as compared to pretreatment levels. The findings suggested significant role of glycosylation changes in identification of high risk group, early detection, staging, prognostication and treatment monitoring of oral cancer and strengthens the clinical significance of glycoproteins in oral carcinogenesis in current era of Glycomics.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Oral)

19. Association between p53 Polymorphisms and Oral Cancer Risk in West Indian Population

Patel K, Vajaria B, Begum R, Shah F, Patel J, Shukla S, Patel P

Division of Biochemistry Research, Cancer Biology

Summary

The tumor suppressor gene TP53 encodes a transcription factor at the center of a network that inhibits cell growth and stimulates apoptosis in response to cellular stresses including DNA damage. *p53* gene variants Arg72Pro in exon 4, 16 bp duplication in intron 3 and *NciI* RFLP in intron 6 have been reported to modulate susceptibility to different types of human neoplasms. Therefore, we carried out this study to evaluate the role of these *p53* SNPs in oral cancer in the population from western region of India. The study comprised of 79 oral cancer cases and 110 healthy controls. Genomic DNA was extracted from WBC and genotype frequencies at these three *p53* loci were determined by PCR-RFLP method. The statistical significance of the data was studied by computing logistic regression to determine Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between genotype and the risk of oral cancer development. Our results suggested that there was a significant difference in the distribution of *p53* codon 72 genotypes between cases and controls. The individuals with Arg/Pro genotype at codon 72 of *p53* were protected from developing oral cancer (OR=0.46, 95% CI: 0.24 – 0.91). No difference was found in the distribution of intron 3 and intron 6 genotypes between cases and controls. Combination analysis revealed that Del/Del and Del/Ins genotypes at intron 3 and Arg/Pro genotype at codon 72 provided protection against oral cancer development (OR=0.45, 95% CI: 0.21–0.98; OR=0.42, 95% CI: 0.17–0.99, respectively). Likewise, the combination of heterozygous genotypes at all three loci conferred protective effect against oral cancer development (OR=0.35, 95% CI: 0.14 – 0.84). When subjects were stratified according to tobacco habits; tobacco habituates with combination of *p53* codon 72 Pro/Pro and Arg/Pro genotype showed marginal protection against oral cancer development (OR = 0.51, 95% CI : 0.23 – 1.13). From these results we conclude that the p53 polymorphisms especially Arg72Pro in exon 4 and its combination with 16 bp duplication in intron 3 and *NciI* RFLP in intron 6 could significantly modify the risk of oral cancer development in this population.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

20. Clinical Significance of Simultaneous Evaluation of M-Protein and Sialylation Changes in Patients with Monoclonal Gammopathies

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Summary

A monoclonal protein (M-protein) has been characterised in multiple myeloma patients. Further, alterations in sialylation of glycoconjugates is among the important molecular changes in multiple myeloma patients. Therefore, we evaluated clinical significance of alterations in serum M-protein and serum total sialic acid (TSA) levels in multiple myeloma patients. Total 200 subjects comprising of 100 controls and 100 cases suspected for multiple myeloma were enrolled for the study. The monoclonal gammopathies were studied by serum protein profiling using high resolution agarose gel electrophoresis and TSA and total protein (TP) levels were estimated using spectrophotometric methods. Data were analysed by SPSS statistical software. The prevalence of M-protein overexpression (P< 0.0001) with significantly altered protein profile i.e. increased alpha globulin levels (P= 0.003), reduced albumin levels as well as reduced A/G ratio (P< 0.0001) were found in patients with monoclonal gammopathies as compared to the controls. The TSA/TP levels were also significantly elevated (P=0.002, P=0.005 for patients with monoclonal gammopathies and multiple myeloma patients respectively) as compared to the controls. ROC curve analysis indicated that gamma globulin levels, A/G ratio and TSA/TP levels could significantly discriminate between controls and patients with monoclonal gammopathies (P<0.0001) as well as between controls and multiple myeloma patients (P<0.0001). The correlation co-efficient analysis revealed significant (r=0.532, P<0.0001) positive correlation between M-protein and TSA/TP levels. The study demonstrated significant clinical usefulness of simultaneous evaluation of monoclonal immunoglobulins and sialylation changes as important biomarkers in patients with monoclonal gammopathies.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

21. Comparison of Isotopic and Non-Isotopic0020methods for Assessing Potency of Immunotoxins Consisting of IL-13 and Truncated *Pseudomonas* Exotoxin in Receptor Positive Human Cancer Cells

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Summary

Previously, we demonstrated that many human tumors overexpress receptors for IL-13 (IL-13R) compared to normal immune cells. These are targets for immunotoxins comprising of IL-13 and a

truncated Pseudomonas exotoxin (IL-13PE), which are tested in various clinical trials for the treatment of human cancers. We wish to develop non-isotopic assays for measuring their biological activity and compare the results with standard isotopic assays that utilize 3H-leucine incorporation or 3H-thymidine . The read-out of the assay is based on reduction of, resazurin, which excites at 544nm and emits at 590nm or MTS at 490nm by metabolites released by dying tumor cells. These techniques in three IL-13R overexpressing and one low IL-13R expressing human tumor cell line showed similar IC50 (the concentration of the immunotoxin that kills 50% of the tumor cells) profiles. Interestingly, the relative end-point read out of the resazurin achieved within first four hours of the assay compared to overnight incubation with 3H-thymidine. All three technologies yielded similar profiles for measuring biological activity of immunotoxin. Because IL-13PE target IL-13R on human tumors, these non-isotopic assays may represent an alternate useful approach for screening immunotoxins. Additional confirmatory studies are currently ongoing to assess these assays.

