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Address for correspondence:

The Editors,
Gujarat Cancer Society Research Journal
The Gujarat Cancer and Research Institute
GCS Journal Office, Research Wing,
Asarwa, Ahmedabad 380016
Gujarat, India
gcsjournal2012@gmail.com

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Editorial

Kothari Kiran C
MS, DNB, PGDHHM, FMAS, FCLS, FIAGES
Dy. Director & Professor, Department of Surgical Oncology
The Gujarat Cancer & Research Institute (GCRI), Ahmedabad

Laparoscopic Surgery for Cancer Patients

Laparoscopic surgery dates back to the 1900s. In Russia in 1901, a German physician performed the first laparoscopic procedure on a human.[1] Ten years later, Jacobaeus used thoracoscopy and laparoscopy to diagnose several disease states, including malignancy. In 1985, Dr. Muhe performed the first successful laparoscopic cholecystectomy (lap chole) in a human. However, this was not well publicized until years later. In 1988, first lap chole was performed in the USA and from 1991it has been accepted as a routine and standard procedure.

Benefits of Minimally Invasive Surgery

There is usually less discomfort following minimally invasive surgery because the incisions are much smaller than those used in traditional surgery. This has been shown to result in a shorter hospital stay, less need for prescription of pain medications, an earlier return to normal activities and less visible scarring. While some experts have suggested a long-term benefit of minimally invasive surgery however it is generally accepted that the primary benefits are seen in the initial recovery from surgery and that the long-term outcomes of traditional and minimally invasive surgery are similar.

Laparoscopy for Diagnosis and Staging of Cancer

Laparoscopy has been shown to decrease significantly the incidence of unnecessary laparotomy for unresectable disease in up to 67% of patients with abdominal malignancies. Many of these studies were done with early-generation computed tomography (CT) scans as part of the noninvasive work-up. Lowy et al[2] and Burke et al[3], however, have shown that diagnostic laparoscopy continues to have significant advantages in preventing unnecessary laparotomy in patients with gastric cancer, pancreatic cancer, etc. even when current-generation CT scanning is used in the diagnostic work-up.

Obtaining biopsies of organs, lymph nodes, and suspicious lesions during laparoscopy is an important part of the diagnosis and staging of malignancies. Laparoscopic guidance of liver biopsy has been shown to be a safe and effective alternative to open liver biopsy, and it significantly decreases hospital stay. A skilled laparoscopic surgeon should be able to perform biopsies of most intra-abdominal areas and organs and recognize suspicious lesions that require biopsy to rule out malignancy.

Laparoscopic Curative Resections

Minimally Invasive Esophagectomy (MIE)

The first esophagectomy performed completely via laparoscopy through a transhiatal approach was in 1995 by DePaul et al. In 1999, Watson et al first described a completely minimally invasive Ivor Lewis technique. Minimally invasive techniques for esophageal resection have been reported to have acceptably reduced procedure-related morbidity without compromising disease-free survival rates. Luketich et al have an extensive reported experience; their initial series of 222 patients has grown to more than 1000.[4] In the initial series, mortality was 1.4% versus 5.5% for an open approach. Furthermore, the survival curve at 19-month follow-up was comparable in the two groups. In their 2012 report of 1011 patients who underwent MIE via either a modified McKeown minimally invasive approach or an MIE Ivor Lewis approach, the authors cited 0.9% mortality for the MIE Ivor Lewis approach.

Van der Sluis et al assessed the long-term oncologic results of robot-assisted minimally invasive thoracolaparoscopic esophagectomy (RAMIE) with two-field lymphadenectomy in 108 patients with potentially resectable esophageal cancer. They found RAMIE to be oncologically effective and capable of providing good local control with a low percentage of local recurrence at long-term follow-up. In a prospective phase II study (coordinated by the Eastern Cooperative Oncology Group) aimed at assessing the feasibility of MIE in a multi-institutional setting, Luketich et al reported the following results:[5]

The 30-day mortality in eligible patients who underwent MIE was 2.1%. The median ICU stay was 2 days. The median hospital stay was 9 days. Adverse events classified as grade 3 or higher included anastomotic leakage (8.6%), acute respiratory distress syndrome (ARDS; 5.7%), pneumonitis (3.8%), and atrial fibrillation (2.9%). The estimated 3-year overall survival (median follow-up, 35.8 months) was 58.4%. Loco regional recurrence occurred in only 7 patients (6.7%)

Laparoscopic Gastrectomy

The laparoscopic surgery was initially applied for early gastric cancer, and adequate evidences have been accumulated in studies on the early gastric

cancer, making it one of the standard treatment options for the early gastric cancer. Along with the maturation of the laparoscopic D2 radical gastrectomy for gastric cancer, the application of laparoscopic techniques for advanced gastric cancer has increasingly been accepted. The learning curve is particularly important during the adoption of the laparoscopic surgery for gastric cancer.

Open gastrectomy with a minimal lymph node dissection of 15 for staging purposes remains an appropriate surgical treatment for gastric adenocarcinoma in the West. With increasing experience and expertise of oncologic surgeons in the minimally invasive approach to gastric resection for cancer, it is becoming evident that laparoscopy as a technique for resection, provides equivalent resections with equivalent lymphadenectomy comparable to the open approach with no compromise in recurrence or long-term survival based on preliminary studies. In addition, based on the known benefits of the minimally invasive approach including reduced surgical trauma, blood loss, pain and quicker recovery for the patient, we are encouraged to expand our indications for this approach.

Laparoscopic Pancreatic Surgery

Laparoscopic pancreatectomy has recently emerged as one of the most advanced applications of surgery, and total laparoscopic pancreaticoduodenectomy (TLPD) has proven to be among the most advanced laparoscopic procedures. Gagner and Pomp were the first to describe the laparoscopic Whipple procedure in 1994. A review of the literature shows that 146 laparoscopic Whipple procedures have been published worldwide since 1994. This review demonstrates that the laparoscopic Whipple procedure is not only feasible but also safe, with low mortality and acceptable rates of complications.[6]

Laparoscopic Resection of Colorectal Cancer

Video-laparoscopic techniques in colorectal surgery were used for the first time in 1990 by Moises Jacobs in Miami, Florida while performing a right hemicolectomy.[7] The development of a circular stapling device for colostomy closure permitted the first laparoscopic colostomy closure to be performed by in 1990 by Joseph Uddo. The development of a laparoscopic intestinal stapler meant that for the first time, the bowel could be transected intraperitoneally. Dennis Fowler successfully demonstrated this in 1990 when he performed the first laparoscopic sigmoid resection. Subsequent years witnessed more technical innovations that could now make laparoscopic surgeries on the colon and rectum feasible.

Laparoscopic colon surgery for cancer has become the gold standard and in experienced hands, can be performed safely and reliably with many short-term benefits to the patients while resulting in at least equivalent long-term outcomes as open surgery. Other potential, but less conclusively demonstrated benefits include better preservation of cell-mediated immune function and reduced tumor cell proliferation. Although a similar level of evidence did not yet exist for the laparoscopic rectal surgery for cancer, the long-term outcomes of the Colorectal Cancer Laparoscopic or Open Resection (COLOR) II trial indicate that laparoscopic surgery is as safe and effective as open surgery in patients with rectal cancers without invasion of adjacent tissues.[8]

Robotic Surgery

"Robotic surgery" or "robotic-assisted surgery" is a newer variation on minimally invasive colon and rectal surgery. The technique is very similar to standard laparoscopic surgery in that instruments are passed into the abdomen through trocars. Rather than manipulate the instruments manually, the surgeon sits at a console, or special computer desk, and manipulates small controllers while observing the inside of the abdomen with a 3-D monitor. A sophisticated computer system translates the movements of the surgeon's hands to the robot, which then moves the surgical instruments. Because the robot is only capable of working in a relatively small part of the abdomen at a time and is difficult to reposition, it is often used for only a portion of an operation. The remainder of the operation is usually performed laparoscopically. Robotic surgery is gaining popularity primarily for rectal operations because the robotic instruments are well suited to operating in the pelvis where laparoscopic surgery is more difficult. Van der Sluis et al assessed the long-term oncologic results of robot-assisted minimally invasive thoracolaparoscopic esophagectomy (RAMIE) with two-field lymphadenectomy in 108 patients with potentially resectable esophageal cancer. They found RAMIE to be oncologically effective and capable of providing good local control with a low percentage of local recurrence at long-term follow-up.

Because it is a newer technique, there is less evidence available to compare the outcomes of robotic surgery with traditional open surgery or the more established minimally invasive techniques. Potential advantages include better visibility and greater ability to perform minimally invasive procedures on the rectum. Disadvantages include the high cost of the robot, which may limit its availability, and the need for additional training.

Conclusion

After the long hibernation of laparoscopic surgery, in the last decade modern technology has

created a spate of laparoscopic techniques and procedures. This new technology coupled with rapidly increasing surgical experience has made laparoscopy a viable tool in the diagnosis and management of abdominal malignancy. For patients devastated by a diagnosis of malignancy, avoiding open surgery with concomitant benefits such as decreased pain and shorter hospitalization can be a bright spot in the battle against cancer.

Laparoscopy, however, has not undergone such rapid development without significant costs. Many surgeons, under economic and competitive pressures, have begun to perform laparoscopic procedures without adequate training or monitoring which has resulted in increase in rate of major complications and rarely death. Credentialing of laparoscopic procedures has varied and is sometimes based only on the experience gained at a 1- or 2-day course. Only when thorough training and preparation are required and strict credentialing is mandated can the risk of complications from laparoscopic surgery can be minimized.

The future of laparoscopic surgery for the management of malignancy holds exciting prospects. However, this bright future is in jeopardy if scientific evaluation, including prospective studies, of these new procedures, especially those for attempted curative resection, is not carried out to determine which procedures benefit the patient. Although surgeons, with pressure from patients, hospitals, and equipment companies, are happy to perform laparoscopic procedures offering their patients less pain and earlier return to normal activities, they must remember first to do no harm.

References:

1. Muhe E: Die erste Cholecystektomie durch das Laparoskop. English summary. Langenbecks Arch Klin Chir 1986;369:804
2. Lowy AM, Mansfield PF, Leach SD, et al: Laparoscopic staging for gastric cancer. Surgery 1996;119:611-614
3. Burke EC, Karpeh MS, Conlon KC, et al: Laparoscopy in the management of gastric adenocarcinoma. Ann Surg 1997;225:262-267
4. Falcone RE, Wanamaker SR, Barnes F, et al: Laparoscopic vs. open wedge biopsy of the liver. J Laparoendosc Surg 1993;3:325-329
5. Luketich JD, Pennathur A, Franchetti Y, et al. Minimally invasive esophagectomy: results of a prospective phase II multicenter trial--the Eastern Cooperative Oncology Group (E2202) study. Ann Surg. 2015; 261:702-707
6. Gagner M, Pomp A: Laparoscopic pylorus-preserving pancreaticoduodenectomy. Surg Endosc. 1994;8:408–410
7. Jacobs M, Verdeja JC, Goldstein HS: Minimally invasive colon resection (laparoscopic colectomy) Surg Laparosc Endosc. 1991;1:144–50
8. Bonjer H, Deijen C, Abis G, et al: A Randomized Trial of Laparoscopic versus Open Surgery for Rectal Cancer. 2 2015 NEJM

Shri R J Kinarivala Research Oration Award, Year - 2016

Bhavana J. Dave,
Ph.D., FACMG
.
Professor of Pediatrics, Pathology and Microbiology,
Munroe Meyer Institute, and Genetics, Cell Biology and Anatomy,
Associate Director of Human Genetics Laboratory
Munroe-Meyer Institute for Genetics and Rehabilitation,
University of Nebraska Medical Center, Omaha, NE, USA.

From Chromosomes to Therapy: A Passage to Translational Medicine in Non-Hodgkin Lymphoma

The genetic profile of an individual plays a crucial role in the causation of cancers and differences in the prevalence of cancers in various geographic regions may be accentuated because of the exposure to various genotoxic agents accidentally, occupationally or by life style. The various cancers that I have scrutinized utilizing genetic studies, oral cancers in India, colorectal and lung cancers in the USA, and non-Hodgkin's lymphoma (NHL) in agricultural areas in the USA, each involve either life style or occupational exposure to carcinogenic/genotoxic agents. Cytogenetics was frequently used in genotoxicity and carcinogenic testing protocols. My scientific journey in research and translational medicine has been in the field of cytogenetics and spans for nearly 30 years and three institutes: The Gujarat Cancer and Research Institute (GCRI), M. D. Anderson Cancer Center (MDACC) and University of Nebraska Medical Center (UNMC).

At GCRI, we utilized all the available techniques for our research. My pre-doctoral work focused on: **(1)Delineation of the genetic causes in the etiology of oral cancers and development of an algorithm utilizing genetic techniques for genotoxicity and genetic susceptibility investigations.** Then and even today, oral cancers are among the most common cancers in India and Southeast Asia. We examined polymorphism in constitutive heterochromatin which correlates with causation of many cancers including oral cancers. Further, a combined application of genotoxicity assays and polymorphism studies provided a deeper insight in neoplastic transformation process. During pre-doctoral years, I investigated the effects of betel nut chewing in oral cancers and oral pre-malignant conditions. The description of oral cancers was made in "*Sushruta-Sanhita*" an ancient Sanskrit text on medicine and surgery written in 600 BC, the mention of betel quid chewing was done in *Mahawamsa* (in Pali language) in 506 BC. The inclusion of tobacco, which was introduced in India around 16th century by the Portuguese, and considered as the major culprit, was a matter of choice implying that other ingredient besides tobacco may be causing the disease. This was reason enough to pursue the effects of areca-nut (betel nut) in the causation of premalignant conditions and oral cancer. Areca nut was erroneously considered safe, constitutes the major amount of betel quid, but there were very few scientific studies since the western world was chasing tobacco and had noted its association with the occurrence of lung cancers. My research findings on cell line and among humans consuming betel nut without tobacco showed that betel nut is implicated in the causation of oral premalignant and malignant conditions. My work on determining genetic aberrations in various in vitro models and among humans with oral premalignant conditions and oral cancers was pivotal and led to the inclusion of regulatory statements and statutory warning on the packets of **Pan Masala** (without tobacco) a popular and widely consumed food product in India. My research stimulated several investigations, and this genetic testing algorithm has since been utilized for many other alleged carcinogenic products.

The main focus of my post-doctoral research at MDACC, Texas, was to: **(2)Identify specific chromosomal breakpoints that determine genetic susceptibility in colorectal and lung cancers,** two of the most common cancers in the U.S.The findings provided a conceptual basis that, lymphocytic chromosomal analyses reflected similar pattern of abnormalities as the tumor tissues and indicated that lymphocytic chromosomal analysis could aid in identifying genetically susceptible individuals. The break points clustered in the regions that harbor either oncogenes or tumor suppressor genes and suggested that the genomic instability represented at the chromosomal level helps to better understand the relationship between genetic susceptibility and environmental carcinogenesis. By challenging the lymphocytes with a known clastogen in vitro, I found that chromosomal fragility is specific for particular

cancers and challenging the cells with mutagens reveals this specificity at a more pronounced rate. Clustering of breaks occurred in specific chromosomal regions and provided clues for further molecular analysis.

After joining UNMC, Nebraska,a world renowned for diagnosis and management of lymphomas, my research emphasis was on NHL.My laboratory participated in multicenter epidemiologic studies that led to the: **(3)Delineation of the epidemiologic factors influencing genetically defined lymphomas** and determined that the risk of NHL with a specific genetic translocation was significantly elevated among farmers who used herbicides and insecticides compared to those who never used any pesticides. Our findings defined the role of various pesticides and elucidated the increased incidence of lymphomas in specific regions of the U.S, including the state of Nebraska, where agriculture is a predominant occupation.

My laboratory has played a prominent role in: **(4)Multicenter investigations for genetic profiling of non-Hodgkin lymphomas:** we participated invarious investigations leading to new categorization of lymphomas based on genetic changes. Based on gene expression profiling the Diffuse large B-cell lymphomas (DLBCL) were categorized into two major categories, the GCB-like and the ABC-like; distinctly different overall survival is observed in these two categories of DLBCL. Molecular profiling can predict survival in DLBCL; and DNA microarrays can formulate a molecular predictor of survival after chemotherapy for DLBCL. We also determined that a distinctly different set of genes are expressed Burkitt's lymphoma as compared to all other NHL. Even among cases containing *CMYC* translocation which could be observed in both DLBCL and Burkitt's lymphoma, the distinction can be made based on gene expression profile. The studies have redefined the prognostic implications of various genetically defined subtypes and their therapeutic stratification. Numerous lymphoma patients have benefited from these studies.

At UNMC, I also continued investigations on: **(5) Defining chromosomal breakpoints in NHL that may be responsible for lymphomagenesis and progression.** My laboratory cytogenetically characterized the largest series of untreated NHL and isolated a then unidentified specific chromosomal abnormality that plays a role in lymphoma progression. The chromosome band 1p36 was frequently disrupted in NHL specifically, the follicular and diffuse large B-cell lymphomas. This seminal definition of the breakpoint and the delineation of molecular consequences of the disruption of that genetic region deleting a putative tumor suppressor gene lead to numerous investigations. The correlation of the chromosomal region and loss of putative tumor suppressor gene with transformation and disease progression was subsequently defined by us and other investigators.

We have continued to work in this area of translational medicine where chromosomal rearrangements play a crucial role in pathogenesis, progression, and prognosis. Rearrangements of the chromosome locus 1p36 with ensuing deletion or disruption of TP73, one of the most distally located putative tumor suppressor genes, is frequent in NHL and confers inferior prognosis. TP73 shares homology to TP53 and is capable of transactivating p53 target genes. We investigated the relationship between rearrangements of chromosome locus 1p36 and p73 expression and examined whether p73 is involved in the regulation of proliferation and survival in common subtypes of NHL. We utilized molecular techniques in conjunction with fluorescence *in situ* hybridization (FISH) in patient specimens and cell lines with and without 1p36 abnormalities. Rearrangements of 1p36 consequently deregulated p73 isoform expression resulting in proliferation and apoptosis in follicular (FL) and DLBCL. *In vitro* studies in DLBCL cells and modulating the expression of the two opposing p73 isoforms (TAp73 and ΔNp73) using expression vectors and siRNA showed altered growth and responses to stress and chemotherapy in DLBCL cells.

Despite the improvement in NHL outcome, recurrence and residual disease are ongoing problems. Based on our observation of the regulation of apoptosis and therapeutic response of NHL cells by p73, we investigated the effect of an NSAID and a COX inhibitor in DLBCL and mantle cell lymphoma (MCL), two clinically aggressive NHL subtypes. *In vitro* treatment of DLBCL or MCL cells demonstrated a dose- and duration-dependent growth inhibition. These results were independent of p53 status. Biological studies showed significantly enhanced cell death and cell cycle arrest and molecular studies confirmed caspase cascade activation and modulation of p73 isoforms. These findings illustrated that modulation of p73 induces apoptosis and expression of pro-apoptotic and cell cycle regulatory targets. Our studies provided a molecular elucidation for the prognostic effect of 1p36 chromosomal rearrangements in NHL, established p73 as a potential therapeutic target, and highlighted the potential of a COX inhibitor as a novel adjuvant therapeutic agent in NHL management.

These investigations emphasize that delineating the functional aspect of genetic alterations and defining clinical targets facilitates therapeutic modulation, and accentuates the significance of comprehensive genetic studies in translational medicine.

A Study of Immune Dysfunction in Patients with Breast Carcinoma

Saiyed Atiya N[1], Raiya Birva N[2], Vora Hemangini H[3]
M.Sc. Student[1], Research Assistant[2], Associate Professor and Head[3]
Immunohistochemistry and Flow Cytometry Division
Corresponding Author : ihcgcri@hotmail.com

Summary

Immune system play a major role in malignant transformation and to understand the role of immune cells in breast carcinoma. We have evaluated lymphocytes, monocytes, neutrophils, ALC (absolute lymphocyte count), AMC (absolute monocyte count), ANC (absolute neutrophil count), ATC (absolute T-cell count), AHTC (absolute helper T-cell count), and ACTC (absolute cytotoxic T-cell count) expression. The flowcytometry method applied for evaluation of CD markers for respective immune cells in peripheral blood, and immunohistochemistry methodology applied for T-cell and neutrophils infiltration in tumor tissue of breast carcinoma patients. The median value of neutrophils ($p = 0.04$) and absolute count of neutrophils was found higher, while median value of lymphocytes was lower in breast carcinoma patients than healthy controls. However no difference in median value of monocytes, absolute lymphocyte counts (ALC), absolute monocyte count (AMC) was noted between these two groups. Further, median value of CD3+ T-cells and CD8+ cytotoxic T-cells was found lower and CD4+ Helper T-cells was found higher in breast carcinoma patients as compared to healthy women. In relation to clinico-pathological parameters, the median value of CD4+ helper T-cells was found significantly lower in grade III and median value of CD8+ cytotoxic T-cells was found significantly increased in BR Score 8 tumors. In tumor microenvironment, CD3+ T-cell infiltration were seen in 98% of patients. A significant higher incidence of CD3+ T-cell infiltration (2+ or 3+ score) was seen in patients with >48 years and BR Score 8 tumors. Further, patients with CD3+ T-cells infiltration in tumor microenvironment with 2+ or 3+ score had low median level of helper T-cells (CD4+) in circulation as compared to patients with 0 or 1+ score. This study observed immune suppression in breast cancer patients and antitumor activity of CD3+ T-cells in tumor microenvironment.