NIH Research Festival, National Institutes of Health, Bethesda, MD, USA October, 2011 (Poster)

22. Evaluation of Isotopic and Non-Isotopic Technologies for Assessing Potency of Chimeric Fusion Immunotoxins Consisting of Ll-13 or IL-4 and Truncated *Pseudomonas* Exotoxin in Receptor Positive Human Cancer Cells

Patel P, Pamela L, Puri R, Joshi B
Division of Biochemistry Research, Cancer Biology

Summary

Previously, we have demonstrated that a variety of human tumors and tumor cell lines overexpress high density receptors for Th2 derived cytokines, IL-13 and IL-4. Because IL-13Rα2 and IL-4Rα chains are primary binding components of the IL-13R and IL-4R system, respectively and they are targets for IL-13PE and IL-4PE immunotoxins, there is a need to develop novel potency assays to test the characteristics of these and other immunotoxins and agents. In the present study, we developed and tested isotopic and non-isotopic assays for measuring biological activity of these two immunotoxins using fluorogenic and chromogenic assays, which measure the release of certain metabolites or DNA from viable cells after incubation with immunotoxins. We compared the data with isotopic techniques that utilize 3H-leucine incorporation and 3H-thymidine uptake. The end-point for flurogenic assay is based on reduction of the fluorogenic chromogen, resazurin, which excites at 544nm and emits at 590nm wavelength by cytosolic dehyrogenases released by dying tumor cells. The read-out for MTS assay is based on increase in the chromogenic intensity at 490 nm wavelength. The fluorogenic (resazurin) and chromogenic (MTS) techniques in three high IL-13R overexpressing and one low IL-13R expressing human tumor cell lines showed similar profile of cytotoxicity. The IC50 (the concentration of the immunotoxin that kills 50% of the tumor cells) obtained by non-isotopic assays positively corroborated with the isotopic assay (3H-Leucine and 3H-thymidine uptake). Interestingly, the end-point read out of the resazurin reduction is achieved within the first four hours of the assay compared to overnight incubation with 3H-thymidine or 4 hours with 3H-leucine incorporation. In addition, these techniques eliminated three lengthy steps involved in isotopic techniques such as freezing and thawing cells, cell harvesting, and drying of filter-mats before counting 3H-leucine or 3H-thymidine incorporation. In addition, these assays did not generate any radio active waste. Our results indicate that non-isotopic assays represent a useful alternative approach for screening potency of these immunotoxins for translational research. These assays may also be applicable for testing cytotoxicity of other agents.

Society for Immunotherapy of Cancer, Rockville, USA, November, 2011 (Poster)

23. Inhibition of Lactate Dehydrogenase(LDH) using Phytochemicals in Patients with Acute Leukemia: Insilico and Invitro Approach

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Summary

All cancer cells rely on changes in metabolism to support their growth and survival. The consequences of this differential metabolism require a detailed understanding of glucose metabolism and its relation to energy production in cancer cells. Alterations in glycolysis pathways and/or enzymes like Hypoxia Inducible factor(HIF-1),Glucose transporter (Glut1- 4), Lactate Dehydrogenase (LDH) produced by tumor cells can be taken up by stromal cells (via the monocarboxylate transporters MCT1-4) to regenerate pyruvate that either can be extruded to refuel the cancer cell or can be used for OXPHOS. LDH has significant role in tumor growth and maintenance. Serum level of LDH has independent prognostic significance and should be determined in all patients with advanced cancer. Increase in the serum concentration of enzymes reflects the tumor burden, growth rate, and cellular proliferation. Our study focused on effect of phytochemicals in modulating LDH activity in acute leukemia. In-silico docking studies with Natural compound library against LDH showed Curcumin, Gingetin, Capsaicin,

Neohespiridine and Tetrandine as potential lead based on their binding free energy. Insilico results were further validated by in-vitro cytotoxicity assay using JURKAT-E6.1 cell line. Inhibition of LDH activity was assessed by direct kinetic spectrophotometric estimation. This was further confirmed by LDH isoenzyme activity using tube gel electrophoresis in serum of acute leukemia patients. Both the results validated the inhibitory effect of curcumin leading to apoptosis in a dose dependent manner. Differential inhibition of LDH isoform by curcumin suggestive of its preferential binding with specific isoform. Present study suggestive of curcumin as potential lead molecule and provide scaffold for further derivatization to obtain better efficacious drug like molecule which may be used as an antileukemic agent.

International Symposium on "Recent Advances in Cancer Research Therapeutics to Chemoprevention", School of Life Sciences, Central University of Gujarat, Gandhinagar, India, February 8-9, 2012 (Poster)

24. Endometrial Stromal Sarcoma: A Rare Presentation in 15 Year Old Girl

Chauhan A, Dave K, Arora R, Parekh B, Bhavsar D, Patel T

Department of Gynecologic Oncology

Summary

Endometrial Stromal Sarcomas are tumor of the uterus very difficult to diagnose because of the rarity & no obvious clinical presentation. A15 year old girl was referred with endometrial polyp report:low-grade endometrial stromal sarcoma(ESS). She had polymenorragia, diagnosed having endometrial collection on sonography. She underwent evaluation under anesthia by the referring doctor which suggested uterine mass protruding from the cervix, biopsy taken reported as low-grade ESS. At our institute, CT-scan reported uterus enlarged - 12x11x9cm. Again EUA & fractional curettage done. Her cervix was looking normal but uterus was enlarged about 14 weeks size of pregnancy. On curettage whole of the uterine cavity was diseased with lots of profuse cheesy material. The HP reported : Suggestive of endometrial stromal tumor. She underwent staging laprotomy, TAH&BSO, bilateral pelvic node dissection & infracolic omental sampling was done. Her histopathology report was as follows: Macroscopic:_Uterus size: 17x13x5 cm. Polypiodial exophytic growth involving the anterior and posterior uterine wall measuring 7x5x3 cm. Growth involves more than ¾ thickness of uterine wall. Microscopic: Endometrial stromal sarcoma, low grade involving anterior and posterior wall of the uterus. Mitosis–10-12/10hpf. Lymphovascular permeation seen. Cervix, nodes, ovary, omentum are negative. Immunohistochemistry diagnosis: Endometrial stromal sarcoma. ER – positive, PR – positive, CD10 – inconclusive, Vimentin – positive, Actin – focal positive, Desmin – negative. She received pelvic radiotherapy followed by chemotherapy (Pacilaxtel & Carboplatin)

International Gynecologic Cancer Society (IGCS) IGCS Regional Meeting on Gynecologic Cancers, New Delhi, India, April 2–3, 2011 (Poster)

Summaries of Presentations at Clinical Meetings

01. A Study of Volume Conductivity Scatter (VCS) Parameters as High Throughput Screening Technique for the Detection of Chronic Lymphoproliferative Disorder

Modi P
Department of Pathology

Summary

The Coulter VCS is a tridimensional flow cytometer designed for differential leukocyte counting on the basis of cell volume, light scatter and conductivity. This study to investigate the detection capability of VCS positional parameters of abnormal lymphocytes in malignant conditions, particularly B chronic lymphatic leukemia, compared to morphologic and immunophenotyping evaluation by using Beckman coulter LH750. Morphology, blood cell counts and immunophenotyping were performed in samples from 40 patients with chronic lymphoproliferative disorders and normal controls for multiparametric flowcytometry. Mean and standard deviation of Volume, conductivity and scatter and immunophenotyping antigen distribution pattern of lymphocytes (score 4 or 5 for B cll) were evaluated as predictors of disease. Their Specificity and Sensitivity were evaluated by ROC curve analysis. Research population data were significantly showed most relevant differences among normal controls and lymphoproliferative disorders, particularly B chronic lymphatic leukemia for volume parameters: criterion: ⩽ 81.4, Sensitivity 81.5, Specificity 90.9, PPV 95.7, NPV 66.7, P=0.0007. conductivity: criterion>101.4, Sensitivity 81.5, Specificity 100.0, PPV 100, NPV 68.7, P=0.0072. Scatter: criterion: ⩽64.4, Sensitivity 44.4, Specificity 90.9, PPV 92.3, NPV 40.0, P=0.7855 (NS). Based on VCS quantitative parameters, this high throughput screening technology could be used to enhance the detection capability of abnormal lymphocytes in malignant conditions, particularly B chronic lymphatic leukemia.

02. "Pain and Palliative Care" Services at GCRI

Joshi G
Department of Anesthesia

Summary

Palliative care is integral part of cancer care. Palliative care aims to enhance the quality of life of patients and their families who are faced with a life-threatening illness like cancer. Palliative care encompasses the whole self, caring for the physical, emotional, and spiritual needs of patients and their families. It provides relief from pain and other symptoms of illness such as fatigue, nausea, shortness of breath, and loss of appetite. The goal is to prevent and relieve these symptoms so you can get on with daily life. To achieve these goals of comprehensive care of cancer patients, a formal "Pain and Palliative Care" services has been started at Gujarat Cancer & Research Institute. Following celebration of "Hospice Day" on 9th October 2010, a separate "Pain and Palliative Care" OPD is functional in Room No 11, in afternoon hours. The presentation is an evaluation of patients attended from October 2010 to January 2011 under this service. Total 263 patients were attended, out of which 45% patients had head & neck cancer, 15% patients had gynec cancer and 6% patients had breast cancer. They were given oral analgesics as per WHO analgesic guidelines for cancer pain. Hundred and five patients had severe pain & required Morphine/Fentanyl patch for pain management. Sixteen point thirty four percent patients required interventional pain management procedures like deep cervical block, coeliac plexus block, superior hypogastric plexus block etc. Other procedures like tracheostomy, percutaneous endoscopic gastrostomy and gastrostomy were done in 32 patients. Psychological evaluation of all patients was done by psychologist. Their social, financial and ethical issues were addressed. Patients and caretakers were given counseling regarding disease status, nutrition, medication and overall care of the patient. Patients' records are maintained in online Icanr software for report and research purpose. Jivdaya foundation, Dallas, USA has supported this project by appointing a dedicated team of a medical officer, psychologist cum data manager and a staff nurse. Dr Geeta Joshi, professor in anesthesiology is co-ordinator of this project. This service has established network with Community Oncology Centre, Vasna and home hospices services as well.