Keywords: AMC, ALC, ANC, ATC, AHTC, ACTC, Flowcytometry, Immunespression.

Introduction

Breast cancer is the first leading malignancy in women worldwide according to Globocon 2012 with an incidence of 27%.[1] In India and at Gujarat Cancer and Research Insitute, similar incidence was noted.

It has long been recognized that immune system plays a role in the development of cancer. Immune cells can suppress tumor development by killing tumor cells or inhibiting their growth. Conversely, they can also promote tumor progression by selecting tumor cells that are fit to grow in an immune competent host or by establishing an immunosuppressive environment. Breast carcinoma are thought to progress in immunocompetent hosts and therefore, it fails to elicit an effective immune response. The host immune response to malignant tumor comprises not only to local response to tumor microenvironment, but also systemic effects. The most frequent systemic alteration detected in solid tumor lymphocytes associated with immunosuppression. T-cell are found to be one of the key factors which coordinate the host immune system to survey and eliminate cells with malignant transformation. T-cell can affect tumor cells directly, or can act indirectly via the production of cytokines that amplify immune response. T-cells broadly classified in to CD8+ and CD4+ cells. Alteration in T-cells correlated with poor patient outcome.[2-8]

In this study, an attempt was made to understand immune dysfunction by evaluation of absolute count of lymphocytes, monocytes, neutrophils, T-cells and their subsets in peripheral blood of breast cancer patients and healthy individuals as well as CD3+ T cells and MPO+ neutrophils in tumor microenvironment of breast cancer patients.

Therefore, a better understanding of immune dysfunction in breast cancer will enable to design novel therapeutic approaches to overcome cancer induced immune dysfunction.

Materials and Methods

Patients: In this study, female with breast carcinoma patients (N=51, age range 35-75 years) diagnosed and treated at Gujarat Cancer and Research Institute, Ahmedabad were enrolled. Peripheral blood samples were collected before surgery from all the patients as well as from aged matched normal healthy women (N= 21, age range 35-75 years) in EDTA vacuette (BD Vacutainer K2 EDTA, BD NJ, USA). Tumor tissue samples of 49 breast carcinoma patients were collected at the time of surgery. The WBC count was recorded from haematology department of the institute. This study was approved by institutional scientific review board and ethics committee.

Flow Cytometry: In peripheral blood lymphocytes, monocytes, neutrophils using CD45 and T-cell subsets using CD3, CD4, and CD8 antibodies were evaluated on flow cytometer. Briefly, 10ul of antibody for respective CD antigen was added to the whole blood (100µl) and incubated for 15 minutes. The antibodies included CD45 (PerCp, clone 2D1) to identify the lymphocytes population and CD3 (APC, clone Sk7), CD4 (PEcy7, clone Sk3), CD8 (APCH7,

clone Sk1) for T-cell and its subsets, respectively. After incubation, 2 ml of erythrocyte lysing solution (1:10 dilution) was added and incubated for 15 minutes at room temperature. Then cells were centrifuge at 400g for 5 minutes and resuspended in 500µl of PBS. All the reagents were obtained from BD Biosciences, San Jose, CA. The samples were acquired in FACS Canto II flowcytometer. The adjustment of PMT voltage and compensation was carried out prior to acquisition using CST beads. The samples were acquired in BD Diva software and at least 30,000 total cells were acquired. Within the lymphocytes, T-cell subsets were identified as: CD3+ cells as T-cells, CD4+ T-cells as Helper T-cells, and CD8+ T-cells as Cytotoxic T-cells. (Figure: 1) Further, percentage of positive cells was calculated using dot plots and absolute cell count using following formulae.

Percentage calculated using dot plots:
(WBC count: Done on cell counter LH750)

$$\text{Absolute Lymphocyte count (ALC)} = \frac{\text{WBC count} \times \text{percentage of lymphocytes}/\mu l}{100}$$

$$\text{Absolute Neutrophil count (ANC)} = \frac{\text{WBC count} \times \text{percentage of neutrophils}/\mu l}{100}$$

$$\text{Absolute Monocyte count (AMC)} = \frac{\text{WBC count} \times \text{percentage of monocytes}/\mu l}{100}$$

$$\text{Absolute T-cell count (ATC)} = \frac{\text{Absolute lymphocytes count} \times \text{percentage of T-cell}/\mu l}{100}$$

$$\text{Absolute Helper T-cell count (AHTC)} = \frac{\text{Absolute T-cell count} \times \text{percentage of helper T-cell}/\mu l}{100}$$

$$\text{Absolute Cytotoxic T-cell count (ACTC)} = \frac{\text{Absolute T-cell count} \times \text{percentage of cytotoxic T-cell}/\mu l}{100}$$

Immunohistochemical localization: Immunohistochemical localization of tumor infiltrating CD3+ T-cells and MPO+ neutrophils were evaluated on formalin fixed paraffin embedded (FFPE) tissue blocks containing primary tumor evaluated by Hematoxylin and Eosin (H&E) staining, on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Vantana, USA). The commercially available antibodies used were anti-CD3 antibody (clone F7.2.38, Dako) and anti-MPO antibody (polyclonal, Thermofisher). The tissue blocks were obtained from the archives of the department of Pathology of the institute. Four µm thin sections were cut on microtome (Leica, Germany) and taken on to 3 Aminopropyltriethoxysilane (APES) coated slides. Briefly, the protocol include following steps of deparafinization using EZ solution, antigen retrieval for 60 minutes using retrieval solution CC1, and incubation with ultra view DAB Inhibitor for 4 minutes, 100µl of anti-CD3 antibody at 37°C for 32 minutes and anti-MPO antibody for 37°C at 60 minutes respectively, ultra view HRP Multimer for 8 minutes, ultra view DAB Detection kit for 8 minutes, counterstained with hematoxylin for 8 minutes and mounted with DPX.

Scoring: Two individual observers scored the sections. Brown color stained lymphocytes positive for CD3 and neutrophils for positive MPO were evaluated. The immunoreactivity scored as negative for (0, no membrane staining), 1+ (<10% cells stained), equivocal 2+ (10%-40% cells stained) and 3+ (≥40%, cells stained).

Statistical analysis: Statistical analysis was carried out using SPSS statistical software version 23 (SPSS Inc, USA). Median value for absolute count of lymphocytes, monocytes, neutrophils, T-cell and their subsets for breast carcinoma patients and healthy women was calculated and compared using Mann-whittney Chi-square test. The median value and correlation with clinico-pathological parameters was done by Mann-Whittney test and Pearson correlation. The P value ≤ 0.05 was considered as significant.

Results

In peripheral blood using CD45 antibody three different populations were enumerated as lymphocytes, monocytes and neutrophils by flow cytometry in breast carcinoma patients and in healthy women (Figure 1). The median value of percentage and absolute count of these three populations of breast carcinoma patients were compared with healthy women.

Comparison of lymphocytes subsets in peripheral blood of breast carcinoma patients with healthy women: In comparison with healthy women, median value of and neutrophils (P=0.04) was higher and lymphocytes value was lower as compared to breast carcinoma patients, however the median value of monocytes was found similar in breast carcinoma patients and healthy women. Median value of CD3+ T-cells, cytotoxic T-cells (CD8+) seen lower and median value of helper T-cells (CD4+) seen higher in breast carcinoma patients as compared to healthy women (Table 1).

Further, a trend of higher median value of ALC and ANC was found in breast carcinoma patients as compared to healthy women. Whereas, median value of AMC, AHTC and ACTC were found similar

in breast carcinoma patients as compared to healthy women (Table 2).

Correlation of lymphocytes and T-cell subsets with clinic-pathological parameters of breast carcinoma patients: In relation to clinico-pathological parameters, the median value of CD4+ helper T-cells was found significantly lower in grade III (P=0.039) compared to their respective counterpart. However, the median value of CD8+ cytotoxic T-cells was found significantly increased (P = 0.04) in BR Score 8 tumors than their counterparts. No significant correlation was noted of these cells with any other clinico-pathological parameters (Table 3).

Incidence of CD3+ T-cells in tumor microenvironment: In tumor microenvironment, CD3+ T-cell infiltration were seen in 98% (47/49) of breast carcinoma patients with 1+ score in 29% (14/49), 2+ score 33% (16/49) and 3+ score in 35% (17/49) patients.

Comparison of CD3+ T-cells infiltration in tumor microenvironment with clinico-pathological parameters: A significant higher incidence of CD3+ T-cell infiltration (2+ 3+ score) was seen in patients with >48 years (p=0.02) and BRscore 8 (p=0.02) tumors as compared to their respective counterpart. (Figures: 2, 3) However, a trend of a higher incidence of CD3 infiltration in tumor microenvironment was noted in patients with post-menopausal status and node positive status as compared to their respective counter parts (Table 4).

Incidence of neutrophils in tumor tissue: Regarding neutrophils infiltration in tumor microenvironment only 9 patients exhibited MPO and therefore statistically analysis was not done. (Figures: 4, 5)

Correlation of CD3+ T cells in tumor microenvironment with peripheral blood cells: Patients with CD3+ T-cell infiltration in tumor microenvironment with 2+ and 3+ score had low median level of Helper T-cell (CD4+) in circulation as compared to patients with 0 and 1+ score (p=0.08; Table 5)

Discussion

In comparison with healthy women, the

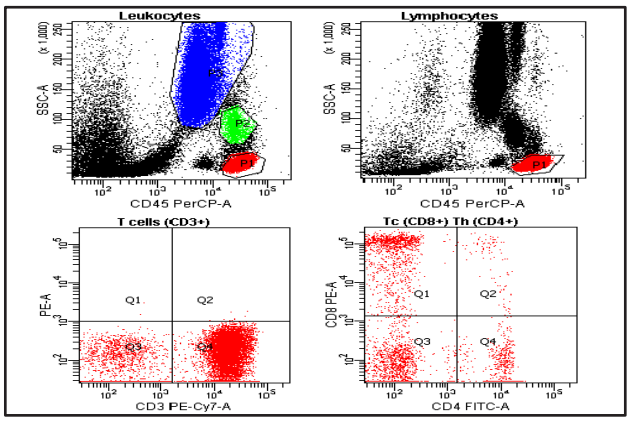


Figure 1: Dot plot depicts lymphocytes, monocytes, neutrophils by CD45 gating and T cells and their subsets

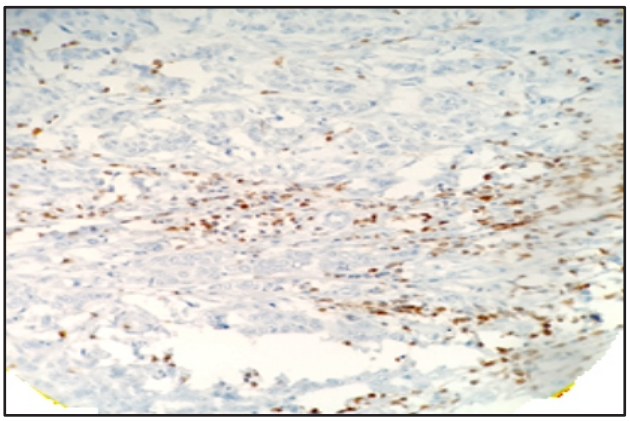


Figure 2: CD3+ T-cells infiltration in tumor microenvironment

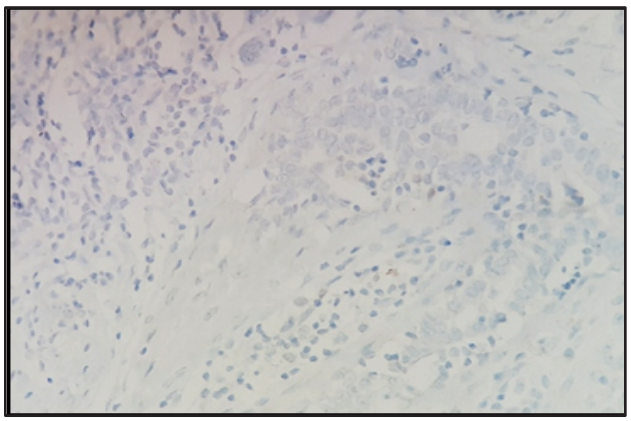


Figure 3: No infiltration of CD3+ T-cells in tumor microenvironment

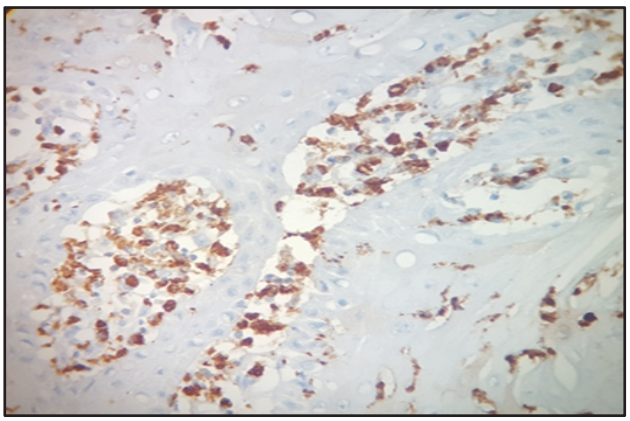


Figure 4: MPO+ neutrophils infiltration in tumor microenvironment

Table 1: Comparison with percentage of lymphocytes subsets between breast carcinoma patients and healthy controls

Cells		Median	Mann-Whitnney (U)	Z- value	P-value
Lymphocytes	HC	41.98	420.500	-1.428	0.153
	BrCA	34.25			
Monocytes	HC	37.21	520.500	-0.192	0.848
	BrCA	36.24			
Neutrophils	HC	28.62	601.00	-2.052	0.040
	BrCA	39.75			
CD3	HC	41.57	429	-1.320	0.187
	BrCA	34.41			
CD4	HC	33.52	473	-0.656	0.512
	BrCA	37.04			
CD8	HC	44.43	348	-2.234	0.25
	BrCA	32.46			

HC, healthy control; BrCA, breast cancer

Table 2: Comparison of absolute counts between breast carcinoma patients and healthy controls

		Median	Mann-Whitnney (U)	Z- value	P-value
ALC	HC	31.24	425	-1.369	0.171
	BrCA	38.67			
AMC	HC	37.52	514	-0.266	0.790
	BrCA	36.08			
ANC	HC	27.19	340	-2.422	0.15
	BrCA	40.33			
ATC	HC	38.22	444	-1.134	0.257
	BrCA	46.44			
AHTC	HC	36.43	516	-0.113	0.910
	BrCA	35.82			
CD8	HC	37.79	487	-0.472	0.637
	BrCA	35.25			

HC, healthy control; BrCA, breast cancer; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; ATC; absolute T cell count; AHTC, absolute helper T cell count; ACTC, absolute cytotoxic T cell count

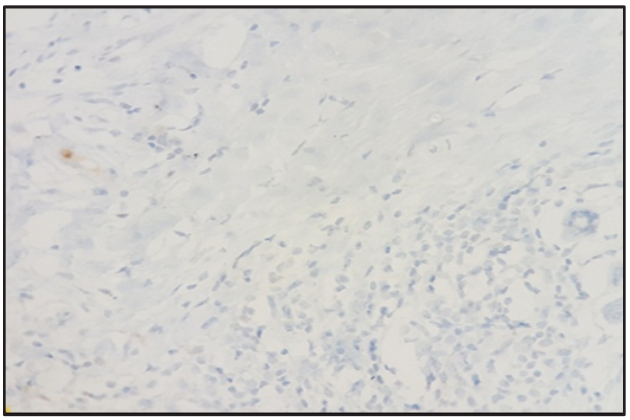


Figure 5: No infiltration of neutrophils in tumor microenvironment

breast carcinoma patient had higher median value of neutrophils and absolute count of neutrophils with a lower median value of lymphocyte percentage. However, no difference in median value of monocytes, ALC, AMC was noted between these two groups. Further, median value of CD3+ T-cells and CD8+ cytotoxic T-cells was found lower and CD4+ helper T-cells was found higher in breast carcinoma patients as compared to healthy women. This suggests an altered immune response in patients with breast carcinoma. Such study was carried out in oral squamous cell carcinoma (OSCC) by Zahorec et al[9] and Birva at al[10] showed increase in neutrophils and monocytes and decrease in lymphocytes reflecting induction of inflammatory response. Elevated counts of neutrophils in blood might be due to production of

Table 3: Comparison of peripheral blood cells of breast carcinoma patients with clinico-pathological parameters

Parameter	Patients (N=51)		ALC	AMC	ANC	ATC	AHTC	ACTC	CD3+	CD4+	CD8+
Age											
≤48	24	1572	321	5737	43.22	600.58	433.80	2.95	39	29.50	
>48	27	1356	264	4480	37.06	264	422.82	1.80	37	27	
Menopausal status											
Pre	21	1568	330	5700	40.80	580	445.20	2.70	38	30	
Post	30	1396	264	4850	37.64	510.63	422.61	1.90	38	27.50	
Tumor size											
T1 ≤2mm	16	1238	322	4851	40.23	489.84	407.55	3.25	37	28	
T2 >2 ≤5mm	25	1578	267	4403	37.64	636.88	434.01	1.70	40	28	
T3 >5mm	08	1771	340	5890	21.85	637.56	530	1.10	36	31	
Nodal status											
N0	21	1518	285	5568	28.60	621	456	1.20	40	29	
N1	27	1406	300	4161	42	580.16	315	2.30	37	28	
Stage											
I	12	1238	302	4851	23.12	423.02	407.55	1.55	36.50	32	
II	25	1567	273	5337	34.22	636.88	443.40	1.60	40	28	
III	11	1590	340	4774	70.84	555.50	456	4.10	36	28	
Histological grade											
I	7	1248	312	6006	24.96	522.72	297	1.60	40	25	
II	17	1425	260	4459	37.05	580.16	364.32	1.60	41	28	
III	20	1587	335	4862	36.04	636.48	459.99	1.30	36.50*	30.50	
BR score											
5	7	1248	312	6006	24.96	522.72	297	1.60	40	25	
6	9	1425	231	5082	47.04	580.16	313.50	2.30	43	28	
7	9	1254	276	4347	12.35	564.30	364.32	0.70	40	26	
8	19	1609	350	5028	36.04	636.48	474.81	1.30	36.50	32#	

(P values: *= 0.03, #= 0.04)

Table 4: Comparison of CD3+ T-cell in tumor microenvironment of breast carcinoma patients with clinico-pathological parameters

Parameter (N=49)	Negative	Positive
Age	17 (35%)	32 (65%)
≤48 (24)	12 (50%)	12 (50%)
>48 (25)	05 (20%)	20 (80%) $
Menopausal status	17 (35%)	32 (65%)
Pre (21)	10 (48%)	11 (52%)
Post (28)	7 (25%)	21 (75%)
Tumor size	17 (35%)	32 (65%)
T1 (16) ≤2mm	8 (50%)	8 (50%)
T2 (25)>2-≤5mm	8 (32%)	17 (68%)
T3 (08)>5mm	1 (12%)	7 (88%)
Nodal status	17 (35%)	32 (65%)
N0 (21)	12 (43%)	16 (57%)
N1 (27)	5 (24%)	16 (76%)

Parameter (N=49)	Negative	Positive
Stage	17 (35%)	32 (65%)
I (11)	7 (58%)	5 (42%)
II (23)	7 (28%)	18 (72%)
III (12)	3 (25%)	9 (75%)
Histological grade	17 (35%)	32 (65%)
I (07)	2 (29%)	5 (71%)
II (17)	11 (52%)	10 (48%)
III (20)	4 (19%)	17 (81%)
BR score	17 (35%)	32 (65%)
5 (07)	6 (54%)	5 (46%)
6 (09)	5 (56%)	4 (44%)
7 (09)	2 (22%)	7 (78%)
8 (19)	4 (20%)	16 (80%) @

(P values: $= 0.03, @= 0.02)

Table 5: Correlation of CD3+T-cells in tumor microenvironment with peripheral blood

CD3 in TM (N=49)	ALC	AMC	ANC	ATC	AHTC	ACTC	CD3+	CD4+	CD8+
r	-0.130	-0.188	-.0104	0.043	-0.247	-0.101	-0.159	-0.043	-0.101
p	0.374	0.195	0.479	0.771	0.087↓	0.479	0.275	0.771	0.492

(p value =0.08 inverse correlation)
r= correlation coefficient; TM= tumor microenvironment

granulocyte-macrophage colony stimulating factor (GM-CSF) seen in several tumors.[11-13] In addition, other cytokine such as granulocyte colony-stimulating factor (G-CSF), interleukin- (IL-)1, and IL-6 produced by tumors seem to contribute elevated neutrophils counts in blood. The neutrophil is associated with poor prognosis in several types of cancer such as lung, melanoma and renal carcinomas.[14-16] Also, neutrophil to lymphocyte ratio was introduced as prognostic factor for colorectal carcinoma.[17] Neutrophils are frequently associated with inflammatory responses to infection and tissue damage which represents evidence for concept of cancer-related inflammation inducing tumor progression.[18] Contrary to that gastric cancer an elevated neutrophil blood count is indicative of better prognosis,[19] means neutrophils directly kill the tumor cells both in vitro and in vivo.[20-22] Also, neutrophil from tumor-bearing animals were reported to enhanced cytotoxicity against several tumors cell lines.[23-25] In the study in tumor microenvironment, neutrophils infiltration was noted in 18% of breast carcinoma patients. Tumor infiltrating neutrophils are more cytotoxic towards tumor cells and produced higher levels of tumor necrosis factor alpha (TNFα), NO and H_2O_2.[26] In contrast, tumor infiltrating neutrophils in established tumors had these functions down regulated and presented a more pro-tumorigenic phenotype. Therefore, the exact role of neutrophils within the tumor is controversial matter.[27,28]

In relation to clinico-pathological parameters, the peripheral blood ALC, AMC and ANC was found lower in patients with older age (>45 years) and post-menopausal patients. Further, in relation to increasing tumor size and lymph node status, median value of ANC, ACTC showed increasing trend while median value of ATC showed decreasing trend with increasing tumor size and positive lymph node status. Further, CD3+ T-cells and ATC was found increasing with disease advancement (stage I to III). Regarding grade of tumor, median value of ALC and ACTC found higher and CD4+ Helper T-cells were significantly lower in grade III tumors and BR score 8 tumors. While ANC found higher in grade I and BR score 5 tumors. Contrary to our finding Ching Liang ho et al[29] showed that ALC and AMC had no correlation with the clinical parameters. A study by Qing Qing li et al[30] found that elevated neutrophil count was associated with poor pathological differentiation, more advanced stage, more metastatic sites, peritoneal metastasis and higher Glasgow prognostic stage (GPS), all of which are suggesting that higher neutrophil count was probably related to great tumor burden. Also as explained by Fondevila et al[31] cancer cells release myeloid growth factor (e.g., granulocyte colony stimulating factor) resulting in production of neutrophils. Circulating neutrophils secrete various cytokines, including vascular endothelial growth factor (VEGF), tumor necrosis factor-α and interleukins, which contribute to progression of cancer.