03. Impact of Obesity on Surgery in Gynecologic Oncology: Our Experience

Chawla H
Department of Gynecological Oncology

Summary

Surgery represents a mainstay in the treatment of gynecological cancers. It is believed to be more difficult and increases the incidence of intraoperative and postoperative complications in obese patients. To estimate the morbidity, adequacy of surgery and complication rate in obese women undergoing gynecological oncology surgery. Patients operated in gynecological oncology unit III, Gujarat Cancer And Research Institute, Ahmedabad from January 2009-June 2011 with BMI >27.5kg/m^2 were

included in the study. During the study period, 20 obese patients were operated amongst which 11 were of endometrial cancer, 5 of ovarian cancer, 2 of cervical and vulval cancer each. Mean BMI was 32kg/m^2. Mean operative time was 3 hours and mean blood loss was 294 ml. Lymphadenectomy was done in 66% of patients. Optimal surgery was possible in all the patients. Mean hospital stay was of 25 days. There was no major intraoperative complication in any case. Wound dehiscence occurred in 7 patients. Obesity per se, without severe comorbid diseases, is not a contraindication to surgical treatment. Such patients should not be considered for suboptimal treatment strategies**.** Special precautions should be taken during preoperative evaluation. A multidisciplinary approach involving experienced surgeon, anaesthetist, physician, physiotherapist and intensivist is mandatory.

04. Study of Morphological and Cytogenetic Features of Acute Promyelocytic Leukemia

Rangwala H
Department of Pathology

Summary

To study morphological and cytogenetical features of acute promyelocytic leukemia (APML) and identify its morphological and cytogenetical variants. A retrospective study of 40 cases of APML diagnosed on morphological basis was done. Morphological study was done on bone marrow aspirate smear stained by Wright stain and cytogenetic study involved evolution by conventional karyotype and/ or Fluorescent in situ hybridisation (FISH) study. Out of 40 cases, on morpholgy 33 cases were of hypergrannular type, 7 cases were of hypogrannular type. Conventional karyotype was done in 38 cases.22 were found positive for t(15;17),1 case was positive each for t(5;17) and t(11;17), karyotype was not informative in 7 cases, normal karyotype was seen in 6 cases, and other abnormality in 1 case. FISH study showed PML-RARA fusion gene product in 35 cases, 1 case each for PLZF-RARA and NPM-RARA and was negative in 3 cases. 37 cases showed morphological and cytogenetical correlations. Among cytogenetic study, FISH study was more sensitive in diagnosing APML as compared to karyotyping. Out of various cytogenetic variants, t(15;17) resulting in PML-RARA fusion gene product which is sensitive to ATRA therapy was the major form while ATRA resistant PLZF-RARA fusion gene product is rare.

05. Intensity Modulated Radiation Therapy (IMRT): GCRI Experience

Patel P
Department of Radiation Oncology

Summary

IMRT in its purest sense is intensity modulation. It has the ability to create customized intensity patterns and is an advanced form of 3D-CRT that uses non-uniform radiation beam intensities. With the ability of IMRT to sculpt the radiation dose, the radiation oncologist has the ability to increase dose to improve tumor control and reduce normal tissue complication. Review of literature justifies the role of IMRT in various sites like head and neck carcinomas, brain tumors, breast tumors, prostate tumors and many other sites where toxicity is also an important concerning area. The basic steps of IMRT treatment are:

- Imaging for staging
- Immobilization
- Imaging for treatment planning
- Treatment Planning – Forward or Inverse
- Plan verification
- Treatment verification
- Treatment delivery

Our experience of IMRT says that IMRT is a very accurate and precise treatment which can help us in enhancing the local control rates and at the same time minimizing toxicities to the important critical structures present in the vicinity. Till date we have treated 550 patients for conformal radiotherapy out of which IMRT cases were 320 and the detailed indications were as follows:

- Conformal Radiotherapy 550
- IMRT 320
- Head and Neck Cancers 186
- Brain Tumors 40
- Ca prostate 52
- Carcinoma Breast 13
- Lymphomas 4
- Carcinoma esophagus 5

Unfortunately we don't have long term follow up of patients so we are unable to comment on the local control and survival rates but acute toxicities were very less compared to conventional therapy.

06. VILI, Visual Inspection with Lugol's Iodine in Cervical Cancer Screening

Prashant B
Department of Gynecological Oncology

Summary

Objectives: To study a feasible alternative strategy for cervical cancer screening & comparing the results of VILI by a retrospective study
Results: VILI does have the potential as an effective, feasible, scientifically correct alternative strategy to population based cervical cancer screening programmes

07. An Abstract of 3 Rare Cases

Sen S
Department of Surgical Oncology

Summary

Three rare cases were presented and discussed at Oncology Training Centre on 12/11/11. A rare case of Von Hippel Lindau Syndrome was presented followed by another rare case of Gastrointestinal Stromal Tumour of the gastroesophageal junction and finally a rare early case of anaplastic carcinoma of body of the pancreas was presented.

08. Unusual Metastatic Malignant Melanoma: Should Stage IV be Treated Aggressively?

Shah S
Department of Surgical Oncology

Summary

Malignant melanoma of penis accounts for less than 1% of all primary penile malignant lesions and less than 0.2% of all malignant melanomas in men. Most common sites are glans penis (82%) followed by prepuce, urethral meatus and the penile shaft. Peak incidence is in sixth to seventh decade. Prognosis of primary mucosal penile melanoma is not worse than that for cutaneous melanoma with comparable tumor thickness. Treatment should be similar to that for cutaneous melanoma, with wide radical excision and sentinel node biopsy in clinically lymph node-negative patients. We report an unusual case of malignant melanoma of penile urethra managed with total amputation of penis and inguinal lymphnodes being observed since they were not palpable; who presented after 3 years of disease free survival with symptomatic solitary splenic metastasis. He was managed with splenectomy and is well thereafter till date.

09. Study of PI3K/AKT/Mtor Pathway Molecules: PTEN and AKT in Breast Cancer

Shah K
Division of Immunohistochemistry and Flow-Cytometry, Cancer Biology

Summary

The PI3K/AKT signaling pathway currently attracts considerable attention as a new target for effective therapeutic strategies in breast cancer. PTEN, a tumor suppressor gene has putative tumor suppressing abilities, including inhibition of PI3K/AKT pathway and its loss in breast cancer confers resistance to therapy. AKT is a serine/thereonine kinase act as a proto-oncogene, when activated promotes cell cycle progression and inhibits apoptosis. The present study evaluated clinical relevance of PTEN and AKT protein expression in breast cancer patients and correlated with clinicopathological parameters and survival. PTEN and AKT expression was studied by Immunohistochemistry in 154 early and advanced breast cancer patients. Reduced expression of PTEN was noted in 40% of breast cancer. AKT expression was seen in 40% breast cancer. PTEN loss is associated with aggressive phenotype as it correlated with advancement of tumor size, disease stage and histological grade in node negative patients. No correlation of AKT was seen with clinicopathological parameters. In PTEN negative tumors higher hormone ER, PR negativity was observed while 45% ER, PR positivity was observed in AKT positive tumors. PTEN positive patients showed a trend of better disease free survival while no correlation of AKT was observed with disease free survival or overall survival. In multivariate survival analysis by Cox-Regression method, PTEN was found to be independent prognostic factor for disease free survival along with other clinicopathological parameters. PTEN loss identifies aggressive phenotype of breast cancer. AKT activation occurs in PI3K dependent and independent manner in breast cancer.

10. Biomarkers in Two Anatomic Sites of the Oral Cavity

Patel S
Division of Molecular Endocrinology, Cancer Biology

Summary

The purpose of the present study was to identify site-specific prognostic biomarkers in patients with oral squamous cell carcinoma (OSCC). For this purpose, Epidermal growth factor receptor (EGFR), Stat3, H-ras, c-myc, p53, cyclin D1, p16, Rb and Bcl-2 were localized immunohistochemically in buccal mucosa (n=74) and tongue carcinoma (n=61) patients. Expression of markers was compared between buccal mucosa and tongue carcinoma and assessed for their prognostic value in site-specific manner. On comparison, only cyclin D1 showed significant difference in expression with higher accumulation in tongue tumors (r=+0.177, p=0.039). Moreover, univariate survival analysis showed that in buccal mucosa patients, loss of p16 and overexpression of H-ras were significant prognosticators for relapse-free survival (RFS) and overall survival (OS), respectively. However, in Cox multivariate analysis, they lost their significance after adjusting for significant clinicopathological parameters. On the other hand, in tongue cancer patients, Cox multivariate analysis showed that for RFS, Stat3 and c-myc, and for OS, Stat3, Bcl-2 and p53 were significant prognosticators after adjusting for significant confounding factors. Our findings

indicated that buccal mucosa and tongue carcinoma exhibit different biological behavior which is reflected in prognosis. Therefore, this approach might be helpful to precisely identify patients for more effectively tailored treatment strategy.

11. Management of Intracranial Metastasis

Patel D
Department of NeuroOncology

Summary

Brain metastasis is the most common brain tumor seen clinically comprising slightly more than half of brain tumors. The incidence of brain metastasis increases in patients with other malignancies. The overall management of the brain metastasis has been discussed. We have discussed our experience with 158 cases of surgically treated brain metastasis at Department of NeuroOncology.