In tumor microenvironment, infiltration of CD3+ T-cell was noted in 98% of breast carcinoma patients, CD3+ T-cell infiltration with 2+ or 3+ score had low median level of helper T-cells (CD4+) in circulation as compared to patients with 0 or 1+ score. No such trend was seen with tumor size, lymph node status, tumor grade and vascular permeation. The intercorrelation of peripheral blood cells with CD3+ T-cells in tumor microenvironment showed an inverse correlation between AHTC of peripheral blood and CD3+ T-cells in tumor microenvironment. Reduction of CD4+ cells in blood inducing tumor infection causes CD4+ helper T-cells to migrate from blood to the tumor site.[32]

Although Rathore et al[33,34] observed significantly high intratumoral CD3+ counts in relation to tumor stage and no significant association of CD3+ and CD8+ TILs with other factors like age, menstrual status, family history, histological grade and lymph node status was noted in TNBC or breast ductal carcinoma patients. High tumor grade, stage and lymph node positivity are known poor prognostic factors in breast cancer. Infiltrating CD3+ TILs could be used as an adjunct to all cases of TNBC and could be useful in stratifying the patients into high-risk or low-risk category at the time of tissue diagnosis on resected specimen.

Conclusion

In patients with breast carcinoma low lymphocytes and CD3+ T-cells with high neutrophils and CD8+ T-cells were seen in peripheral blood than

healthy women indicates immune suppression. Further, high cytotoxic T-cells in peripheral blood of grade III tumors with lower CD4+ helper T-cells was observed which indicate altered immune status of breast carcinoma patients. Also, CD3+ T-cells infiltration in tumor microenvironment was noted in 98% of cases suggesting its anti-tumor activity. Hence, patients with altered immune status can be treated with interferon α and interleukin to boost up the immune response to help to kill cancer cells.

References

1. Ferlay J, Soerjomataram I, Ervik M, et al: Globocan 2012
2. Standish LJ, Sweet ES, Novack J, et al: Breast Cancer and the Immune System. Journal of the Society for Integrative Oncology 2008; 6(4):158-168
3. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. Cell 2010; 140:883-899
4. Carvalho M I, Pires I, Prada J, Queiroga F, L; A role for T-lymphocytes in human breast cancer and in canine mammary tumors. Biomed Research International 2014;130894
5. Al-Tameemi M, Chaplain M, d' Onofrio A. Evasion of tumours from the control of the immune system: consequences of brief encounters. Biology Direct 2012; 7:31
6. Matkowski R, Gisterek I., Halon, A, et al: The prognostic role of tumor-infiltrating CD4 and CD8 T lymphocytes in breast cancer. Anticancer Research 2009; 29: 2445-2451
7. Maher J, Davies ET: Targeting cytotoxic T lymphocytes for cancer immunotherapy. British Journal of Cancer 2004; 91:817-821.
8. U.S. Department of Health and Human Services National Institutes of Health: Understanding the immune system how it works. [Online] Available at: http://www.imgt.org September, 2003
9. Zahorec R: Ratio of neutrophil to lymphocyte counts-rapid and simple parameter of systemic inflammation and stress in critically ill Bratislavské lekárske listy 2001; 102: 5-14
10. Birva B, Hemangini V: Study of Immune Effector Cells in Leukoplakia and Oral Cancer. Journal of Cancer Research and Treatment 2014; 28-40
11. Uribe-Querol, E., & Rosales, C: Neutrophils in Cancer: Two Sides of the Same Coin. Journal of Immunology Research 2015; 11:983698
12. McGary CT, Miele ME, Welch DR: Highly metastatic 13762NF rat mammary adenocarcinoma cell clones stimulate bone marrow by secretion of granulocyte-macrophage colony-stimulating factor/interleukin-3 activity. The American Journal of Pathology 1995; 147:1668-1681
13. Lechner MG, Daniel Esptecnal: et al: Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. The Journal of Immunology 2010; 185.:2273-2284
14. Schmidt H, Bastholt L, Geertsen P, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. British Journal of Cancer 2005; 93:273-278
15. Atzpodien J, Reitz M: Peripheral blood neutrophils as independent immunologic predictor of response and long-term survival upon immunotherapy in metastatic renal-cell carcinoma. Cancer Biotherapy & Radiopharmaceuticals 2008; 23:129-134
16. Bellocq A, Antoine M, Flahault A, et al. Neutrophil alveolitis in bronchioloalveolar carcinoma: induction by tumor-derived interleukin-8 and relation to clinical outcome. The American Journal of Pathology 1998; 152:83-92
17. Walsh SR, Cook EJ, Goulder F, et al: Neutrophil–lymphocyte ratio as a prognostic factor in colorectal cancer. Journal of Surgical Oncology 2005; 91:181-4
18. Hanahan D, Weinberg RA: Hallmarks of cancer: the next generation. Cell 2011; 144:646-674
19. Caruso RA, Bellocco R, Pagano M et al: Prognostic value of intratumoral neutrophils in advanced gastric carcinoma in a high-risk area in northern Italy. Modern Pathology 2002; 15:831-837
20. Pickaver AH, Ratcliffe NA, Williams AE et al: Cytotoxic effects of peritoneal neutrophils on a syngeneic rat tumour. Nature. 1972; 235:186-187
21. Gerrard TL, Cohen DJ, Kaplan AM: Human neutrophil-mediated cytotoxicity to tumor cells. Journal of the National Cancer Institute 1981; 66:483-488
22. Katano M, Torisu M: Neutrophil–Mediated Tumor Cell Destruction in Cancer Ascites. Cancer 1982; 50:62-68
23. Ishihara Y, Fujii T, Iijima H et al: The role of neutrophils as cytotoxic cells in lung metastasis: suppression of tumor cell metastasis by a biological response modifier (PSK). In vivo Athens, Greece 1997; 12:175-182
24. Ishihara Y, Iijima H, Matsunaga K: Contribution of cytokines on the suppression of lung metastasis. Biotherapy 1998; 11:267-275
25. Yan J, Kloecker G, Fleming C et al: Human polymorphonuclear neutrophils specifically recognize and kill cancerous cells. Oncoimmunology 2014; 3:950163
26. Mishalian I, Bayuh R, Levy L, et al: Tumor-associated neutrophils (TAN) develop pro-

tumorigenic properties during tumor progression. Cancer Immunology, Immunotherapy 2013; 62:1745-1756
27. Fridlender ZG, Albelda SM. Tumor-associated neutrophils: friend or foe? Carcinogenesis 2012; 33:949-955
28. Brandau S, Dumitru CA, Lang S: Protumor and antitumor functions of neutrophil granulocytes. In Seminars in immunopathology Springer-Verlag 2013; 35:163-176
29. Ho C-L, Lu C-S, Chen J-H, et al: Neutrophil/Lymphocyte Ratio, Lymphocyte/Monocyte Ratio, and Absolute Lymphocyte Count/Absolute Monocyte Count Prognostic Score in Diffuse Large B-Cell Lymphoma: Useful Prognostic Tools in the Rituximab Era. Wei. L, ed. Medicine 2015; 9:3017-3023
30. Li QQ, Lu ZH, Yang L, et al: Neutrophil count and the inflammation-based glasgow prognostic score predict survival in patients with advanced gastric cancer receiving first-line chemotherapy. Asian Pacific Journal of Cancer Prevention 2014; 15:945-50
31. Fondevila C, Metges JP, Fuster J, et al: p53 and VEGF expression are independent predictors of tumour recurrence and survival following curative resection of gastric cancer. British Journal of Cancer 2004; 90:206-215
32. Kristian T, Ilkka L, Juuso J, Aila KK, Minna O, Riku T, Nina L, Johan L, Ari R, Anna K, Anniina K, et al: T-cell Subsets in Peripheral Blood and Tumors of Patients Treated With Oncolytic Adenoviruses. Molecular Therapy 2015; 23:964-73
33. Tsoutsou PG, Bourhis J, Coukos G: Tumor-infiltrating lymphocytes in triple-negative breast cancer: A biomarker for use beyond prognosis. Journal of Clinical Oncology 2015;33:1297-1298
34. Rathore AS, Kumar S, Konwar R, et al: CD3+, CD4+ & CD8+ tumour infiltrating lymphocytes (TILs) are predictors of favourable survival outcome in infiltrating ductal carcinoma of breast. The Indian Journal of Medical Research 2014; 40:361-369

Expression of RARβ Gene in Invasive Ductal Carcinoma of Breast

Bhavnagari Hunayna M[1], Patel Kinjal D[2], Patel Prabhudas S[3], Shah Franky D[4]
M.Sc Student[1], Junior Research Fellow[2], Professor and Head[3], Senior Scientific Officer[4]
Biochemistry Research Division
Department of Cancer Biology
Corresponding Author: frankyjha@yahoo.com

Summary

Signalling through RARβ plays an important role in breast carcinogenesis. A growing body of evidence supports the hypothesis that RARβ gene is a tumor suppressor gene and chemo preventive effect of retinoids are due to induction of RARβ gene expression which is lost or reduced in many malignant tumors. Breast cancer is a leading malignancy among the female world wide. Therefore, this study was conducted to determine the expression of RARβ in breast cancer. The expression of RARβ mRNA was examined in 24 paired tissue specimen from patient with invasive ductal carcinoma using semi quantitative reverse transcriptase polymerase chain reaction (RT-PCR) method. Statistical analysis of data was done by SPSS software (version 15).The result depicted significantly reduced RARβ mRNA expression in malignant tissues as compared to normal tissues (p=0.003). Receive Operative characteristic Curve (ROC) curve analysis also revealed that RARβ could significantly discriminate between normal and malignant tissue (p=0.009). Further, analysis showed that RARβ expression was lower in old age and post-menopausal woman. It was also decreased in early stage tumor, infiltration with no LN and organ metastasis and ER positive status. Interestingly, RARβ transcript levels were lowest in tumors with Luminal B subtype followed by TNBC. HER2 enriched tumors showed higher RARβ expression than other two subtypes. Collectively, observations suggest that reduction of RARβ gene expression is an important event in breast carcinogenesis.

Keywords: Breast cancer, Invasive ductal carcinoma, Retinoic acid receptor beta

Introduction

Breast cancer is a leading malignancy among women world wide with 1.7 million cases and 5, 21,900 deaths reported in 2012.[1] It is rising steadily in all developing countries including India. The incidence rate of breast cancer in India is 27% from all female cancer cases and is second leading malignancy among the female. At The Gujarat Cancer & Research Institute (GCRI), Ahmedabad incidence rate of breast cancer is 20.29% from all the female cancer cases (40.81%). It is a complex disease involving multiple etiological factors and various signaling pathways.[2] Retinoid signaling pathway is one of the most important pathway in breast cancer.

Retinol is taken up from the blood and bound to cellular retinol-binding protein (CRBP) in the cytoplasm. The retinol dehydrogenase (RoDH) enzymes metabolize retinol to retinal, and then retinal is metabolized to retinoic acid (RA) by the retinaldehyde dehydrogenases (RALDHs). RA is bound in the cytoplasm by cellular RA-binding protein (CRABP). RA enters the nucleus and binds to the RA receptors (RARs) and the retinoid X receptors (RXRs), which themselves heterodimerize and bind to a sequence of DNA known as the retinoic acid response elements (RARE). This activates transcription of target genes that leads differentiation, apoptosis and inhibits proliferation and migration. RAR and RXR both contain three subunits α, β and γ.[3] Among the three RARs, RARβ has been well known for its tumor suppressive effects in epithelial cells.[4] Exogenous expression of the RARβ gene can cause RA-dependent and RA-independent apoptosis and growth arrest. RARβ induced growth arrest and apoptosis are mediated through RARα. As RA ligand-bound RARα binds to the RARE on the RARβ promoter, multiple activator proteins assemble at the site and result in the up regulation of the RARβ gene.[5] The expression of RARβ results in the transactivation and expression of a number of its target genes that mediate cell differentiation and death. All trance retinoic acid (ATRA) has ability to initiate differentiation of promyelocytic leukemic cells to granulocytes. On the basis of the dramatic success of retinoic acid therapy of acute promyelocytic leukemia harboring the RAR/PML translocation confirms the important role of RARβ in tumor growth inhibition. It is also becoming increasingly clear that RARβ expression is lost early in carcinogenesis or is epigenetically silenced[6] in many solid tumors, providing an opportunity for novel treatment strategies to be investigated using retinoid together with epigenetic modifiers that promote re-expression of silenced genes. Previous study from our lab showed increased level of retinol in breast cancer and speculated that the key factor for aberrant RA signalling may be due to loss of RARβ and CRBP.[7] Based on this fact the present study was designed to analyse RARß expression in breast cancer.

Materials and Methods

Study population: In the present study, histoptahologically confirmed and previously untreated breast cancer patients (n=24) were included. The patients were collected from the out patient department of GCRI, Ahmedabad, India. The study design was approved by the institutional review board of the Institute. Written consents were obtained from all the patients prior to tissue collection. The demographic details of the subjects are summarized in Table 1. Stage (tumor node metastasis (TNM)) of

malignant disease was determined according the American joint committee on cancer (AJCC) criteria. Clinicopathological characteristics including stage, tumor grade, invasion, infiltration, lymph node metastasis, organ metastasis, hormone receptors status and molecular subtypes are mentioned in Table 2.

Sample collection and processing: Twenty-four paired (malignant and normal) tissue samples from breast cancer patients were collected at the time of surgery. The obtained tissue samples were washed with sterile phosphate buffer saline (PBS, pH: 7.4) and stored in RNA later (Qiagen, USA) at -80°C until analyzed.

RARβ mRNA by semi-quantitative RT-PCR: Total RNA was isolated from tissue using trizol reagent (Sigma, USA) and RNeasy mini kit (Qiagen, USA) and stored at -80°C until analysis.[8] Quantitative and Qualitative Analysis of RNA was done by spectrophotometer and agarose gel electrophoresis respectively. mRNA expression level of RARβ was estimated using semi-quantitative RT-PCR. RT-PCR was performed using commercially available amfirivert one step RT-PCR kit (GenDEPOT, USA). mRNA expression levels were analysed using specific pair of primers for RARβ forward primer (5'TGCCTTTGGAAATGGATGACAC3') and reverse primer (5'TGACTGACCCCACTGTTTTCC3'). GAPDH was used as housekeeping gene. The primers for GAPDH are forward primer (5'GACCCCTTCATTGACCTCAACTACATG3') and reverse primer(5'GTCCACCACCCTGTTGCTGTAGCC3').[9] RT-PCR reaction was carried out in a total volume of 25µl and contained 0.5 µg of RNA, 12.5µl RT-PCR buffer mix (3.5 mM Mgcl2 and 0.4 mM of each dNTP), 0.6µM of each primer, 0.75µl RT-PCR enzyme mix and diethylpyrocarbonate (DEPC) treated water. After amplification, the PCR products were separated on a 1.5% agarose gel containing 0.05µg/ml of ethidium bromide (EtBr). DNA ladder (100 bp) was used as a size marker. Gel images were visualized and analysed using gel documentation system (Alpha Innotech, USA).

Statistical analysis: Data was analyzed statistically using the SPSS statistical software (Version 15). Student paired't' test was performed to assess difference of RARβ mRNA expression levels between normal and breast cancer tissues. Receiver operating characteristic curve (ROC) was constructed to evaluate discriminatory efficacy of RARβ mRNA expression levels between malignant and normal tissues. Student's independent't' test was performed to compare RARβ mRNA expression levels according to different clinicopathological parameters. All the values were expressed as the mean ± standard error of the mean (SEM). Two sided p values were calculated and p values ≤0.05 were considered to be significant.

Results

Details of the patients:

In the present study, pretreated breast cancer patients were included and their demographic details of breast cancer patients were collected using standard questionnaires. The details of the patients like age, menopausal status and family history of cancer are provided in Table 1. The group of breast cancer patients presented a mean age of 48 years, with an age range of 27-75 years. Clinicopathological details of the patients were collected from available hospital records and shown in Table 2. All the patients had invasive ductal carcinoma. Most of the patients were in early stages and estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER2) positive status.

Semi quantitative analysis of RARβ mRNA expression:

Figure 1 shows representative pattern of RARβ mRNA expression in normal and malignant breast tissue. Relative expression of RARβ was obtained by normalization with GAPDH. Paired student't' test was used to compare mean levels of RARβ in malignant and normal tissues. The mean level of RARβ mRNA expression was significantly reduced in the malignant tissues as compared to their normal counterparts (p = 0.003) (Figure 2; Table 3).

ROC curve analysis for RARβ mRNA expression in normal and malignant tissues:

ROC curve provides comparison of sensitivity and specificity of the parameters simultaneously. Area under curve (AUC) is a measure of the overall performance of the diagnostic test and is interpreted as the average value of the sensitivity for all possible value of specificity. ROC curve was constructed for RARβ mRNA levels to evaluate their efficacy to distinguish between normal and malignant tissues. As shown in Table 4 and Figure 3, RARβ mRNA levels could significantly (p=0.009) discriminate between normal and malignant tissues. The AUC for RARβ mRNA levels was 0.813.

Comparison of RARβ mRNA levels according to various clinicopathalogical parameters

Comparison of RARβ mRNA expression in different clinicopathological parameters are given in Table 5. RARβ mRNA expression levels were found to be reduced in older age group as compared to younger age group, post-menopausal women as compared to pre-menopausal women, early stages as compared to advanced stages, lymph node negative tumor as compared to lymph node positive tumor, invasive tumor as compare to non invasive tumor, infiltrative tumor as compare to non infiltrative tumor, ER positive

tumor as compare to ER negative tumor ,without organ metastasis tumor as compared to organ metastasis tumor. The difference of RARβ mRNA expression levels was almost comparable between groups of grade tumor well, moderate and poor, PR negative and PR positive, HER2 negative and HER2 positive. RARβ mRNA expression levels were lowest in tumors with Luminal B subtype followed by TNBC. HER2 enriched tumor shows higher RARβ mRNA expression than other two subtypes. However, no statistical significance was found for RARβ mRNA expression levels among different clinicopathological parameter.

Discussion

Inactivation of tumor suppressor gene; RARβ is a key event in initiation and progression of cancer. Our results showed reduced expression of RARβ in malignant tissue as compared to normal breast tissue. Secondly, RARβ could significantly discriminate between normal and malignant tissue. In accordance to our results, RARβ gene expression is frequently lost in primary tumors and their metastasis compared to adjacent non-cancerous tissues.[10] Loss of RARβ, therefore, may contribute to the tumorigenicity of human mammary epithelial cells. The expression of the RARβ mRNA is absent or down-regulated in most human breast cancer cell lines.[11] Sun et al have showed that RARβ expression was lower in the breast cancer tissue compared to normal tissue and fibroadenoma.[12] Several studies have showed lower or loss of RARβ expression in malignant breast tissues as compared to normal breast tissues.[13-15] In addition, various studies have showed loss of RARβ in solid tumor cells, including non small cell lung carcinoma, head and neck cancer and cervical cancer.[16-18]

Various mechanisms of action for silencing RARβ in breast cancer includes epigenetic modification like methylation at the promoter region of the gene and a compacted chromatin structure, DNA deacetylation and loss of heterozygosity.[6] Among all these, methylation of RARβ gene may be the important mechanism by which RARβ gene expression is silenced.[19] Epigenetically silenced RARβ has been shown to be re-expressed in the presence of DNA methyltransferase inhibitors (DNMT) inhibitors and histone deacetylase (HDAC) inhibitors in RARβ2 silent breast cancer cells.[2] Alterations in RARβ gene reduced expression may be due to the endogenous receptors contain point mutations or polymorphisms that disrupt function of RARβ gene. However, apart from epigenetic modifications, a number of alternative mechanisms have been proposed for silencing of RARβ including the loss of coactivators and impaired RARα signaling.

In our study, RARβ transcript levels were lowest in tumors with Luminal B subtype followed by TNBC. HER2 enriched tumor shows higher RARβ expression than other two subtypes In contrast to our results HER2 tumor showed significantly higher level of RARβ methylation compared to luminal breast tumor subtypes.[20] Further in the current study, reduced RARβ was found in postmenopausal compared to premenopausal women. In contrast to our result inverse association was reported between RARβ hypermethylation among postmenopausal women.[21] RARβ was reduced in early stage of cancer compared to advance stage; this result suggests that reduction of RARβ expression is early event in breast cancer. Thus, along with study of RARβ gene expression, epigenetic modifications should be incorporated in the study in order to identify accurate biomarkers of response to retinoid therapy for breast cancer.

Further, detailed analysis by inclusion of large number of patients in combination with epigenetic study of RARβ gene is warranted to identify its role in breast carcinogenesis.

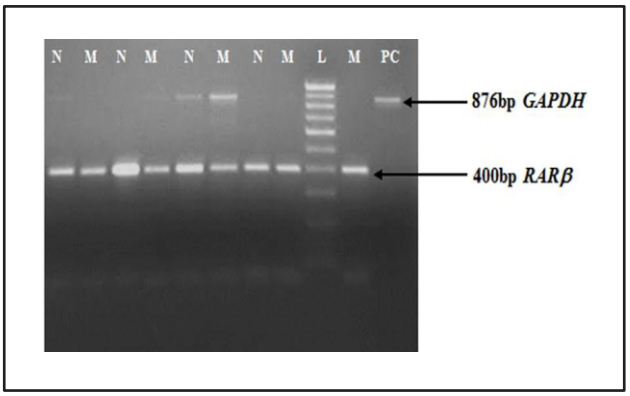


Figure 1. Representative pattern of RARβ mRNA expression: Lanes N and M represents the amplicon pairs of RARβ (400bp) and GAPDH (876bp) from malignant and normal tissues respectively. Lane L represents DNA Ladder (100-1000 bp) and Lane PC represents positive control. Lower mRNA expression of RARl was observed in malignant tissues as compared to their normal counter parts.

Table 1. Demographic details of the patients

No. of Breast Cancer Patients	**24 (100%)**
Age (Years)	
Mean	48
Median	45
Range	27-75
Menopausal Status	
Premenopausal	6 (25%)
Postmenopausal	18 (75%)
Family History	
Yes	2 (8.3%)
No	22 (91.7%)

Table 2. Clinicopathological details of the breast cancer patients

Characteristics	N (%)
Histological Type	
IDC	24 (100 %)
Others	-
Stage	
Early (I + II)	18 (75%)
Advance (III + IV)	6 (25%)
Tumor Grade	
I+II	6 (25%)
III	18 (75%)
Invasion	
Yes	12 (50%)
No	12 (50%)
Infiltration	
Yes	1 (4.2%)
No	23 (95.83%)
Lymph node Metastasis	
Yes	18 (75%)
PNS*-Yes	8 (33.3%)
PNS-No	10 (41.6%)
No	6 (25%)
Organ Metastasis	
Yes	2 (8.3%)
No	22 (91.7%)

Characteristics	N (%)
Estrogen Receptor	
Yes	12 (50%)
No	10 (41.6%)
Undefined	2 (8.3%)
Progesterone Receptor	
Yes	10 (41.6%)
No	12 (50%)
Undefined	2 (8.3%)
HER2	
Yes	16 (66.6%)
No	4 (16.6%)
Undefined	2 (8.3%)
Molecular Subtypes	
Luminal B	10(41.6%)
Basal like	4 (16.6%)
HER 2 enriched	6 (25%)
Undefined	4 (16.6%)
Hormone Receptor	
Yes	18 (75%)
No	4 (16.6%)
Undefined	2 (8.3)

Table 3. RARβ mRNA expression levels in normal and malignant breast tissues

	Mean	SEM	Significance
Normal tissue	0.362	0.049	**p=0.003**
Malignant tissue	0.209	0.029	

Table 4. Area under curve for RARβ mRNA expression levels in normal and malignant breast tissues

Parameter	AUC	Significance	95% Confidence Interval (CI)	
			Lower Bound	Upper Bound
RARβ	0.813	**p=0.009**	0.642	0.983

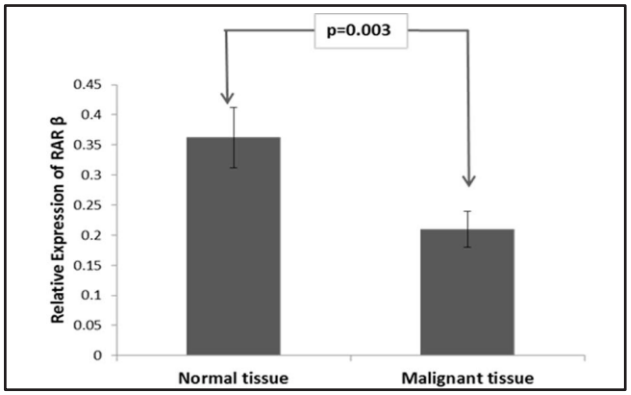


Figure 2. Graphical representation of RARβ mRNA expression levels in normal and malignant tissues

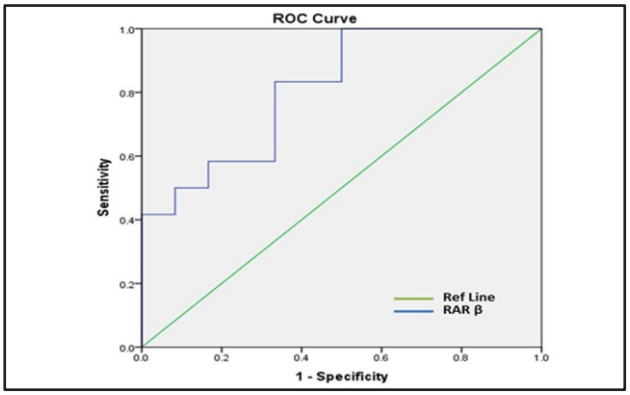


Figure 3. ROC curve for RARβ mRNA expression levels in normal and malignant tissues

Table 5. Comparison of RARβ mRNA expression levels according to the various clinicopathalogical parameters

Groups	Mean ± SEM
Pre menopausal	0.237±0.0763
Post menopausal	0.200±0.033
Early stage(I+II)	0.190±0.032
Advance stage (III+IV)	0.267±0.067
Lymph node Negative	0.167±0.020
Lymph node Positive	0.223±0.038
Perinodal spread Negative	0.210±0.050
Perinodal spread Positive	0.240±0.068
Well and Moderate (I+II) grade	0.190±0.067
Poor (III) grade	0.215±0.035
Non Invasive	0.226±0.042
Invasive	0.192±0.045
Non Infiltrative	0.215±0.032
Infiltrative	0.140±0.00
Without organ metastasis	0.199±0.033
Organ metastasis	0.260±0.082
ER Negative	0.242±0.048
ER Positive	0.193±0.044
PR Negative	0.220±0.045
PR Positive	0.210±0.050
HER2 Negative	0.186±0.008
HER2 Positive	0.207±0.041
Luminal B	0.164±0.040
HER2 enriched	0.279±0.077
TNBC	0.186±0.008

References

1. Torre LA, Bray F, Siegel RL et al: Global cancer statistics, 2012.CA: a cancer journal for clinicians 2015; 65:87-108
2. Connolly RM, Nguyen NK, Sukumar S: Molecular pathways: current role and future directions of the retinoic acid pathway in cancer prevention and treatment. Clinical Cancer Research 2013; 19:1651-1659
3. Maden M: Retinoid signaling in the development of the central nervous system. Nat Rev Neurosci 2002; 3: 843-853
4. Freemantle SJ, Spinella MJ, Dmitrovsky E: Retinoids in cancer therapy and chemoprevention: promise meets resistance. Oncogene 2003; 20:7305-7315
5. Bushue N, Wan YJ: Retinoid pathway and cancer therapeutics. Adv Drug DelivRev , 2010; 62:1285-1298
6. Sirchia SM, Ferguson AT, Sironi E, et al: Evidence of epigenetic changes affecting the chromatin state of the retinoic acid receptor beta2 promoter in breast cancer cells. Oncogene 2000; 19: 1556-1563
7. Shah FD, Patel JB, Shukla SN, et al: Evaluation of plasma non-enzymatic antioxidants in breast cancer etiology. Asian Pac J Cancer Prev 2009;10: 91-96
8. Cirera S: Highly efficient method for isolation of total RNA from adipose tissue. BMC Res Notes 2013;18, 6, 472
9. Pasquali D, Bellastella A, Valente A, et al: Retinoic acid receptors alpha, beta and gamma, and cellular retinol binding protein-I expression in breast fibrocystic disease and cancer. Eur J Endocrinol 1997;137: 410-414
10. Mehrotra J, Vali M, McVeigh M, et al: Very high frequency of hypermethylated genes in breast cancer metastasis to the bone, brain, and lung. Clin Cancer Res 2004; 10:3104-3109
11. diMasi A, Leboffe L, De Marinis E, et al: Retinoic acid receptors: from molecular mechanisms to cancer therapy. Mol Aspects Med 2015; 41: 1-115
12. Sun J, Xu X, Liu J, et al: Epigenetic regulation of retinoic acid receptor β2 gene in the initiation of breast cancer. Med Oncol 2011; 28: 1311-1318
13. Yang Q, Mori I, Shan L et al: Biallelic Inactivation of Retinoic Acid Receptor β2 Gene by Epigenetic Change in Breast Cancer. Am J Pathol 2001; 158: 299-303
14. Widschwendter M, Berger J, Müller HM, et al: Epigenetic down regulation of the retinoic acid receptor-beta2 gene in breast cancer. J Mammary Gland BiolNeoplasia 2001; 6: 193-201
15. Pestalozzi BC, Zahrieh D, Mallon E, et al: Distinct clinical and prognostic features of infiltrating lobular carcinoma of the breast: combined results of 15 International Breast Cancer Study Group clinical trials. J ClinOncol 2008; 26: 3006-3014
16. Olasz J, Juhász A, Remenár E, et al RAR beta2 suppression in head and neck squamous cell carcinoma correlates with site, histology and age. Oncol Rep 2007;18: 105-112
17. Picard E, Seguin C, Monhoven N, et al: Expression of retinoid receptor genes and proteins in non-small-cell lung cancer. J Natl Cancer Inst 1999; 91:1059-1066
18. Ivanova T, Petrenko A, Gritsko T, et al: Methylation and silencing of the retinoic acid receptor-beta 2 gene in cervical cancer. BMC Cancer 2002;2:4
19. Cote S, Momparler RL: Activation of the retinoic acid receptor b gene by 5-aza-28 deoxycytidine in human DLD-1 colon carcinoma cells. Anticancer Drugs 1997; 8: 56–61
20. Lee JS, Fackler MJ, Lee JH, et al: Basal-like breast cancer displays distinct patterns of promoter methylation. Cancer Biol Ther 2010; 9:1017-1024
21. Tao MH, Shields PG, Nie J, et al: DNA hypermethylation and clinicopathological features in breast cancer: the Western New York Exposures and Breast Cancer (WEB) Study. Breast Cancer Res Treat 2009;114: 559-568

Significance of Salivary Glutathione-S-transferase and Glutathione Reductase Enzymes in Oral Cancer

Bhimani Dhara V[1], Patel Kinjal R[2], Patel Jayendrakumar B[3]*, Patel Prabhudas S[4]
Junior Research Fellow[1] Junior Research Assistant[2] Senior Scientific Officer[3]* and
Incharge Professor and Head[4], Biochemistry Research Division
Department of Cancer Biology
Biochemistry Research Division,
Corresponding Author: jayendra_p@rediffmail.com

Summary

Oral cancer is a major health burden in India. Therefore, it is important and necessary to apply the vast knowledge that has been obtained regarding salivary biomarkers in oral cancer in the clinical area. Therefore, the aim of the study was to analyse salivary enzyme activity of glutathione-S-transferase (GST) and glutathione reductase (GR) as well as polymorphism in GSTM1 and GSTT1 genes from healthy individuals and oral cancer patients. Clinically diagnosed 57 oral cancer patients and 38 controls were enrolled for the study. Saliva samples were collected from the subjects. GST and GR enzyme activities were analyzed by spectrophotometric methods. PCR method was performed to study GSTM1 and GSTT1 polymorphisms. Statistical analysis was carried out by using SPSS software (Version 15). Mean salivary GR activity was significantly higher in oral cancer patients as compared to controls. Mean salivary GST activity was significantly lower in healthy individuals with tobacco habits as compared to healthy individuals without habit of tobacco. Mean salivary GST and GR activities were associated with clinico-pathological parameters including tumor site, tumor differentiation and stage of the disease. In pilot study, GSTM1 null genotypes were higher (41.6%) in oral cancer patients as compared to controls (36.8%), whereas GSTT1 null genotypes were higher in controls (26.3%) as compared to patients (16.7%). Present study suggested that salivary analysis of antioxidant is simple and non-invasive technique which may be helpful in screening and as early diagnostic marker for oral cancer patients.

Keywords: Glutathione-s-transferase, Glutathione reductase, GSTM1, GSTT1, Saliva, Oral Cancer

Introduction

India has been identified as one of the high risk countries where 77,000 new oral cancer cases are reported which constitute one third of world burden.[1] It has been recognized that tobacco is the major risk factor found to be associated with oral cancer. Epidemiological study was also reported that genetic factors are also play a major role for oral carcinogenesis. Both genetic and environmental factors involved in the development of oral cancer. Its interaction on carcinogenesis has been well demonstrated by Phase I and Phase II enzymes that are involved in the metabolism of carcinogenesis and major players for detoxification.[2] The metabolic activation of carcinogens is catalysed by Phase I enzymes which cause biotransformation of carcinogens into reactive intermediate metabolites that can cause DNA damage. Protection can be accomplished by inhibition of activating enzymes and/or induction of Phase II enzymes which leads to detoxification and excretion of carcinogens.[3] Thus, Phase II enzymes are very important mainly glutathione-S-transferase (GST) family of enzymes and glutathione reductase (GR) enzyme. GST further sub-divided into five main classes: alpha, beta, mu, pi, theta and zeta. Among subtypes of GST genes, GSTM1 and GSTT1 genes are very important to study for better understanding and pathogenesis of oral cancer. GST and GR are very important enzymes in achieving protection against carcinogenesis, mutagenesis and other forms of toxicity from reactive forms of carcinogens and found to be of prime importance in carcinogenesis.[4]

Saliva may be used as a potential diagnostic tool for detection of various diseases because of its non invasiveness, easy collection procedure, less chances of contamination, decreased patient discomfort and cost effectiveness.[5] Further, use of saliva as a diagnostic aid in oral cavity cancer is gaining immense popularity due to the close anatomic proximity of saliva to both pre-malignant and malignant neoplasms making it ideal for screening of these lesions.[6]

There is scarcity of data on GST and GR activity as well as GSTM1 and GSTT1 polymorphisms simultaneously from saliva as a sample source from oral cancer patients.[7,8] Therefore, study regarding salivary GST and its associated biomarkers might help in screening as well as early diagnosis of oral cancer and could be established as a screening and as an early diagnostic marker. Hence, present study is aimed to analyze GST and GR enzyme activities as well as polymorphism of GSTM1 and GSTT1 in oral cancer patients and controls to understand the utility of saliva, a non-invasive tool for early diagnosis of oral cancer.

Materials and Methods

Subjects :

The present study was approved by institutional review board of The Gujarat Cancer and Research Institute and informed consent was taken from all the participants. The details of age and tobacco habits in healthy individuals and oral cancer patients are shown depicted in Table 1. In oral cancer cohort, there were 57 males with age range of 20-75 years (mean 43.3 years). In controls, there were 38 males with age range of 16-50 years (mean 28.9 years). Tobacco habits were highly prevalent (93.0%) in oral cancer cases whereas 39.5% controls were tobacco users. Table 2 represents clinico-

pathological details of oral cancer patients. Among oral cancer cases, majority of the patients were of buccal carcinoma (63.2%) and tongue carcinoma (26.3%). It was also found that majority of the cases had moderate differentiated tumor (42.1%) and with tumor size less <4 cms (52.6%). A total of 38.6% of the patients were in early stage at diagnosis and majority of the patients did not have lymph-node metastasis (43.9%).

Table 1: Details of age and tobacco history in healthy individuals and oral cancer patients

Subjects	Age Mean (Range)	Status of Tobacco Habits	
		Having habits of tobacco No. (%)	Having no habits of tobacco No. (%)
Controls (38)	28.9 (16-50) Years	15 (39.5)	23 (60.5)
Oral cancer patients (57)	43.3 (20-74) Years	53 (93.0)	04 (07.0)

Table 2: Clinico-pathological parameters of oral cancer patients

Clinico-pathological characteristics (N=57)	No. (%)
Site:	
Buccal Mucosa	36 (63.2)
Tongue	15 (26.3)
Others	06 (10.5)
Tumor Differentiation:	
Well	22 (38.6)
Moderate	24 (42.1)
Undefined	11 (19.3)
Tumor's size:	
<4 cms	30 (52.6)
≥4 cms	10 (17.6)
Undefined	17 (29.8)
Stage:	
Early [I + II]	22 (38.6)
Advanced [III + IV]	18 (31.6)
Undefined	17 (29.8)
Lymph Node Metastasis:	
Negative	25 (43.9)
Positive	15 (26.3)
Undefined	17 (29.8)

Collection and processing of samples: In the present study, saliva was used as a source for measuring GST and GR enzyme activities as well as to analyze GSTM1 and GSTT1 polymorphisms. The standard protocol was used for saliva collection.[9] Briefly the subjects rinsed their mouth with water and then threw off that water. Unstimulated whole saliva was collected in 50 ml falcon tube kept on ice, and was processed immediately. The saliva was centrifuged at 2600 g for 15 minutes at 4°C. The supernatant was collected in different aliquots and protease inhibitor was added as follows: 1 µl aprotinin (1mg/ml), 3 µl sodium orthovananadate (40mM), and 1 µl phenyl methyl sulfonyl fluoride (PMSF) (1mg/ml) were added in 100 µl of saliva supernatant.[9] After the addition of protease inhibitors, the saliva supernatants were immediately stored at -80°C until analyzed. Before supernatant separation, 1 ml whole saliva was stored at -80°C for DNA isolation. All the patients were taken in the study with oral malignancy confirmed by a registered histopathologist. Only those patients were selected who had not gone through any type of anticancer therapy like surgery, chemotherapy or radio therapy. Subjects suffering from major illness in the past and HIV, HBsAg and HCV positive subjects were excluded from the study.

Analysis of GST and GR enzyme activities: Spectrophotometric methods (UV-1800 Spectrophotometer, Shimadzu) were performed for the estimation of GST and GR enzyme activities. Salivary GST activity was assayed at 37°C using 1-chloro-2-4-dinitrobenzene by measuring the increase in absorbance at 340 nm.[10] Salivary GR activity was determined at 37°C by following NADPH oxidation at 340 nm. The decrease in absorbance was measured at 340 nm.[11]

Analysis of GSTM1 and GSTT1 genotyping: Genomic DNA was isolated from whole saliva using DNA isolation kit (Qiagen, USA) according to manufacturer's instructions and stored at -20°C until analysis. Analysis was performed by spectrophometric method to check purity and quantity of DNA. The GSTM1 and GSTT1 genetic polymorphisms were determined by PCR followed by analysis on agarose gel. β-globin gene was co-amplified as an internal control using primers 5'-CAA CTT CAT CCA CGT TCA CC-3' (forward) and 5'-GAA GAG CCA AGG ACA GGT AC-3' (reverse).

For GSTM1 gene, PCR was performed in a 25 µl reaction mixture containing 12.5 µl PCR master mix (Fermantas), 100 ng of template DNA and 250 ng of each primer (Bangalore Genei) having sequence 5'-GAA CTC CCT GAA AAG CTA AAG C-3' (forward) and 5'-GTT GGG CTC AAA TAT ACG GTG G-3' (reverse)[12]. PCR conditions were the following: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 1 min, 1 min annealing at 59.2°C, 1 min extension at 72°C and final extension for 5 min at 72°C. The amplifications were performed on thermal cycler (Eppendorf master cycler gradient, Hamburg, Germany). The PCR products were then analysed by electrophoresis on ethidium bromide stained 1.5% agarose gel. The presence or absence of GSTM1 gene was detected by the presence or absence of a band at 215 bp. A band at 268 bp (β-globin) was documented as successful amplification. For GSTT1,

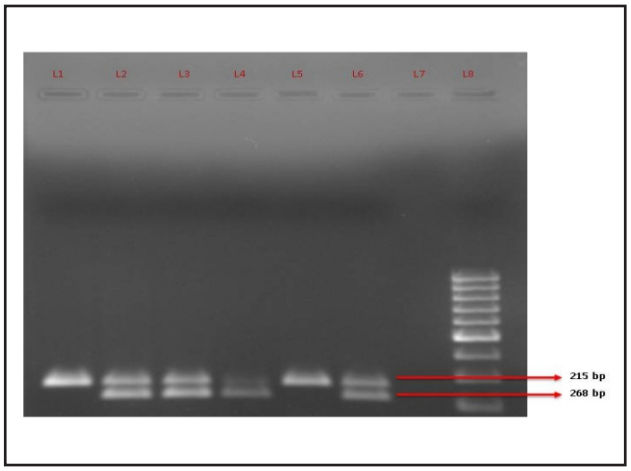
PCR was performed in a 25 µl reaction mixture containing 12.5 µl PCR master mix (Fermantas), 100 ng of template DNA and 250 ng of each primer (Bangalore Genei) having sequence 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' (forward) and 5'-TCA CCG GAT CAT GGC CAG CA-3' (reverse) .12 PCR conditions were the following: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 1 min, 1 min annealing at 59.2°C, 1 min extension at 72°C and final extension for 10 min at 72°C. The PCR products were then analysed by electrophoresis on ethidium bromide stained 1.5% agarose gel. The presence or absence of GSTT1 gene was detected by the presence or absence of a band at 480 bp. A band at 268 bp (β-globin) was documented as successful amplification.