Presentations at the Clinical Meetings (January 2011 to December 2011)

Sr.	Date	Speaker/Department	Title
1	22.01.11	Prashant B Gynecological Oncology, Unit II	Visual Inspection with Lugol's Iodine: An Alternative Strategy for Cervical Cancer Screening
2	12.03.11	Rachh S Nuclear Medicine	PET-CT in Management of Lung Cancer
3	26.03.11	Shah S Surgical Oncology, Unit I	Unusual Metastatic Malignant Melanoma. Should Stage IV be Treated Aggressively?
4	14.05.11	Joshi G Anaesthesia	Pain and Palliative Care Services – A Lamp is Lighted
5	28.05.11	Shah M Musculo-Skeletal Oncology	Showcase – 3 Cases of Difficult Limb Salvage Surgeries done at GCRI with Long Term Follow up
6	11.06.11	Patel P Radiation Oncology	Intensity Modulated Radiation Therapy: GCRI Experience
7	25.06.11	Deshpande G Surgical Oncology, Unit VI	Surgical Management of Metastasis to Lung & Our Experience at GCRI
8	09.07.11	Batra T Surgical Oncology, Unit III	Cancer of Esophagus – Minimal Invasive Esophagectomy Versus open Esophagectomy – An Honest Experience at GCRI
9	23.07.11	Chawla H Gynecological Oncology, Unit III	The Impact of Obesity on Surgery in Gynecological Oncology: Our Experience
10	06.08.11	Rajvik K Immunohistochemistry & Flow Cytometry, Cancer Biology	Study of P13K/AKT/Mtor Pathway Molecules: PTEN and AKT in Breast Cancer
11	27.08.11	Sethi S Radiodiagnosis	Ultrasonography of Thyroid Nodules – Benign versus Malignant Disease
12	10.09.11	Patel S Molecular Endocrinology, Cancer Biology	Biomarkers in Two Anatomic Sites of the Oral Cavity
13	24.09.11	Patel D Neuro Oncology	Management of Intracranial Metastasis
14	08.10.11	Maka V Medical Oncology, Unit III	Study of Central Venous Catheter – GCRI Experience
15	08.10.11	Modi P Pathology	Study of VCS Parameters as High Throughput Screening Technique for Detection of Chronic Lymphoproliferative Disorders
16	12.11.11	Sen S Surgical Oncology, Unit IV	Three Rare Case Presentation from Surgical Oncology Unit IV
17	10.12.11	Hussain A Pathology	Study of Morphological and Cytogenetic Features of Acute Promyelocytic Leukemia

Journal club / Guest lecture / Review lecture Presentations (January 2011 to December 2011)

Sr.	Date	Presenter/Department	Topic	Author	Citation
1.	12.03.11	Gauba Y Surgical Oncology, Unit V	Gastrointestinal Stromal Tumors: Current Management	Pisters PWT and Patel SR	Journal of Surgical Oncology 2010; 102: 530-538
2.	09.04.11	Kothari P Plastic Surgery	Breast Reconstruction With SGAP And IGAP Flaps	LoTempio MM and Allen RJ	Plast Reconstr Surg 2010; 126(2): 393-401
3.	09.04.11	Goswami P Microbiology	Patient Safety: Challenges in Health Care Associated Infections	Overview	
4.	14.05.11	Avinash CB Medical Oncology, Unit I	Response Assessment by Combined PET-CT Scan Versus CT Scan alone using RECIST in Patients with Locally Advanced Head and Neck Cancer Treated with Chemoradiation	Passero VA, Branstetter BF, Shuai y, Heron DE et al	Annals of Oncology 2010; 21(11): 2278-2283
5.	11.06.11	Sinha V Gynecological Oncology, Unit I	Safety and Immunogenicity of a Vaccine Targeting Human Papillomavirus Types 6, 11, 16 & 18: A Randomized, Placebo-Controlled Trial in 176 Korean Subjects	Kang S, Kim KH, Kim YT, et al	Int J Gynecol Cancer 2008; 18: 1013–1019
6.	09.07.11	Patankar P Physiotherapy	Treat Your Own Back	Overview	
7.	06.08.11	Jain D Surgical Oncology, Unit II	Delayed Colo-Anal Anastomosis is an Alternative to Prophylactic Diverting Stoma after Total Mesorectal Excision for Middle and Low Rectal Carcinomas	Jarry J, Faucheron JL, Moreno W et al.	Eur J Surg Oncol 2011; 37: 127-133 Epub 2010 Dec 24.
8.	10.09.11	Batra T Uro Oncology	Renal Cell Carcinoma	Overview	
9.	08.10.11	Patel K	Polymorphisms in	Anantharama	Oral Oncology

Sr.	Date	Presenter/Department	Topic	Author	Citation
		Biochemistry Research Division	Tobacco Metabolism and DNA Repair Genes Modulate Oral Precancer and Cancer Risk	n D, Samant TA, Sen S, Mahimkar MB	2011; 47: 866–872
10.	22.10.11	Dave P Gynecological Oncology	SOP – Where do we Stand at the End of Four Months? And our Experience with Clinical Trial Runs	Review	
11.	12.11.11	Raval A Receptor And Growth Factor Laboratory	Insulin-Like Growth Factor – Dependent Proliferation and Survival of Triple Negative Breast Cancer Cells: Implications for Therapy	Davison Z, Blacquière GE, Westley BR et al	Neoplasia 2011; 13: 504–515
12.	12.11.11	Mayank M Nuclear Medicine	Interesting Cases of PET-CT		
13.	10.12.11	Patel D Cell Biology Division	Variant Philadelphia Translocation: Molecular-cytogenetic Characterization and Prognostic Influence on Frontline Imatinib Therapy	Marzocchi G, Castagnetti F, Luatti S et al	Blood 2011; 117: 6793-6800