The standard nomenclature used for the functional GST alleles was GSTM1*1 and GSTT1*1 to designate samples containing at least one copy of the GSTM1 or GSTT1 genes, respectively; the GST null alleles were designated GSTM1*0 and GSTT1*0. Figure 1 is representative patterns of GSTM1 and GSTT1 genotypes. The presence of GSTM1 band was indicated by the presence of 215 bp. The presence of 215 bp and 268 bp band which corresponds to GSTM1 gene and β-globin gene respectively, indicates GSTM1 not null genotype (GSTM1*1), while the absence of the 215 bp with the presence of 268 bp were taken as GSTM1 null genotype (GSTM1*0). The presence of GSTT1 band was indicated by the presence of 480 bp. The presence of 480 bp and 268 bp band which corresponds to GSTT1 gene and β-globin gene respectively indicates, GSTT1 not null genotype (GSTT1*1), while the absence of the 480 bp with the presence of 268 bp were taken as GSTT1 null genotype (GSTT1*0).

Statistical analysis: All data were analysed statistically using SPSS statistical software version 15. The enzyme activities of GST and GR were represented as Mean± Standard error of mean (S.E.M.). Student t-test was performed to estimate statistical significance of the parameter. Receiver operating characteristic (ROC) curves was plotted for GST and GR enzyme activities to estimate its efficacy to discriminate between oral cancer patients and controls. Chi square analysis was performed to find out difference in the distribution of GSTM1 and GSTT1 genotypes between oral cancer patients and controls. Odd ratios (ORs) and 95% Confidence Intervals (CI) were also calculated to estimate the oral cancer risk associated with GSTM1 and GSTT1 genotypes.

Results

Comparison of salivary GST and GR activities between controls and oral cancer patients: Mean salivary GST and GR activities in controls and oral cancer patients are represented in Figure 2. Mean salivary GST activity was higher in oral cancer patients as compared to controls. It was also found to be significantly higher mean salivary GR activity in oral cancer patients as compared to controls (p=0.014).



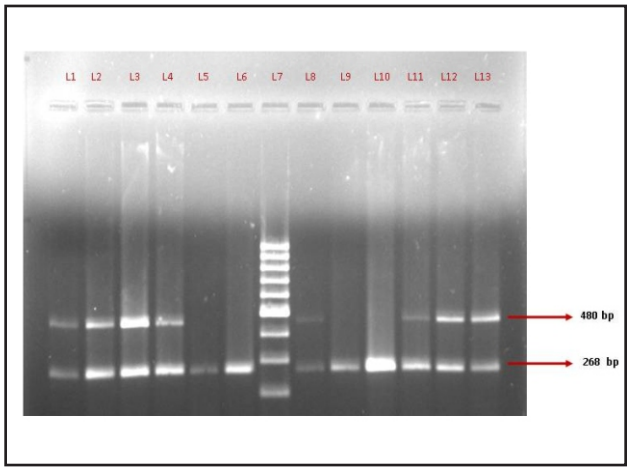


Figure 1:
A: GSTM1 polymorphism
Representative pattern for GSTM1 polymorphism
All the lanes show a constitutive band of β-globin house keeping gene (268 bp); Lanes 2,3,4,6 show an additional band (215 bp) of the GSTM1 gene; Lanes 1,5 show only 268bp band which indicates loss of GSTM1 gene in these subjects; Lane7: Negative control; Lane 8:100 bp DNA ladder.

B: GSTT1 polymorphism
Representative pattern for GSTT1 polymorphism
All the lanes show a constitutive band of β-globin house keeping gene (268 bp); Lanes 1,2,3,4,8,11,12,13 show an additional band (480 bp) of the GSTT1 gene; Lanes 5,6,9,10 show only 268 bp band which indicates loss of GSTT1 gene in these subjects;Lane 7: 100 bp DNA ladder.

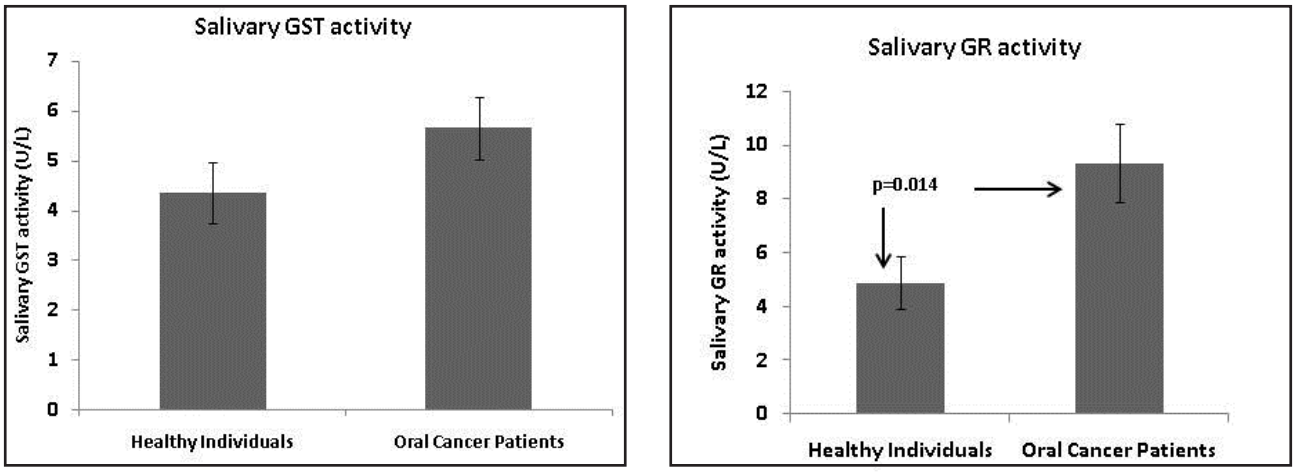


Figure 2: Comparison of salivary GST and GR activities between controls and oral cancer patients

Association of salivary biomarkers with tobacco habits: Mean salivary GST and GR enzyme activities in controls with no habit of tobacco, controls with habit of tobacco and oral cancer patients with habit of tobacco are presented in Table 3. Mean salivary GST activity was significantly lower in healthy individuals with habit of tobacco as compared to healthy individuals with no habit of tobacco (p=003). Mean salivary GST activity was lower in oral cancer patients with habit of tobacco as compared to healthy individuals with no habit of tobacco. However, it was significantly higher in oral cancer patients with habit of tobacco as compared to healthy individuals with habit of tobacco (p=0.010). Further, salivary GR activity was higher in oral cancer patients with habit of tobacco as compared to controls with no habit of tobacco.

Comparison of salivary biomarkers with different sites of oral cancer: Mean salivary GST and GR enzyme activities in controls, buccal mucosa and tongue carcinoma patients with squamous cell carcinoma are presented in Figure 3. Mean salivary GST activity was found to be higher in buccal carcinoma as well as tongue carcinoma patients as compared to controls. It was also observed slightly higher mean GST activity in buccal carcinoma patients as compared to tongue carcinoma patients. Mean salivary GR activity found significantly higher in buccal carcinoma patients as compared to controls (p=0.04). Further, GR activity was also higher in tongue carcinoma patients as compared to controls; however, difference was not statistically significant. Comparable GR activity was observed between patients with buccal carcinoma and tongue carcinoma patients.

Association of salivary biomarkers with different clinico-pathological parameters: Mean salivary GST and GR enzyme activities in controls, patients with well and moderately differentiated tumors are presented in Figure 4, Mean salivary GST activity was higher in patients with well and moderately differentiated tumors as compared to controls. However, Mean salivary GST activity was comparable between patients with well and moderately differentiated tumors. It was observed that mean salivary GR activity was significantly higher in patients with well differentiated tumors as compared to controls (p=0.018). Mean salivary GR activity was also higher in patients with well differentiated tumors as compared to controls. However, it was comparable in patients with well and moderately differentiated tumors.

Mean salivary GST and GR enzyme activities in controls and patients with early and advanced stage are represented in Figure 5. Mean salivary GST activity was found to be higher in early stage patients as compared to controls and patients with advanced stage. Whereas, mean salivary GR activity was found to be significantly higher in patients with early stage as compared to controls (p=0.032) and advanced stage patients.

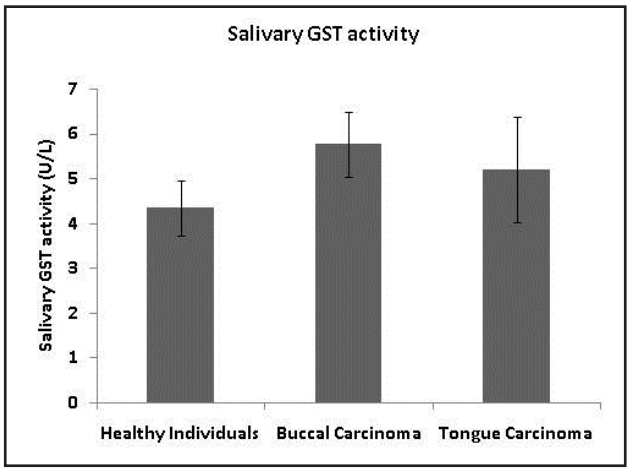
Table 4 represents mean salivary GST and GR activity in patients with positive and negative lymph node metastasis. Mean salivary GST and GR activities were found to be slightly higher in patients with negative lymph-node as compared to patients with positive lymph-node. However, difference was not statistically significant.

Table 3: Mean salivary biomarkers levels in controls with no habit of tobacco, controls with habit of tobacco and oral cancer patients with habit of tobacco

Biomarkers	Controls having habits of tobacco Mean ± S.E.M	Controls having habits of tobacco Mean ± S.E.M	Oral cancer patients having habits of tobacco Mean ± S.E.M
Salivary GST (U/L)	6.16 ±0.98	2.6 ±0.45*	5.17 ±0.60@
Salivary GR (U/L)	4.96 ±1.39	4.78 ±1.47	9.60 ±1.65

*p=0.003 compared with controls with no habits of tobacco

@ p=0.010 compared with controls with habits of tobacco



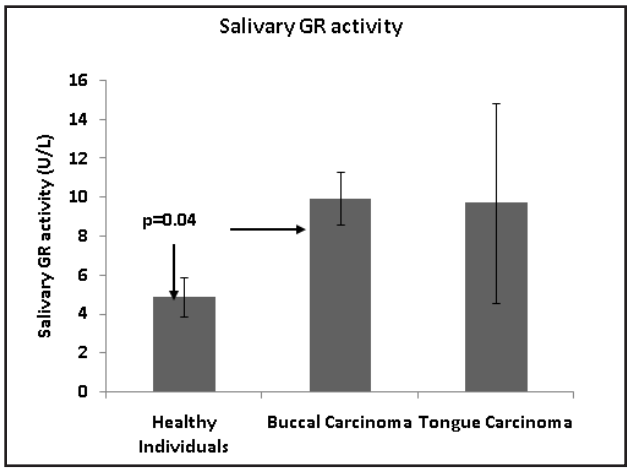
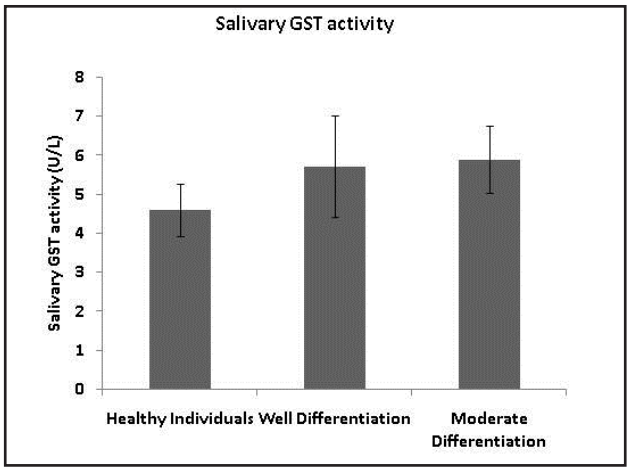


Figure 3: Comparison of salivary GST and GR activities according to site of primary tumor



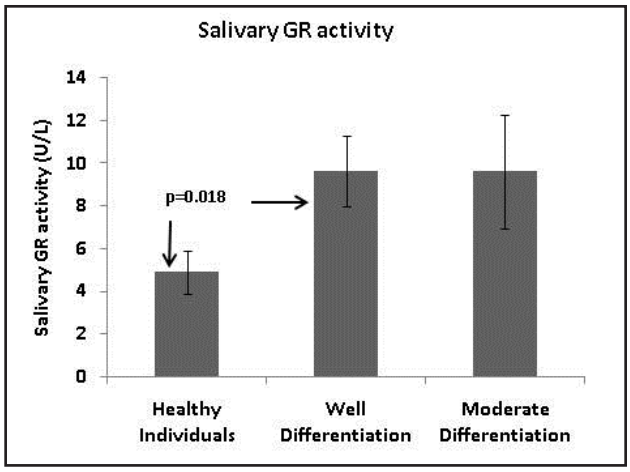
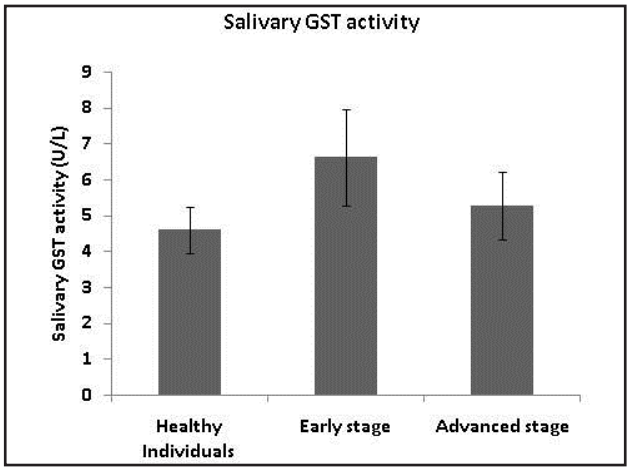


Figure 4: Comparison of salivary GST and GR activities according to tumor differentiation



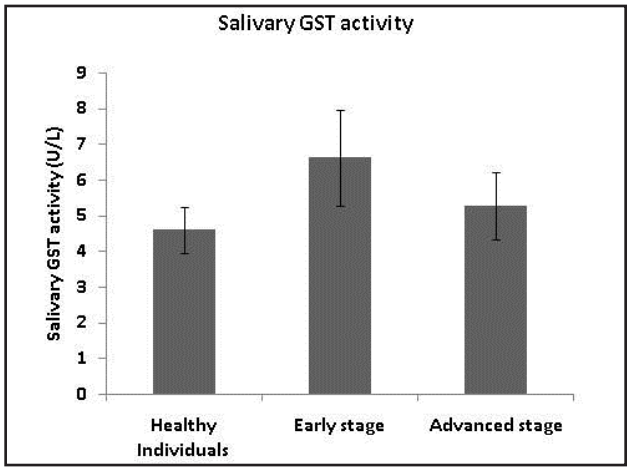


Figure 5: Comparison of salivary GST and GR activities according to stage of disease

Table 4: Comparison of salivary biomarkers according to Lymph nodes metastasis

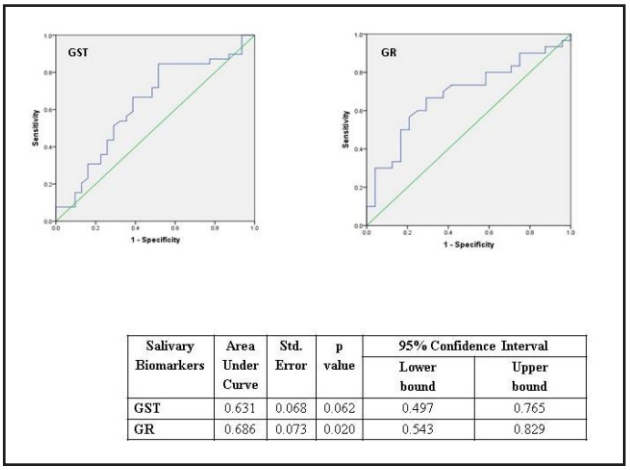
Biomarkers	Lymph Node Negative Mean ± S.E.M	Lymph Node Positive Mean ± S.E.M
Salivary GST (U/L)	6.43 ±1.26	5.40 ±1.02
Salivary GR (U/L)	11.48 ±2.60	8.45 ±2.35

Receiver's Operative Characteristic (ROC) curve analysis for salivary biomarkers: ROC curve analysis is a meaningful statistical approach to find discriminatory efficacy of the biomarker as it consider sensitivity as well as specificity of the biomarker simultaneously. ROC curves were plotted for salivary GST and GR activities as shown in Figure 6. It was observed that salivary GR activity could significantly discriminate between oral cancer patients and controls with area under curve of 0.686 (p=0.020). Salivary GST

activity could also discriminate between oral cancer patients and controls with area under curve of 0.631 (p=0.062), however could not achieve the statistical significance.

Frequency distribution of GSTM1 and GSTT1 genotypes in controls and oral cancer patients-A pilot study

Genotype frequency of GSTM1 and GSTT1 gene in controls and oral cancer patients are represented in Table 5. It was observed that 36.8% controls and 41.7% oral cancer patients represents GSTM1*0 genotype. Frequency of GSTM1 null genotype was slightly higher in oral cancer patients with moderate risk with an odds ratio of 1.2 (95% CI = 0.3-5.4). It was also observed that 26.3% controls and 16.7% oral cancer patients represents GSTT1*0 genotype. Frequency of GSTT1 null genotype was higher in controls as compared to oral cancer patients. However, GSTT1 genotypes could not associate with oral cancer risk (odds ratio :0.06, 95%CI: 0.1-3.5).



Salivary Biomarkers	Area Under Curve	Std. Error	p value	95% Confidence Interval	
				Lower bound	Upper bound
GST	0.631	0.068	0.062	0.497	0.765
GR	0.686	0.073	0.020	0.543	0.829

Figure 6: ROC curve for salivary GST and GR activity between healthy individuals and oral cancer patients

Table 5: Frequency distribution of GSTM1 and GSTT1genotypes in controls and oral cancer patients

Polymorphims	Controls No. (%)	Oral cancer patients No. (%)	$\chi 2$ p value	OR (95%CI) p value
GSTM1 genotypes				
GSTM1*1	12 (63.2%)	07 (58.3%)	0.0121 p=0.912	1 (Referent)
GSTM1*0	07 (36.8%)	05 (41.6%)		1.2 (0.3-5.4) p=0.788
Total	19 (100%)	12 (100%)		
GSTT1 genotypes				
GSTT1*1	14 (73.7%)	10 (83.3%)	0.0342 p=0.853	1.0 (Referent)
GSTT1*0	05 (26.3%)	02 (16.7%)		0.6 (0.1-3.5) p=0.535
Total	19 (100%)	12 (100%)		

Discussion

Free-radical associated damages play a major role in causation of cancer in tobacco habituates.[13] The damages are reflected through antioxidant enzymes activities in body fluid and have become interesting research area. An effective defence against oxidative damage is the glutathione cycle, which includes oxidation of reduced glutathione to oxidized glutathione during detoxification by GST. The maintenance of reduced glutathione levels depends on the activity of the GST and GR which have attracted interest in the fields of diagnosis and monitoring the different types of malignancy.[14]

Oral cavity is destined with an unconventional salivary antioxidant system that also contains anti nitrate and anti oxygen species. This salivary antioxidant coordination is based on enzymatic and non-enzymatic antioxidants.[15] It also includes another crucial anticancer salivary enzyme, GST, which catalyzes glutathione conjugation to the carcinogen electrophilic epoxide intermediates to protect against DNA damage.[16] However, there is limited study on GST and GR from saliva samples of oral cancer patients. Thus, present study has analyzed GST and GR enzyme activity as well as GSTM1 and GSTT1 polymorphisms from saliva samples of oral cancer patient. Our results depicted that salivary GR activity was significantly higher in oral cancer patients as compared to controls. Also, mean salivary GST activity was observed higher in oral cancer patients as compared to controls.

There are many studies in the literature which have analyzed GST and GR activity from body fluid and tumor tissues.[4,13,17] However, it is important to mention that there are scarce of data in the literature on GST and GR activity from saliva of oral cancer patients. However, one recent study has analysed salivary GST activity, suggested that GST activity was significantly higher in oral cancer patients as compared to controls.[8] On the contrary, Bahar et al have observed that salivary GST levels were significantly lower in oral cancer patients as compared to controls.[7] However, in the present study, ROC curve analysis also suggested that salivary GST and GR activities have good discriminatory efficacy between oral cancer patients and controls which further strengthen our results.

Present study also analyzed salivary GST and GR activity in subjects with habit of tobacco and non

habit of tobacco. Mean salivary GST activity was significantly lower in controls with habit of tobacco as compared to controls with no habit of tobacco. However, it was significantly higher in oral cancer patients as compared to controls with habit of tobacco. The mean GR activity was marginally higher in oral cancer patients as compared to controls with habit of tobacco. Previous blood based studies from our laboratory have also observed the association of GST and GR activity with tobacco habit.[4,13] However, there are no earlier reports on the association of these salivary GST and GR activities with tobacco habit.

Present study was also observed significantly higher GR activity in buccal carcinoma patients as compared to controls. This observation suggests that antioxidant alteration might play role in specific site involved in oral cancer, also buccal mucosa is highly exposed to carcinogens present in tobacco. Further, it was suggested that changes in the salivary antioxidant enzymes may be appropriate marker for the prognosis of oral diseases compare to conventional invasive serum antioxidant enzyme.[18] When present study has analyzed association between these salivary biomarkers with various clinico-pathological parameters, it was observed that mean salivary GR activity was significantly higher in patients with well differentiated tumors as well as patients with early stage as compared to the controls. Singh et al suggested that salivary GST activity was significantly higher in oral cancer patients with moderately differentiated tumors as compared to patients with poorly differentiated tumors.[8] However, in the present study we did not observe significant difference in the GST activity according to degree of differentiation. Furthermore, it is important to mention that there are no much data in the literature regarding the association of salivary GST and GR activities with different clinico-pathological parameters in oral cancer patients. However, it can be suggested that salivary GR activity play an important role in oral carcinogenesis.

Recent molecular epidemiological studies revealed that genetically inherited polymorphisms also play important role in susceptibility of various malignancies. The contradictory results of GST activity may be due to this polymorphism as it results in complete deletion of GSTM1 and GSTT1 gene. Significant associations between the GSTM1 deletion and increased cancer incidence in case/control studies have been reported for oral cancer.[13] Both positive and negative results have been reported for associations between GSTM1 null genotype and cancer risk.[19] Several Indian studies from various regions had conducted and most of them have identified GSTM1 null genotype as a risk factor for oral cancer development, though the level of significance varied. [3,20] Present study also observed that salivary GSTM1 null genotype was associated with moderate risk of oral cancer with an odds ratio of 1.2, 95% CI = 0.3-5.4. However, results could not reach statistical significance. This may be due to small sample size in the present study. For GSTT1, the distribution of null genotype ranged from 7.3% to 19.0% in India.[20,21] The comparisons of the frequency distribution across various studies showed that differences do exist in the Indian population according to geographic regions.[3,20,21] In the present study, salivary GSTT1 null genotypes were higher in controls as compared to oral cancer patients. This result is not in accordance with previous blood based study in oral cancer patients.[2] However, it is important to mention that there are no previous reports on GSTM1 and GSTT1 polymorphisms from saliva as a sample in oral cancer patients. Also, small sample size may be one of the contributing factors. However, being a pilot study, this study suggests that saliva can be used for genotyping assays.

In the present study, it was shown that salivary GST and GR enzyme activities were altered in oral cancer patients. Further, salivary based GST and GR activities have been associated with tobacco habits and clinico-pathological parameters. In pilot study, it was also observed that salivary GSTM1 null genotype was associated with moderate risk of oral cancer. These results suggested salivary analysis of antioxidant is simple and non-invasive technique which may be helpful in screening and as early diagnostic marker for oral cancer patients. It can also reveal that salivary antioxidant alteration may contribute to the pathogenesis of oral cancer.

References:

1. Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics 2002 CA Cancer J Clin 2005; 55: 74-108
2. Singh RD, Haridas N, Shah FD, et al: Gene polymorphisms, tobacco exposure and oral cancer susceptibility: a study from Gujarat, West India. Oral Disease 2013; 20: 84-93
3. Soya SS, Vinod T, Reddy KS, Gopalakrishnan S, Adithan C: Genetic polymorphism of glutathione-S-transferase genes (GSTM1, GSTT1 and GSTP1) and susceptibility to upper aerodigestive tract cancers in a South Indian Population. Eur J Cancer 2007; 43: 2698-2706
4. Rawal RM, Patel DD, Patel PS, et al: Assessment of glutathione-S-transferase and glutathione reductase in patients with squamous-cell carcinoma of buccal mucosa. Int J Cancer 1999; 83: 727-731
5. Bigler LR, Steckfus CF: Salivary biomarkers for the detection of malignant tumors that are remote from the oral cavity. Clin Lab Med 2009; 29: 71-85
6. Bonne N, Wong D: Salivary biomarker development using genomic, proteomic and metabolomic approaches. Genome Med 2012;4: 82
7. Bahar G, Feinmesser R, Shpitzer T, et al: Salivary analysis in oral cancer patients. Cancer 2007; 109: 54-59

8. Singh H, Shetty P, Sreelatha SV, Patidar M: Analysis of salivary antioxidant levels in different clinical staging and histological grading of oral squamous cell carcinoma: Non invasive technique in dentistry. Journal of Clinical and Diagnostic Research 2014; 8: ZC08-11
9. Hu S, Wang J, Meijer J, et al: Salivary proteomic and genomic biomarkers for primary sjorgen's syndrome. Arthritis Rheum 2007; 56: 3588-3600
10. Habig WH, Jacoby WB: Assays for differentiation of glutathione-S-transferase. Methods Enzymol 1981; 17: 398-405
11. Carlberg I, Mannerwick B: Glutathione reductase. Methods Enzymol 2 1985; 113: 484-490
12. Sivoňová M, Waczuliková I, Dobrota, D, et al: Polymorphisms of glutathione-S-transferase M1, T1, P1 and the risk of prostate cancer: a case-control study. J Exp Clin Cancer Res 2009; 28: 32
13. Patel BP, Rawal RM, Patel PS, et al: Tobacco, antioxidant enzymes, oxidative stress, and genetic susceptibility in oral cancer. Am J Clin Oncol 2008; 31: 454-459
14. Patel BP, Rawal RM, Patel JB, et al: Serum glutathione-S-transferase and glutathione reductase activity in head and neck cancer patients. Neoplasma 2002; 49: 260-266
15. Nagler RM, Lischinsky S, Diamond E, Klein I, Reznick AZ: New insights into salivary lactate dehydrogenase of human subjects. J Lab Clin Med 2001; 137: 363-369
16. Shiboski CH, Schmidt BL, Jordan RC: Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 20-44 years. Cancer 2005; 103: 1843-1849
17. Patel BP, Rawal UM, Shah PM, et al: Study of tobacco habits and alterations in enzymatic antioxidant system in oral cancer. Oncology 2005; 68: 511-509
18. Greabu M, Battino M, Totan A, et al: Effect of gas phase and particulate phase of cigarette smoke on salivary antioxidants. What can be the role of vitamin C and pyridoxine? Pharmacol Rep 2007; 59: 613-618
19. Zhang ZJ, Hao K, Shi R, et al: Glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1) null polymorphisms, smoking and their interaction in oral cancer: a HuGE review and meta-analysis. Am J Epidemiol 2011; 173: 847-857
20. Singh M, Shah PP, Singh AP, et al: Association of genetic polymorphisms in glutathione S-transferases and susceptibility to head and neck cancer. Mutant Res 2008; 638: 184–194
21. Sam SS, Thomas V, Reddy KS, Surianarayanan G, Chandrasekaran A: Gene environment interactions associated with CYP1A1 MspI and GST polymorphisms and the risk of upper aerodigestive tract cancers in an Indian population. J Cancer Res Clin Oncol 2010; 136: 945-951
22. Anantharaman D, Samant TA, Sen S, Mahimkar MB: Polymorphisms in tobacco metabolism and DNA repair genes modulate oral precancer and cancer risk. Oral Oncol 2011; 47: 866-872

Morphine: Myths and Facts in Cancer Care

Joshi Geeta M,
Professor and Head,
Department of Palliative Medicine
Corresponding Author: dr.geetajoshi@gmail.com

1. It is too early to start morphine. It is given only when disease is advanced and not curable.

It is not stage of the disease, but severity of pain to cancer patients is indication to start morphine. In general, the prevalence of pain at the time of cancer diagnosis and early in the course of disease is estimated to be approximately 50%, increasing to 75% at advanced stages.[1] If patient has severe pain, not responding to step II opioids of WHO analgesic ladder, Morphine can be given.

2. Patient will get addicted or dependent.

Fears about psychological dependence (addiction) are often exaggerated when morphine is used to treat severe pain. Drug addicts are people who have drug seeking behavior and habits. Cancer patients requiring morphine for their pain management are less likely to become addict, as when their pain is in good control, they don't try to increase the dose. Sometimes it is required even to decrease the dosage. Addiction only occurs when people have no pain and they abuse opioid medicines.

3. Once started, it will be difficult to discontinue.

Physical dependence is a physical condition caused by chronic use of a tolerance forming drug, in which abrupt or gradual drug withdrawal causes unpleasant physical symptoms.[2] morphine doesn't cause physical dependence as long as patient has pain. Hence; it is easy to discontinue and change over to some other pain medication. E.g. Cancer patient receiving morphine for his pain control is easily weaned off the drug after giving effective nerve block.

4. Patient will remain drowsy, when on morphine.

Patients given morphine for severe pain management may remain drowsy for 2 to 3 days initially, as they get good pain relief and they catch up with the lost sleep of many days, because of pain! Once they are on correct dose and stable pain, they are not excessively sedated. Drowsiness is temporary problem and sometimes with excessive dose.[3] It disappears when patient takes medicine continuously.

5. Morphine is given p.r.n. (pro re nata) or as-needed basis.

Morphine has an elimination half-life of around 120 minutes.[4] Morphine, immediate release tablets, when given for cancer pain relief, it should be given four hourly, to avoid pain from occurring again and again. Management of chronic pain may require using medication on a regularly scheduled basis. Morphine may be scheduled "around the clock" at regular times, with a dose given in between if patient has episodes of "breakthrough pain".

6. Morphine side effects, particularly constipation is difficult to manage.

A series of studies conducted in Florida hospice evaluated opiate-induced constipation and found that 40% to 64% of hospice patients with cancer have been found to have constipation.[5]Constipation is common side effect of oral morphine, but one that can often be managed with an ounce of prevention. Advice on dietary change, 8-10 glasses of water a day, Warm or hot fluids, Increase physical activity when possible, can be helpful. A prescription of laxative and counseling for this side effect helps the patient to deal with constipation. The finding of constipation is relevant because studies have shown that it is negatively related to overall quality of life.[6]

7. Morphine is given only in terminal stage of cancer when death is imminent.

Morphine does not have this meaning today and is not used only for terminal care. The correct use of morphine improves the quality of life of the patient with pain and helps the patient maintain his/her level of self-care and independence, mental awareness and dignity. Life is enhanced because pain is reduced to a tolerable level and the patient is able to take interest in life, rest, sleep and eat.

8. Morphine causes respiratory depression, hence; should not be given in presence of breathlessness or in patients of lung metastasis.

Patients in pain also respond differently to opioids than do persons without pain.[7] Pain acts as a natural antagonist to the respiratory depressant effect of opioids. Respiratory depression does not occur if morphine is given for pain. Morphine reduces inappropriate and excessive respiratory drive and substantially reduces ventilatory response to hypoxia and hypercapnia. It reduces sensation of breathlessness and helps patients to breathe comfortable. Oral and parenteral opiates are widely accepted as providing good symptom relief, and the risk of significant respiratory depression appears to be negligible.[8]

9. Morphine causes delirium, hence; should not be given in patients with brain metastasis.

Presence of brain metastasis is not absolute contraindication for using morphine. Severe pain is also an important contributing factor for delirium and withholding opioid medications for fear of risk of delirium is clinically inappropriate, but the lowest dose consistent with pain control should be used. One should treat other causes like dehydration, infection and metabolic abnormalities before stopping morphine, which may be necessary for other distressing symptoms. Haloperidol and chlorpromazine (Thorazine) were found to be effective in treating delirium in this population.[9]

10. Only Anesthesiologists and oncologists can prescribe morphine.

Morphine dispensing, stocking & prescribing is governed by Narcotic Drugs and Psychotropic Substances Act, (NDPS) 1985. As per Amendments NDPS 2014, any hospital, hospice or other institution providing pain relief and palliative care to patients can apply for recognition as RECOGNIZED MEDICAL INSTITUTION (RMI) for allotment of MORPHINE for stocking and dispensing. No individual shall be allowed as Recognized Medical Institution. As per standard operating procedures for RMIs, a MBBS doctor with 10 days "Hands On" training in pain and palliative care can prescribe morphine.

11. Patients on morphine will need higher doses on long term use, as they develop tolerance to drug.

This is common fear of Physicians as well as Patients. In fact, 90% of patients never need more than 60mg of oral morphine every 4 hours, irrespective of duration of use. Increase in dose requirement is because of increase in pain due to disease worsening and not because of tolerance to drug. Sawe J studied pharmacokinetics of high dose of morphine, given for long term treatment. With 10 to 20 fold Increase of oral dose over a period of 6 – 8 months, it doesn't change kinetic of morphine.[10]

12. There is an upper safe limit for total oral dose of morphine in 24 hours.

The correct dose of morphine is the dose that relieves the pain without causing drowsiness. Increase the dose in steps until the pain is relieved. Use the increments of 5mg, 10mg, 20mg, 30mg, 45mg, 60mg, etc. Increase the dose every four hours, if there is still pain. 90% of patients will have relief within that 5mg to 60mg range. Occasionally, the dose may need to be 400mg or higher per day.

13. Morphine given by parental route has better effect than oral morphine.

Oral morphine is successful in more than 90% of cancer pain patients.[11] There is no difference in efficacy between oral tablets, rectal suppositories and parental injections and between controlled release and intermittent release morphine tablets.[12] Patients who cannot take morphine by mouth, can be given subcutaneous morphine infusion.

References

1. Epidemiology of Cancer Pain: Fact sheets, Global Year against Cancer Oct 2008-Oct 2009
2. https://en.wikipedia.org/wiki/Physical_dependence
3. McNicol E, Horowicz-Mehler N, Fisk RA, et al: Management of opioid side effects in cancer-related and chronic noncancer pain: a systematic review. J Pain 2003; 4:231-256
4. www.palliativecare.org.au/portals/46/resources/FactsAboutMorphine.pdf
5. Weitzner MA, Moody LN, McMillan SC: Symptom management issues in hospice care. Am J HospPalliat Care 1997; 14:190-195
6. McMillan SC, Small BJ: Symptom distress and quality of life in patients with cancer newly admitted to hospice homecare. OncolNurs Forum. 2002; 29:1421-1428
7. Hill CS: The barriers to adequate pain management with opioid analgesics. Semin Oncology 1993; 20:1-5
8. Clemens KE, Quednau I, Klaschik E: Is there a higher risk of respiratory depression in opioid-naive palliative care patients during symptomatic therapy of dyspnea with strong opioids? J Palliat Med 2008;11:204-216
9. Jackson KC, Lipman AG: Drug therapy for delirium in terminally ill patients. Cochrane Database Syst Rev 2004; 2:CD004770
10. Sawe J: High-dose morphine and methadone in cancer patients. Clinical pharmacokinetic considerations of oral treatment. Clin Pharmacokinet 1986; 11:87-106
11. Zylicz Z, Twycross RG: Oral opioids in the treatment of cancer pain. Oral opioids in the treatment of cancer pain. Neth J Med 1991; 39:108-114
12. Rom A. Stevens, MD, Ghazi SM: MD Routes of Opioid Analgesic Therapy in the Management of Cancer Pain. Cancer Control 2000;7:132-141

Solitary Breast Tumour, Leptomeningeal Carcinomatosis and Bone Metastasis: A Rare Presentation of Metastatic Ovarian Carcinoma

Mehta Dhruv P[1], Anand Asha S[2], Talele Avinash[1], Parikh Sonia[3], Shah Sandip[3], Patel Apurva[2], Panchal Harsha[2]
Resident[1], Professor[2], Associate Professor[3]
Department of Medical and Pediatric Oncology
Corresponding Author: ashaanand1757@yahoo.com

Summary

Breast metastasis from primary ovarian carcinoma is rare occurrence with only 40 cases reported in literature till date. Accurate differentiation between extra mammary metastasis and primary breast cancer is difficult without a good clinical history as majority present with a solitary nodule and lack pathognomonic pathologic features. Also, prognosis and therapies differ significantly with ovarian metastasis to breast conferring poor prognosis. Leptomeningeal carcinomatosis is extremely rare complication of ovarian cancer with sparse literature on it. We report a case of 53 year old female with stage IV ovarian carcinoma who presented initially with skeletal metastasis, and later developed breast metastasis and leptomeningeal carcinomatosis on chemotherapy.

Keywords: Leptomeningitis, Mammary tumours, Bony metastases, Ovarian adenocarcinoma, Intrathecal platin, Mammosonography

Introduction

Breast cancer is one of the most common primary malignancies in women, yet metastatic tumours to the breast account for about 2% of breast cancer cases.[1] The most common source of metastasis to the breast is a contralateral primary breast tumour. However only 0.07% of metastatic disease originate from a primary ovarian tumour. To date, a total of only 40 such cases have been reported in the English-language literature.[2] The task of distinguishing primary breast cancer and papillary serous ovarian carcinoma is challenging, yet carry important therapeutic and prognostic consequences. Leptomeningeal carcinomatosis (LMC), also known as carcinomatous leptomeningitis, is dissemination and growth of malignant cells throughout the pia mater and the arachnoid membrane. LMC occurs in only 3-8% of all cancer patients, but is associated with devastating neurologic complications with high mortality.[3] Till 1994, only 14 cases of LMC due to complication of ovarian cancer have been reported.[4] Incidence of bony metastasis from ovarian cancer is rare (0.1-0.12%)[5] and its presentation during time of diagnosis is still rarer. Herein, we report a case of ovarian carcinoma who presented with skeletal metastasis at the time of diagnosis and developed solitary breast metastasis and leptomeningeal carcinomatosis on chemotherapy.

Case Report

A 53 years old married female with no familial cancer history and an unknown BRCA-status, presented with pelvic discomfort, lack of appetite, weight loss and malaise. On examination she was found to have hard, immobile, nontender, nodular pelvic mass, probably of ovarian origin, which was confirmed by per vaginum and per speculum examination. Her routine investigations including complete blood counts, liver and renal function tests were within normal limits. CA125 level was 139.5 units/ml. Serum CEA level was 3.13 ng/ml. CT scan thorax, abdomen and pelvis revealed 9.8*cm x 10.3 cm. right ovarian mass with extension to serosal surface of sigmoid colon, peritoneal and omental metastasis, minimal ascites and multiple sclerotic lesions involving vertebrae, bilateral ribs, pelvic bones and sternum. Ultrasound guided biopsy of mass lesion revealed diagnosis of ovarian serous papillary adenocarcinoma (Figure 1). She was given radiotherapy for painful dorsal vertebral metastasis initially. She was then started on paclitaxel (175 mg/m^2) – carboplatin (AUC 5) chemotherapy every 21 days. After 5 cycles of paclitaxel – carboplatin chemotherapy, she developed a lump in her right breast. CA125 level was 62.66 units/ml. CT scan revealed right residual ovarian lesion 7.2 x 6.2 x 6.6 cm., 15 x 15 mm soft tissue mass in LIQ of right breast (Figure 2), 24 x 26 mm mildly enhancing lesion at umbilical region in anterior abdominal wall with multiple bone metastasis. Mammosonography revealed 12 x 11 mm spiculated lesion with microcalcifications causing adjacent parenchymal distortion in LIQ of right breast (BIRAD IVc) (Figure 3). Breast biopsy revealed poorly differentiated adenocarcinoma, morphologically similar to previously diagnosed ovarian carcinoma (Figure 4). Immunohistochemistry revealed positive ER status with negative PR/Her-2 neu. In view of ER positivity, she was started on tamoxifen 20 mg once a day. After 6 months of intake of tamoxifen, patient presented to us with severe headache, dizziness and vomiting. On examination, she was oriented to time, place and

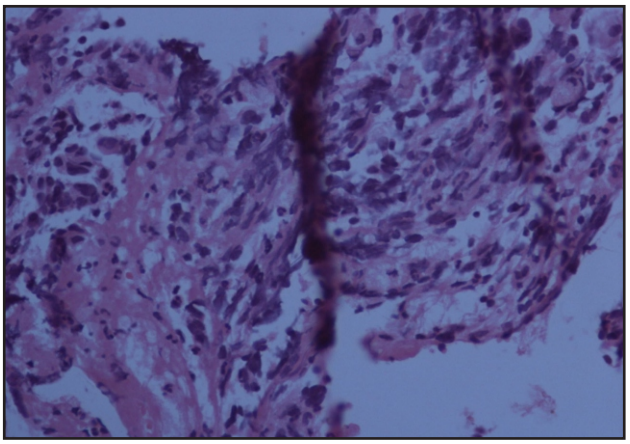


Figure 1: H & E section (40*) of pelvic mass biopsy demonstrating ovarian serous papillary adenocarcinoma.