Case Presentations for Morbidity, Mortality at Clinical Meetings (January 2011 - December 2011)

Sr No.	Date	Presenter/Department	Case Discussion
1	22.01.11	Patel D Anesthesiology	Critical Review of Mortality Morbidity Data of Previous Year - 2010
2	26.02.11	Avinash CB Medical Oncology, Unit I	A Case Discussion of Acute Proliferative Myeloid Leukemia (Mortality)
3	26.03.11	Gauba Y Surgical Oncology, Unit V	A Case of Ca Esophagus (Mortality)
4	23.04.11	Tank T Anesthesiology	Mortality and Morbidity Data Presentation for Surgery and Medical Oncology
5	28.05.11	Gupta P Surgical Oncology, Unit III	A Case of Ca Lung with Post-Operative B P Fistula (Morbidity)
6	25.06.11	Lingutia A Medical Oncology, Unit II	A Case of Neutropenic Enterocolitis in AML (Morbidity)
7	23.07.11	Tank T Anesthesiology	Mortality and Morbidity Data Presentation for Surgical and Medical Oncology
8	27.08.11	Gauba Y Surgical Oncology, Unit V	A Case of Retroperitoneal Sarcoma (Morbidity)
9	24.09.11	Parida P Medical Oncology, Unit III	A Case of ALL Pancreatic and Renal Infiltration (Mortality)
10	22.10.11	Sen S Surgical Oncology, Unit IV	A Case of Periampullary Carcinoma (Mortality)
11	26.11.11	Parikh A Paediatric Surgery	A Case of Ruptured Hepatoblastoma – Emergency Surgery (Morbidity)
12	24.12.11	Parekh C Gynecological Oncology, Unit II	A Case of Growing Teratoma (Mortality)

About the Journal and Instructions for Authors

Gujarat Cancer Society Research Journal is a biannually (April and October) peer-reviewed journal published by the Gujarat Cancer Society (formerly published as GCS Research Bulletin). The journal's full text is available online at http://www.cancerindia.org

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A manuscript will be reviewed for possible publication with the understanding that it is being submitted to Gujarat Cancer Society Research Journal at that point in time and has not been published anywhere, simultaneously submitted, or already accepted for publication elsewhere. The journal expects that authors would authorize one of them to correspond with the journal for all matters related to the manuscript. On submission, editors review all submitted manuscripts initially for suitability for formal review. Manuscripts with insufficient originality, serious scientific or technical flaws, or lack of a significant message are rejected before proceeding for formal peer-review. Manuscripts that are unlikely to be of interest to the Gujarat Cancer Society Research Journal readers are also liable to be rejected at this stage itself.

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3. Submit one copy printed on A4 size papers.
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- Title Page
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- Text (Introduction including Aims and Objectives, Materials and Methods, Results and Analysis, Discussion with Conclusions)
- Tables (separate page, Number Arabic numerals (e.g. 1,2,3) as it comes in results)
- Figures and Illustration (separate page, JPEG print version, Number Arabic numerals (e.g. 1,2,3) as in results, If photographs of persons are used, the subjects or patients should not be identifiable).
- Legends to Figures and Illustration: Present the legends for illustrations on separate page using double-spacing, with Arabic numerals corresponding to the Illustrations.
- References (separate page, number references consecutively in the order in which they are first mentioned in the text. Identify references in the text in numerals in superscript). Only recent (not more than fifteen years old unless historical) references should be used.
- Acknowledgement

Units and Abbreviations

Avoid abbreviations in the title and abstract. All unusual abbreviations should be fully explained at their first occurrence in the text. All measurements should be expressed in SI units. ***Drug names*** Generic drug names should be used.

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The title page should have the following:

- Type of manuscript (article/case report)
- The title of the article, which should be concise, but informative; (Title case, not ALL CAPITALS, not underlined)
- The name by which each contributor is known (Last name, First name and initials of middle name), with institutional affiliation
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- The name, address, phone numbers and e-mail address of the contributor responsible
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Language and Grammar

- Uniformly American English
- Abbreviations spelt out in full for the first time
- Numerals from 1 to 10 spelt out
- Numerals at the beginning of the sentence spelt out

Summary: Summary no more than 250 words for original article and 150 words for Case Report. Should have following headings: Introduction (state the purposes of the study or investigation), Materials and Methods (selection of study subjects/patients, observational and analytical methods), Results (give specific data an their statistical significance, where ever possible), and Conclusion (succinct emphasis of new and important aspects of the study or observations). Do not use symbols in the Summary; rather, spell out what they stand for in full.

Text: This should consist of Introduction (including Aims and Objectives), Materials and Methods, Results, Discussion with Conclusions. Cite every Reference, Figures and Tables mentioned in the text in Arabic numerals (e.g. 1,2,3).