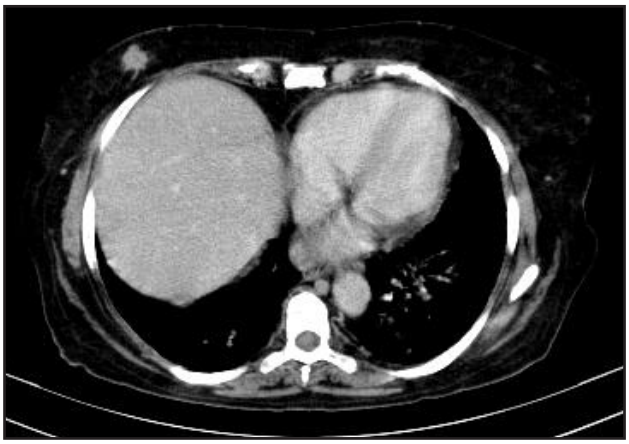


Figure 2: CT thorax showing soft tissue mass in right breast.

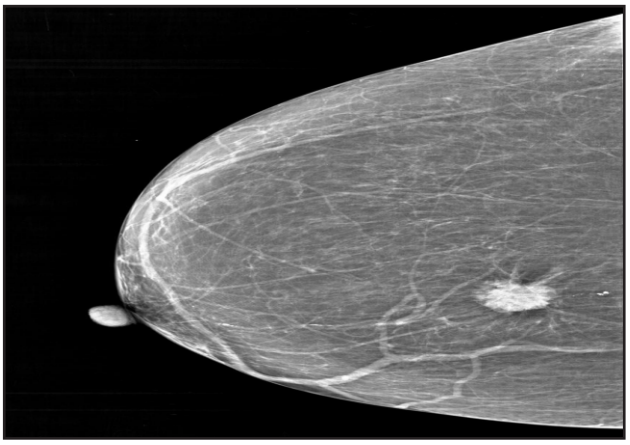


Figure 3: Mammosonographic image showing speculated lesion in LIQ of right breast.

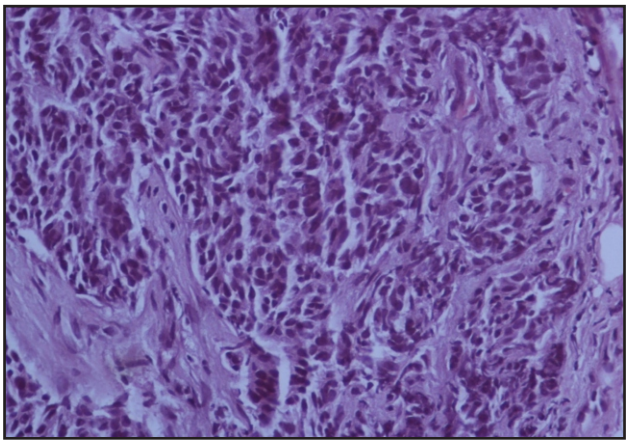


Figure 4: H &E section (40*) of breast biopsy showing adenocarcinoma morphologically similar to previously diagnosed ovarian carcinoma.

person. Her vitals were within normal range. No lymph nodes were palpable. On neurological examination, there were no focal neurological signs or abnormal reflexes. There was no neck stiffness or nystagmus. The sensory examination was normal. There was no evidence of spinal tenderness or cord compression. Fundus examination was normal with no evidence of papilledema. Rest of the systemic examination was normal. CA125 level was 60.67 units/ml. The MRI of brain showed no abnormalities. In view of persistent intractable headache with nausea and vomiting, a lumbar puncture with analysis of cerebrospinal fluid (CSF) for routine, microscopy and cytology was performed. The CSF glucose concentration was 23.4 mg/dl, protein content of 21 mg/dl, chloride concentration of 118.8 mmol/l, leukocyte count of 4 cells/mm^3 with 100% polymorphs. CSF cytological analysis was positive for metastatic adenocarcinoma. The diagnosis of leptomeningeal carcinomatosis (LMC) due to metastatic ovarian disease was made. Patient was started on supportive care, steroids and mannitol. On stabilization, patient was given biweekly intrathecal chemotherapy with methotrexate 15mg, cytarabine 70mg and hydrocortisone 50mg. However, patient's relatives refused further treatment and follow ups and they took discharge against medical advice.

Discussion

Ovarian cancer is a major cause of morbidity and mortality among gynecological malignancies. The current standard treatment of epithelial ovarian cancer of all histological subtypes involves primary optimal debulking surgery followed by platin-based chemotherapy. Significant advances in surgery and chemotherapy have been achieved over the past decades, but overall 5 year survival rate of advanced and metastatic disease remains dismal. Non-hematologic metastasis to breast are rare occurrences and they usually develop in fifth or sixth decade, with most patients having documented history of metastatic spread of their tumour.[1] Our patient was in her 5th decade with documented history of metastatic spread of ovarian cancer to peritoneum, omentum and bone. Majority of secondary tumours to breast are from contralateral breast, with lymphomas, melanomas and gastrointestinal malignancies being other causes.[1] Breast metastasis from primary ovarian

carcinoma is uncommon with liver and lung being the most common sites of distant ovarian carcinoma metastasis. The absence of hereditary breast–ovarian cancer, a history of recurrent/advanced ovarian carcinoma, and a shorter interval between ovarian carcinoma diagnosis and development of breast mass are the factors favouring metastasis to breast as was in our case.[6] In 85% of patients, the most common form of clinical presentation is a solitary tumour as was in our case. The most common location is upper outer quadrant in 62% of patients[2] while in our patient it was in lower inner quadrant. Mammographically, metastatic tumours to the breast generally lack speculation, microcalcifications, architectural distortion and other skin changes. Microcalcifications can be seen with ovarian metastasis due to presence of psammoma bodies associated with some ovarian cancers, as was in our case. Breast metastasis from a primary ovarian tumour generally is diagnosed an average of two years after the initial diagnosis of ovarian cancer.[2] However, our patient developed breast mass within 4 months of initial diagnosis. Papillary serous adenocarcinoma is the most common histologic variant of ovarian cancer associated with breast metastases[2] as in our case. Secondary breast involvement from an ovarian tumour suggests widespread dissemination and is associated with a poor prognosis, with most patients dying within 1 year (survival times range from 13 days to 3.5 years).[2]

Leptomeningeal carcinomatosis (LMC) is dissemination and growth of malignant cells throughout the pia mater and the arachnoid membrane by propagation in CSF. Meningeal involvement as presenting symptom of malignancy is rare with most patients diagnosed with LMC have prior cancer diagnosis.[7] LMC occurs in only 3-8% of all cancer patients.[3] The rate of LMC in breast cancer, lung cancer and melanoma are 12-14%, 10-26% and 17-25% respectively.[8] The various routes of spread of tumour cells to leptomeninges suggested are arterial circulation, retrograde flow in Batson's venous plexus, spread via perineural spaces, perivascular spaces, or lymphatics, and direct infiltration from bone metastases.[9] 28-75% of patients with LMC have synchronous or pre-existing brain metastasis.[10] However, our patient had no evidence of brain metastasis. The main diagnostic procedures for LMC are CSF cytological examination and neuroimaging. CSF cytological examination is required for definitive diagnosis of LMC but it is invasive procedure and its sensitivity is suboptimal. It has been reported that sensitivity of single lumbar puncture is only 54% which increases to 91% on multiple repeated taps.[11] Among neuroimaging studies, MRI is the main choice for the diagnosis of LMC as it has 1.5-2 times higher specificity and sensitivity than CT scans, however false negative MRI findings are seen in 30% of cases[12] (as was in our first case too). Meningeal contrast enhancement is not a specific finding as it may be seen in various infectious and inflammatory processes, but focal areas of linear enhancement, especially in a nodular pattern, in the appropriate clinical setting is highly suggestive of LMC.[13] Treatment strategy for LMC include intrathecal chemotherapy with methotrexate, cytarabine,thiotepa and hydrocortisone,[14] radiotherapy and best supportive care. Some reports have shown that intrathecal chemotherapy may prolong the survival compared to those on best supportive care,[15] but the median overall survival for LMC with either methotrexate or cytarabine remains dismal at 3-4 months only.[16] Epithelial ovarian cancer is biologically radiosensitive disease, and some studies support giving radiotherapy for LMC.[17]The incidence of skeletal metastasis in ovarian cancer as reported in an autopsy series was 0.06-0.19% with epithelial histology reflecting its rarity.[5] The most common metastatic sites are vertebral column and sternum, followed by the ribs, pelvic and cranialbones, with pain being reported as the most common symptom, as was in our case. Median time to development of bone metastasis is 13-49 months.[18] Median overall survival after bone manifestation is 7.2 months after diagnosis. This negative impact on overall survival is most apparent when bone spread is diagnosed at anearly time point or with primary diagnosis. Late onset of bone metastatization or the late diagnosis of bone spread, respectively, does not affect the overall prognosis of these patients.[19] Also, platinum sensitivity of the disease loses its prognostic significance on the diagnosis of bone metastases. In our case, breast metastasis occurred during ongoing platinum – based chemotherapy. Early spread of the disease to bones reflects aggressive nature of the disease, resistance to standard chemotherapy and poor prognosis.[19]

Conclusion

Extramammary tumours and primary breast tumours should be distinguished from each other as prognosis and therapies differ and to avoid any unnecessary surgical procedures. Ovarian metastasis to the breast should be treated as a systemic disease with palliative intent and with appropriate chemotherapeutic agents. Leptomeningeal carcinomatosis is rare but extremely fatal complication of ovarian cancer that commonly manifests as headache. At present, screening of bone metastases is not part of routine workup in ovarian cancer. However, in view of different prognostic impact on overall survival, early recognition with high index of suspicion is needed to ameliorate the symptoms and initiate early appropriate treatment.

References:

1. Georgiannos SN, Chin J, Goode AW, et al: Secondaryneoplasms of the breast: a survey of the 20th Century. Cancer 2001;92: 2259–2266
2. Klein RL, Brown AR, Gomez-Castro CM, et al: Ovarian cancer metastatic to the breast presentingas inflammatory breast cancer: a case report andliterature review. J Cancer 2010;1: 27-31
3. De Angelis LM: Current diagnosis and treatment of leptomeningeal metastasis. J Neurooncol 1998;38: 245-252
4. American Cancer Society, Inc. [USA]. Available from URL: https://csn.cancer.org/node/143982. Published Jul 10, 2004
5. Rose PG, Piver SM, Tsukada Y, Lau TS: Metastatic patterns in histologicvariants of ovarian cancer: An autopsy study. Cancer 1989;64: 1508-1513
6. Cormio G, Rossi C, Cazzolla A, et al: Distant metastases in ovarian carcinoma. Int J Gynecol Cancer 2003;13: 125-129
7. Olson ME, Chernik NL, Posner JB: Infiltration of the leptomeninges by systemic cancer. A clinical and pathologic study. Arch Neurol 1974;30: 122-137
8. Oh SY, Lee SJ, Lee J, et al: Gastric leptomeningeal carcinomatosis: multi-centre retrospective analysis of 54 cases. World J Gastroenterol 2009;15: 5086-5090
9. Kokkoris CP: Leptomeningeal carcinomatosis. How does cancer reach the pia arachnoid? Cancer 1983;51: 154-160
10. Groves MD: Leptomeningeal disease. Neurosurg Clin N Am 2011;22: 67-78
11. Wasserstrom WR, Glass JP, Posner JB: Diagnosis and treatment of leptomeningeal metastases from solid tumours: experience with 90 patients. Cancer 1982;49: 759-72
12. Yousem DM, Patrone PM, Grossman RI: Leptomeningeal metastases: MR evaluation. J Comput Assist Tomogr 1990;14: 255-261
13. Lisenko Y, Kumar AJ, Yao J, Ajani J, Ho L: Leptomeningeal carcinomatosis originating from gastric cancer: report of eight cases and review of the literature. Am J Clin Oncol 2003;26: 165-170
14. Groves MD: New strategies in the management of leptomeningeal metastases. Arch Neurol 2010;67: 305-312
15. Beauchesne P: Intrathecal chemotherapy for treatment of leptomeningeal dissemination of metastatic tumours. Lancet Oncol 2010;11: 871-879
16. Cole BF, Glantz MJ, Jaeckle KA, Chamberlain MC, Mackowiak JI: Quality-of-life-adjusted survival comparison of sustained-release cytosine arabinoside versus intrathecal methotrexate for treatment of solid tumour neoplastic meningitis. Cancer 2003;97: 3053-3060
17. Teckie S, Makker V, Tabar V, et al: Radiation therapy for epithelial ovarian cancer brain metastases: clinical outcomes and predictors of survival. Radiation Oncology 2013;8 :36
18. Dauplat J, Hacker NF, Nieberg RK, Berek SJ, Rose PT, Sagae S: Distant metastasis in epithelial ovarian carcinoma. Cancer 1987;60: 1561-1566
19. Sehouli J, Olschewski J, Schotters V, Fotopoulou C, Pietzner K: Prognostic role of early versus late onset of bone metastasis in patients with carcinoma of the ovary, peritoneum and fallopian tube. Annals of oncology 2013;24: 3024-3028

Anesthetic Management of a Child with Tuberous Sclerosis Posted for Ventriculo-Peritoneal Shunt – A Case Report

Jansari Amita H[1], Sanghavi Priti R[2] , Gauswami Nileshgiri N[3], Patel Bipin M[4]
Junior Lecturer[1], Professor[2], Resident[3], Professor and Head[4]
Department of Anesthesiology
Corresponding author: dramitajansari@gmail.com

Summary

Tuberous sclerosis is autosomal dominant condition that affects multiple system and causes benign tumor to grow in brain, kidneys, heart, eyes, lungs and skin. A combination of symptoms may include seizures, intellectual disability, developmental delay, behaviour abnormalities and problems in skin, abdomen, lung and kidneys. It has a classical triad of symptoms including epilepsy, skin lesions and mental retardation. An eight year old male pediatric patient was admitted in neuro-oncology department with complains of headache, drowsiness, vomiting, convulsion with Glassgow Coma Scale 8. He was diagnosed as having tuberous sclerosis with giant cell astrocytoma causing obstructive hydrocephalus. He was posted for ventriculo-peritoneal shunt to relieve obstruction. The patient had several components from tuberous sclerosis including cardiac rhabdomyoma, skin lesion, hypertension, brain astrocytoma. Providing anesthesia in such patients is very challenging.

Keywords: Tuberous Sclerosis, Anesthesia, Children

Introduction

Tuberous sclerosis is an autosomal dominant condition that affects multiple systems in the body affecting one million people worldwide. Incidence is 1:6000 children born with the disease and affects all ethnic groups and both genders have equal chances.[1,2,3] Most common features of tuberous sclerosis complex are: (1) benign tumours having abnormal lesions in many organ systems including brain, skin, heart, eyes, kidneys, lungs. (2) Seizures (3) learning disabilities, developmental and behavioural disorders, including autism spectrum disorders. This triad is present in only 30% of cases and about 6% have none of the triad.[4,5] Patient with such genetic disorders with or without congenital anomalies presents unique challenges for administering anesthesia. It is important to recognize risk features and potential perioperative complications which require unique anesthesia consideration to avoid morbidity and mortality.

Case Report

An eight years, 25 kg male patient with giant astrocytoma causing obstructive hydrocephalus was admitted in neuro-oncology department for his complaints of headache, vomiting, drowsiness since 7-8 days. He was known case of tuberous sclerosis since birth and had history of seizure since six years for which he was on antiepileptic drug. Emergency ventriculo-peritoneal shunt was planned. On preoperative examination he had hypertension, seizure disorder, and cardiac rhabdomyoma. He has difficulty in speech. On physical examination multiple whitish, watery, papular lesion present over axilla, inguinal region over scrotal skin. Baseline heart rate was 114/minute and blood pressure was 130/80 mm of Hg. Cardio-respiratory system was normal except tachycardia confirmed by ECG. All other investigations were normal except platelets count of 77000/cmm. Chest x-ray, coagulation profile, renal function test, liver function test, serum electrolytes were normal. Indirect laryngoscopy wasdone to rule out any oropharygeal and laryngeal hamartoma and papiloma. His CT scan brain showed subependymal giant cell astrocytoma, subependymal nodules, hydrocephalus suggestive of tuberous sclerosis. MRI brain showed 3.8x3.3cm area of blooming involving choroid plexus in the left lateral ventricle, suggestive of bleeding with extension into both lateral and third ventricles along with diffuse cerebral edema. 2D ECHO showed concentric hypertrophy with enlargement of left ventricle, small to moderate size solitary rhabdomyoma over anterolateral wall of left ventricle in connection with anterior to anterolateral papillary muscle. After baseline vital signs were assessed the patient was given inj glycopyrolate 0.01 mg/kg IV, inj. fentanyl 50 mcg IV. Then anaesthesia was induced with inj. thiopentone sodium 160 mg IV, inj. succinyl choline 30 mg IV then intubated with flexometallic endotracheal tube no-5. Anesthesia was maintained with O_2 40%+N_2O 60% with sevoflurane 1.5% + inj. atracurium 0.5 mg/kg IV. Before emergence, a 650 mg acetaminophane suppository was inserted. The inhaled anaesthetic was terminated and 100% oxygen was given during emergence. After reversal patient was extubated. Intraoperative patient had hypertension mean arterial blood pressure of 131mm of Hg. which was treated with inj. labetalol 0.2 mg/kg. Patient was transferred to neuro ICU and monitored. There were no reported complications during hospitalisation.

Discussion

Tuberous sclerosis is genetic condition caused by mutation in two genes TSC1 (Tuberous sclerosis complex 1) and TSC2 the genes that encode hamartin and tuberlin. In about 80% of people who have tuberous sclerosis genetic testing can detect mutation in one of these genes.[6,7] Tuberous sclerosis is one of the several conditions that are called neurocutaneous syndrome; all these disorders are genetic conditions that cause tumours and other types of abnormal tissue to grow in the brain, skin and other organs.[5]

In our case we found the presence of 3 major features and hence the definite diagnosis of tuberous sclerosis.

Perioperative management of patient with tuberous sclerosis is complicated by presence of cardiovascular, neurological and renal tumours. Tuberous sclerosis affects many systems and the anaesthesiologist must be ready to meet these challenges. Careful evaluation of each systems and the extent and severity of the disease process and assessment of individual is essential for safe anaesthesia plan for patient having tuberous sclerosis. Preoperative chest x-ray is indicated to exclude pulmonary and mediastinal nodules.[6] These patients may present for many surgical procedure due to widespread hamartoma and abnormal growth of many normal tissue.

Cardiovascular manifestation can have major anaesthesia implications. Cardiac rhabdomyoma are usually asymptomatic but should be suspected in patients with arrhythmia. Up to 50-60% of patients with tuberous sclerosis have cardiac disease, mainly rhabdomyoma which may be single or multiple and may occur in any chamber. These leads to mechanical problems because of their size or disturbance in the conduction system caused by their infiltrating nature. Cardiac arrhythmia is a significant problem in tuberous sclerosis complex (slow to irregular to fast rhythm).[3,6,8] Hypertension can occur due to renal tumour or renal artery stenosis, and cardiac anomaly.[9,10] Cardiovascular assessment is essential part of preoperative work up even in asymptomatic patients.

Neurological problems include epilepsy, focal lesions, and communication problems. Neurological lesions include single or multiple cerebral nodules, astrocytomas and multiple subependymal periventricular nodules. These nodules cause obstructive hydrocephalus. Astrocytomas cause focal neurological deficit, increase intracranial pressure, behavioural changes.[11] In 60% cases mental retardation is observed.[5] Eighty to Ninety percent has seizure and seizure activity is difficult to control. Epilepsy is common in 80-90% [10,12,13] of patients and these patients are usually on antiepileptic drugs and should be continued in preoperative period so possible drug interaction should be kept in mind before anesthetising the patients. Hypoglycaemia, hypocarbia and hyponaetremia should be avoided.

Premedication is helpful because many patients with tuberous sclerosis have behaviour problems.[5,16] Our patient was premedicated with glycopyrrolate as it has less chronotropic effect than atropine and short acting opioid inj. fentanyl which has sedative, analgesic, cardiostable property and is easily metabolised by liver.[3,15,16] An oral midazolam 0.5mg/kg gives amnesia and anxiolysis within 15 min.[17] As our patient drowsy we have not used was anxiolysis. Barbiturates causes seizure suppression and decreased cerebral metabolism of oxygen.[17] We have used short acting depolarising muscle relaxant succinyl choline for intubation because hamartoma and papilloma may present in larynx and oropharynx and may obscure vision and may lead to difficult intubation and bleeding.[4,5] Although succinyl choline can produce increase intracranial pressure, it need not be viewed as contraindicated. Kovaric and co-authors observed no change in intracranial pressure after the administration of succinyl choline 1mg/kg to ten non paralyzed ventilated neurosurgical ICU patients, six

Clinical Diagnostic criteria:

Major features

1. Hypomelanotic macules (≥3, at least 5-mm diameter),
2. Angiofibromas (≥3) or fibrous cephalic plaque,
3. Ungual fibromas (≥2),
4. Shagreen patch,
5. Multiple retinal hamartomas
6. Cortical dysplasias,
7. Subependymal nodules,
8. Subependymal giant cell astrocytoma,
9. Cardiac rhabdomyoma,
10. Lymphangioleiomyomatosis
11. Angiomyolipomas (≥2)

Minor features

1. "Confetti" skin lesions,
2. Dental enamel pits (>3),
3. Intraoral fibromas (≥2),
4. Retinal achromic patch,
5. Multiple renal cysts,
6. Nonrenal hamartomas

Definite diagnosis: 2 major features or 1 major feature with ≥2 minor features. Possible diagnosis: either 1 major feature or ≥2 minor features.[14,15]

of whom had sustained head injury.[18] As with many anesthetics, the concern should be not weather it is used but how it is used. Ketamin should be avoided because it decreases seizure threshold and increases intracranial pressure.[17] If cardiac pathology is significant etomidate 0.3 mg/kg is a drug of choice.[3,16,17] Anesthesia can be maintained with sevoflurane, desflurane, or isoflurane.[16] Renal and hepatic function of patients can affect the selection of neuromuscular blocking drug. Atracurium and mivacurium is safest choice due to low percentage of renal clearance.[16]

Post operatively patients of tuberous sclerosis should be monitored closely for seizure activity. High level of carbon dioxide levels may increase the potential for seizure and arrythemia. Adequate pain relief and control of tachypnea and preservation of a normal respiratory rate decreases risk of seizure. So thorough assessment of the patient is important for safe anesthesia.[17]

Conclusion

Thorough preoperative assessment, careful perioperative management and close monitoring be required in a patient with tuberous sclerosis for safe anaesthetic outcome.