Introduction including Aims and Objective: State the purpose of the article. Summarize the rationale for the study or observation. Give only strictly pertinent information and references, and do not review the subject extensively. Do not include data or conclusions from the work being reported.

Materials and Methods: Describe precisely your selection of the observational or experimental subjects (patients, including controls). Identify the methods, apparatus (including manufacturer's name and address in parenthesis), and procedures in sufficient detail to allow others to reproduce the method. Give references to established methods, including statistical methods; provide references and brief descriptions for methods that have been published but are not well-known. For new or substantially-modified methods, describe and give reasons for using them and evaluate their limitations.

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Illustrations (Figures) and Legends for Illustrations: All Illustrations must be submitted in JPEG print version that is ready for reproduction. Figures should be numbered consecutively in Arabic numerals (e.g. Fig. 1, 2, 3) according to the order in which they have been first cited in the text. If photographs of persons are used, the subjects or patients must not be identifiable. Present the legends for illustrations using double-spacing, with Arabic numerals corresponding to the Illustrations.

Acknowledgement: State contributions that need to be acknowledged.

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Standard Journal:
You CH, Lee KY, Chey RY et al: Electrogastrographic study of patients with unexplained nausea, bloating, and vomiting. Gastroenterology 1980; 79:311-314

Online journal article:
Miyamoto O, Auer RN: Hypoxia, hyperoxia, ischemia and brain necrosis. Neurology [serial online] 2000; 54:362-71. Available at: www.neurology.org. Accessed February 23, 2000.

Chapter in a book:
Weinstein L, Swartz MN: Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: Saunders, 1974: 457-472

Online book or website:
Garrow A, Weinhouse GL: Anoxic brain injury: assessment and prognosis. In: UpToDate Cardiovascular Medicine [online]. Available at: www.UpToDateInc.com /card.Accessed February 22, 2000.

In press:
Lillywhite HB, Donald JA: Pulmonary blood flow regulation in an aquatic snake. Science. In press.

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GCS Medical College and Hospital

Gujarat Cancer Society

The Gujarat Cancer Society (GCS) was formed on April 21, 1961 under the patronage of the then H.E. Governor of Gujarat, Shri Mehdi Navaz Jung, who laid the foundation stone on January 26, 1962. It all started with an initial donation of Rs.5000/- by the Inner Wheel Club (Ladies' Wing of the Rotary Club). This club collected money through a fund raising fashion fhow. The founders of the society visualized a need for developing modern facilities for needy patients suffering from cancer of the newly formed State of Gujarat. The M.P. Shah Trust of London donated pound 55000 and the dream of creating a cancer hospital was realized. With the constant support of the Government of Gujarat, help from the Government of India and wholehearted financial support from philanthropists of Gujarat, the M.P. Shah Cancer Hospital was commissioned in the year 1965. On December 14, 1966, the then Prime Minister of India, Smt. Indira Gandhi dedicated the M.P. Shah Cancer Hospital to people. The Government of India granted 175% tax exemption on all donations to the Gujarat Cancer Society. This tax exemption has helped rapid development of the M.P. Shah Cancer Hospital. For sustaining growth and development of the Cancer Hospital, the Gujarat Cancer and Research Institute (GCRI) was formed as an autonomous body in the year 1972 as per an agreement between the Government of Gujarat and the Gujarat Cancer Society, which came into force from February 1, 1972. The Gujarat Cancer Society became an active member along with the state authorities in spearheading cancer care activity in the State of Gujarat. Community Oncology Centre started as Late Harigangadas Dhwarkadas Cancer Detection Centre & Hospice Complex on June 21, 1986 and it is developed to the present date Community Oncology Centre. We are indebted to the Late Jitendra Mehta, Founder Secretary of the Gujarat Cancer Society and to Dr. T.B. Patel, the founder director of GCRI for their selfless efforts in establishing a strong foundation for our organization.

Gujarat Cancer and Research Institute

Gujarat Cancer and Research Institute is now a 650 bedded Regional Cancer Centre and a front line major oncology facility active in the field of diagnosis, therapeutics of cancer, prevention, community research, basic research and oncology training etc.

Community Oncology Centre

Community Oncology Centre has developed various services not only the Hospice Centre but also cancer detection actively in the form of health check up facility and permanent education exhibition on cancer and de-addiction of tobacco etc.

GCS Medical College and Hospital

The process of establishing GCS Medical College was started in January 2009 and when the MOU was signed with Govt. of Gujarat. The full fledged Medical College and hospital were formally inaugurated by Hon'ble Chief Minister of Gujarat Shri Narendrabhai Modi on 27th August 2011.

GCS Medical College has already started admitting students and in the first batch, 150 medical students are already enrolled and out of the proposed 1000 beds for the hospital facility, around 450 beds are now functional and caters to poor and lower middle income people in the eastern part of Ahmedabad.

All donations are exempted from Income Tax under IT Act 35(i)(ii)(175%), 35AC(100%) and 80G(50%) and donations in Foreign Currencies accepted approval vide Reg. No.041910257, dated 22-03-2001.
Visit us at http://cancerindia.org ; E-mail: gcriad1@sancharnet.in

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GCS Medical College, Hospital and Research Center

All donations are exempted U/S 35(1)(2) [up to 175%]

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