References:

1. Weiner DM, Ewalt DH, Roach ES, Hensle TW: The tuberous sclerosis complex: a comprehensive review. J Am Coll Surg 1998;187:548-561
2. Dinno ND: Griffith's 5-minute clinical consult.10th ed. Philadelphia, Pa: lippincott williams and Wilkins; 2002:1124-1125
3. Steve Septer, Edward S: Thompson, Ann willemsen-Dunlap. Anesthesia concerns for children with tuberous sclerosis. AANA Journal 2006;74:219-225
4. Lee JJ, Imrie M, Taylor V: Anaesthesia and tuberous sclerosis. Br J Anaesth 1994; 73:421-425
5. Bosenberg A: Tuberous sclerosis Anaesthetic considerations. Southern African Journal of Anaesthesia and Analgesia 2003;4-6
6. Slegtenhorst V, Hoogt D, Hermans C, et al: Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. Science. 1997;277:805–808
7. Whittemore VH: Unlocking a Cure for Tuberous Sclerosis Complex: An Assessment of Scientific Progress and Research Needs. Tuberous Sclerosis Alliance. 2011:1-8
8. Smith HC, Watson GH, Patel RG, et al: Cardiac rhabdomyomata in tuberous sclerosis: their course and diagnostic value. Arch Dis Child 1989; 64: 196-200
9. Schweiger JW, Schwartz RE, Stayer SA: The anaesthetic management of the patient with tuberous sclerosis complex. Pediatric Aneshesia. 1994;4:339-342
10. Hussain A,Al-Abri S: Taberous sclerosis complex: Sedation-Anaesthetic Considrations. The internet Journal of Anesthesiology 2007;16:1-6
11. Bryson E ,Neustein S: Tuberous Sclerosis: A Case Report and Review: The Internet Journal of Anesthesiology 2007;13:1-4
12. Sparagana SP, Delgado MR, Batchelor LL, Roach ES: Seizure remission and antiepileptic drug discontinuation in children with tuberous sclerosis complex. Arch Neurol 2003;60:1286-1289
13. Karenfort M, Kruse B, Freitag H, Pannek H, Tuxhorn I: Epilepsy surgery outcome in children with focal focal epilepsy due to tuberous sclerosis complex. Neuropediatrics 2002;33:255-261
14. Hinton R, Prakash A, Romp L, Krueger D, Knilans T: Cardiovascular Manifestations of Tuberous Sclerosism Complex and Summary of the Revised Diagnostic Criteria and Surveillance and Management Recommendations From the International Tuberous Sclerosis Consensus Group. Journal of the American Heart Association 2015:1-12
15. Northrup H, Krueger DA: International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 International Tuberous Sclerosis Consensus Conference. Pediatr Neurol 2013;49:243–254
16. Cote CJ, Todres ID, Ryan JF, Goudsouzian NG. A Practice of Anaesthesia for Infants and Children. 3rd ed. Philadelphia, Pa: WB Saunders; 2001:45, 184-185,573
17. Diaz JH: Perioperative management of children with congenital phakomatoses. Peditr Anesth 2000;10:121-128
18. Kovaric WD, Mayberg TS, Lam AM, et al : Succinyl cholin does not change intracranial pressure, cerebral blood flow, velocity, or the electroencephalogram in patients with neurologic injury. Anesth Analg 1994;78:469-473

Vertebral Metastasis a Rare Phenomenon in Gall Bladder Carcinoma - A Case Report

Kalaskar Preetam B[1], Anand Asha S[2], Panchal Harsha P[3], Patel Apurva A[3], Shah Sandip S[4], Parikh Sonia S[4], Patel Alpesh[1], Talele Avinash[1], Joshi Kshitij[1], Mekha Mangesh[1], Madhabhavi Irrapa[1], Kukkar Sandip[1], Makadia Gautam[1], Choudhary Mukesh[1], Agare Suhas[1]
DM Resident[1], Professor and Head[2], Professor[3], Associate Professor[4]
Department of Medical Oncology
Corresponding Author: ashaanand1757@yahoo.com

Summary

Carcinoma gall bladder is a highly fatal malignancy in metastatic setting. An uncommon entity in western world is a frequent entity in northern parts of India. Liver is the most common site of gallbladder cancer metastasis and skeletal metastasis being extremely rare. Only few cases have been reported in literature. We are reporting a case of carcinoma gall bladder with vertebral metastasis, to emphasise the importance of evaluating symptomatic bony sites.

Keywords: Carcinoma gall bladder, Vertebral metastasis, Northern parts of India

Introduction

Carcinoma gall bladder an uncommon entity in western world, is a frequent entity in northern parts of India[1] mostly presenting as advance disease. Most common site of metastasis being liver and skeletal metastasis is extremely rare. Only few cases have being reported in literature. We are reporting a case of carcinoma gall bladder with vertebral metastasis.

Case Report

Fifty-two years old female without any comorbidities with a performance scale of 2, presented to our institute with chief complaints of abdominal discomfort and weakness of bilateral lower limb for last 20 days. On examination abdomen was soft with tenderness in right hypochondriac region, power in bilateral lower limbs was 3/5. Complete blood count, renal function test, liver function test were within normal limits. Ultrasonography of the abdomen showed hypoechoeic lesions in liver, one of which in the gall bladder fossa was not distinguishable from liver parenchyma and this lesion was not biopsible. Tumour markers: CA 19-9 was slightly elevated wherears CA-125, CA 15-3, and CEA were within normal range. MRI spine and abdomen was suggestive of multiple dorso lumbar metastases and bilateral liver metastasis, one of which was in gall bladder fossa (Figure 1,2). Evaluation for primary was done including colonoscopy, bilateral mammography; per speculum examination with Pap smear. None of them showed any evidence of disease. An USG guided biopsy from liver was reported as malignant poorly differentiated carcinoma, further IHC was advised which suggested as primary from gall bladder, which was CK7+, AE+, CK20-ve. (breast, ovary, colon cancers were ruled out using specific IHC markers for these sites). Biopsy from spinal lesion suggested metastatic carcinoma poorly differentiated. Patient was initially treated with palliative RT to spine, followed by one cycle of gemcitabine plus oxaliplatin with zolendronic acid. However patient's performance scale worsened to 4

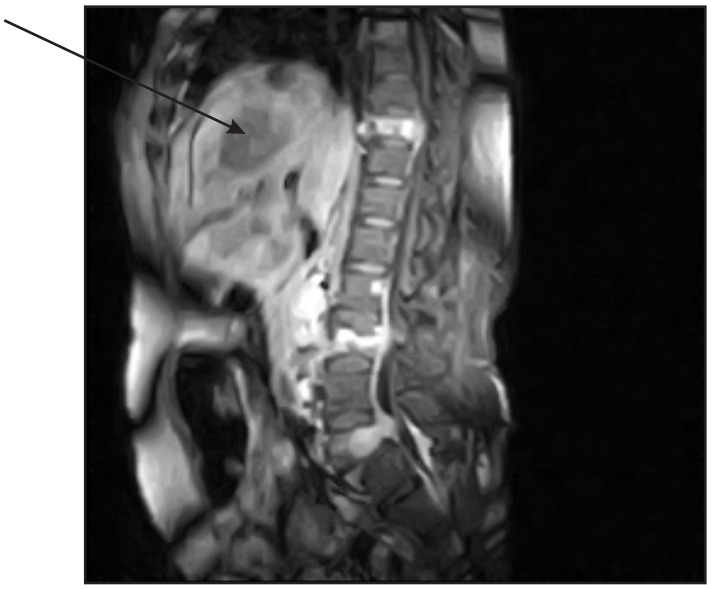


Figure 1: MRI showing Liver metastasis

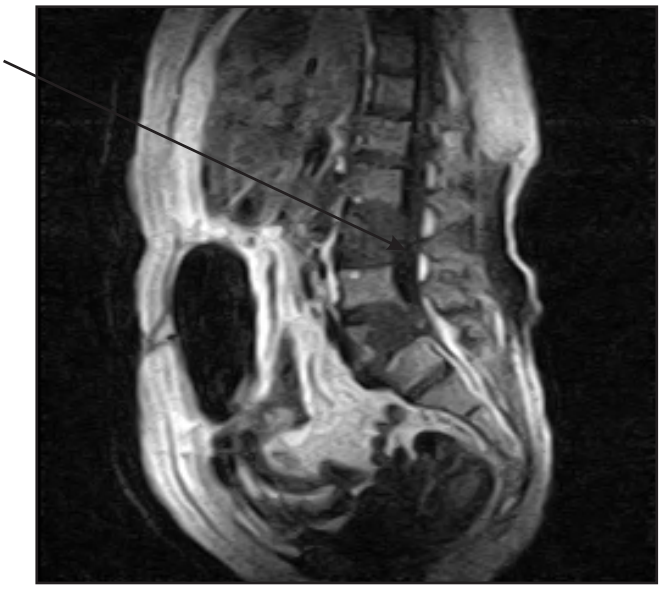


Figure 2: Vertebral metastasis

and later was treated with supportive care.

Discussion

Carcinoma gall bladder is a very fatal malignancy.[3] Most common histology is adenocarcinoma; other being adenosquamous, squamous, small cell carcinoma, sarcoma, lymphoma.[4] Presentation is either as incidental detection post cholycystectomy, on imaging or as advanced disease. Liver is the most common site of gallbladder cancer metastasis[5] and skeletal metastasis are rare.[6] There are only 5 prior reports of gallbladder cancer metastasis to the bone in the English language literature.[7] The rarer presentation in this report is skeletal metastasis at diagnosis. In our case the presenting complaints of bilateral lower limb weakness led to an MRI imaging, however patients may present with non specific symptoms related to back which should not be ignored. Morbidity related to bony disease can be reduced by early detection. We are reporting this case to emphasise the importance of screening skeleton in carcinoma gall bladder with pain in bony sites or neurological symptoms due to bony disease.

Conclusions

Carcinoma gall bladder rarely metastasizes to bone. It should be considered in differential diagnosis of occult primary metastasis to bone and one should evaluate symptomatic bony sites at the earliest in patients of carcinoma gall bladder.

References:

1. Kim WS, Jang K-T, Choi DW, et al: Clinicopathologic analysis of adenosquamous/squamous cell carcinoma of the gallbladder. J Surg Oncol 2010;103:239-242
2. Serra I, Calvo A, Báez S, Yamamoto M, Endoh K, Aranda W: Risk factors of gallbladder cancer: An international collaborative case–contol study. Cancer 1996;78:1515-1516
3. Lai CE, Lau W: Gallbladder cancer- A comprehensive review. The Surgeon. 2008;6:101-110
4. Kim WS, Jang K-T, Choi DW, Choi SH, Heo JS, You DD, et al: Clinicopathologic analysis of adenosquamous/squamous cell carcinoma of the gallbladder. J Surg Oncol 2010;103:239-242
5. Pandey M, Shukla VK. Diet and gallbladder cancer: a case–control stud European Journal of Cancer Prevention 2002;11:365-368
6. Prakash M, Aiyappan SK, Kumar A, Sreenivasan R, Yadav T, Khandelwal N: Solitary skeletal metastasis in carcinoma gallbladder: two case reports. Cancer Imaging. 2010;10:121-123
7. Youssef F, Khan A, Davidson B: Disseminated bony metastases following incidental gallbladder cancer detected after laparoscopic cholecystectomy. HPB. 2003;5:258-260

Monoblastic Sarcoma / Myeloid Sarcoma of Paraspinal Region Presenting as Acute Paraparesis in Aleukemic Patient- A Rare Case Report from Western India

Kikani Alpeshkumar[1], Modi Mitul[1*], Panchal Harsha[2], Trivedi Priti[2*], Lakum Nirmal[1#], Parikh Sonia[2], Makadia Gautam[1:]
Resident[1], Professor[2]
Department of Medical and Pediatric Oncology
*Department of Pathology
#Department of Radiology

Summary

Myeloid sarcoma (MS), also known as extramedullary acute myeloid leukemia, extramedullary myeloid tumor, and granulocytic sarcoma, myeloblastoma or chloroma, is a rare manifestation which is characterized by the occurrence of 1 or more tumor myeloid masses occurring at an extramedullary site. We are reporting a rare case of monoblastic sarcoma in a 50 year male patient without history of acute myeloid leukemia with involvement of paraspinal region, abdominal wall and tongue, who presented with acute paraparesis. The patient was treated with decompression laminectomy followed by Radiotherapy. After confirming diagnosis of monoblastic sarcoma, patient was treated with acute myeloid leukemia chemotherapy regimen to control systemic disease, but outcome was poor.

Keywords: Myeloid sarcoma, Monoblastic sarcoma ,Granulocytic sarcoma , Myeloblastoma, Compressive myelopathy

Introduction

Myeloid sarcoma (MS) is a rare malignant condition comprising of immature myeloid cells and developing at an extramedullary site that most commonly involves the bone, skin, or lymph node, although any part of the body may be affected.[1] MS may develop de novo or simultaneously with acute myeloid leukemia (AML), myeloproliferative neoplasm (MPN), or myelodysplastic syndrome (MDS). MS may be the first manifestation of AML, precede it by months or years, or equally represent the initial manifestation of relapse in a previously treated AML in remission.[2,3] The incidence of myeloid sarcoma in the course of acute myeloid leukaemia has been reported to range from 3-4.7%. The occurrence of extra-medullary lesions before the onset of overt disease is rare.[4] Here we are reporting a case of monoblastic sarcoma in a patient without overt myeloid leukemia.

Case Report

A 50 year male presented with gradual onset of both lower limb weakness over two month which progressed to complete paralysis of both lower limb with urinary incontinence. At the same time patient had noted lump in right iliac region. Initially patient consulted local physician who evaluated patient with USG pelvis and MRI dorso-lumbar spine with whole spine screening. Ultrasonography of pelvis suggested large well defined hypoechoic lesion measuring 45 mm×30 mm in subcutaneous tissue and musculature in anterior abdominal wall, possibility of metastatic deposit , biopsy of which was suggestive of malignant round cell tumor. MRI of spine revealed altered signal intensity lesion in intraduralextramedullary compartment extending from D3 to D7 level in post contrast T1 weighted SAG images (Figure 1) and in T2 weighted images showed altered signal intensity (hyperintense) lesion in intraduralextramedullary compartment at D3 level (Figure 2). There were multiple other similarly enhancing lesions seen along visualized posterior aspects of ribs and posterior elements of the other distal dorsal vertebrae and adjacent soft tissue, possibility of metastases. On presentation at GCRI, patient had complete paraplegia with bladder dysfunction. On physical examination patient was having macroglossia, right iliac lump and paraspinal mass, bilateral complete paraplegia (power grade 0) with bowel bladder involvement. Patient was posted for decompression D6/D7 laminectomy.

Surgical pathology report was suggestive of Monoblastic sarcomas (MBLS) composed of a large population (> 80%) of monoblasts. The neoplastic cells were large, with abundant eosinophilic cytoplasm, round or oval nuclei with dispersed chromatin and one or more prominent nucleoli. Promonocytes showed more irregular, delicately convoluted nuclear features. Immunohistochemistry was done to confirm the diagnosis which was positive for LCA, CD43 (strong positive), CD 117, vimentin, Bcl2, CD79a (weak positive) and negative for CD2, CD3, CD10, CD20, CD19, CD21, Bcl6, CD1a, S100, EMA, CD23, CD30, CD13. CD 99 was diffuse stained. MIB 1 was 80%. From this IHC panels diagnosis of monoblastic sarcoma/ myeloid sarcoma was confirmed. There were no blasts in peripheral smear and bone marrow aspiration and biopsy was done which was normocellular and uninvolved by any primary or secondary malignancy. Patient was treated with post operative radiotherapy to spine D6/D7

region with 30G in 10 fractions. Radiotherapy was not given to tongue and chest wall because they were asymptomatic. Patient was referred to medical oncology department for further management. Patient was advised PET CT which was suggestive of non avid soft tissue density lesion in costal pleura, few peritoneal nodules and subcutaneous nodules anterior to urinary bladder. There was no FDG avid disease anywhere in body. Biopsy of the tongue was done which revealed same pathology as in paraspinal mass. As such myeloid sarcoma is chemotherapy sensitive disease so patient was treated with chemotherapy. Patient was started for 5+2 induction with daunorubicin and cytarabine but expired due to sudden cardiac arrest after one day of chemotherapy, the cause of cardiac arrest was not found.

Discussion

Myeloid sarcomas were first described in the early 19th century. They were initially described as "chloroma" due to their greenish external appearance. This is a because of the presence of myeloperoxidase enzymes in the premature myeloid cells. Later on many cases of chloroma were described that were not green and had the gross features of a sarcoma, so the terminology was changed to " granulocytic sarcoma". As we now know that not all myeloid leukemias are derived from granulocytes , the preferred term is "myeloid sarcoma". The clinical presentation of myeloid sarcoma varies and is dependent on the site of involvement. Commonly involved sites of occurrence include subperiosteal bone structures of the skull, paranasal sinuses, sternum, ribs, vertebrae and pelvis; lymph nodes and skin are also common sites.[4] Rare sites reported in the literature include the pancreas, heart, brain, mouth, breast, gastrointestinal and biliary tract, prostate, urinary bladder and gynecologic tract and more.[6] A single tumor or sometimes multiple nodular masses of various sizes may occur. Myeloid sarcomas may be found in one of four settings: 1. In patients with known acute myeloid leukemia (AML) in the active phase of the disease. 2. In patients with a chronic myeloproliferative disorder (CMPD) or a myelodysplastic syndrome (MDS), in whom myeloid sarcoma may be the first manifestation of blastic transformation. 3. As the first manifestation of relapse in patients previously treated for primary or secondary acute leukemia. 4. De novo in healthy subjects, in whom a typical form of AML may occur after an interval of weeks, months or even years.[4,7] Rarely no leukemia develops. No age group is immune; however, some reports suggest that two thirds of the cases occur before the age of 15 years. Grossly the neoplastic tissue usually appears firm with a fish-flesh appearance. FDG PETCT is negative in 10 % cases in myeloid sarcoma.

Microscopically monoblastic sarcomas are composed of a large population (>80%) of monoblasts. The neoplastic cells are large, with abundant eosinophilic cytoplasm, round or oval nuclei with dispersed chromatin and one or more prominent nucleoli. Promonocytes show more irregular, delicately convoluted nuclear features. On immunohistochemistry , Monoblastic sarcomas are strongly positive for CD43, lysozyme, CD68, CD163, weakly for CD4, and negative for CD34.[5]

The WHO Classification of tumours; pathology and genetics of tumours of hematopoietic and lymphoid tissues[6] recognizes monoblastic sarcomas as less common variants that is composed of monoblasts and associated with acute monoblastic leukemia and also tumors with bilineage or trilineage hematopoiesis, predominant erythroid precursors or predominant megakaryocytes that may occur in conjunction with transformation of a CMPD, but isolated MS without overt leukemia is very rare.[6]

Giemsa or Wright/Giemsa stains on imprints are the best way to see the morphology of the blasts. Cytochemical stains such as a positive sudan black or myeloperoxidase stain are helpful if touch imprints are available to identify the myeloid lineage. The definitive diagnosis today is usually based on immunohistochemistry. [5,9] The best immunohistochemical stains used for this include MPO and lysozyme. MPO immunostain is positive in most myeloblastic variants (as well as in some cells myelomonocytic variants) while lysozyme is frequently expressed in monoblastic variants. Megakaryoblastic cells are characterized by the expression of factor VIII, CD 61, and CD 31[8] while Glycophorin C and/or blood group proteins occur in the rare erythroblastic variant. A variable percentage of non-differentiated blasts may be positive for CD13, CD33, CD 34, CD117 (cKit), or CD99.[8] Sometimes expression of aberrant markers such as B-cell-, T-cell-, or NK-associated antigens including CD30 may be seen. Positivity of tumor cells for CD43, a T-cell marker, without coexpression of CD3 should always prompt consideration of a myeloid tumor and not be misinterpreted as a neoplasm of T-cell lineage. The use of only four antibodies (MPO, CD68, Lysozyme and CD34) has been proposed to distinguish the more common variants of myeloid sarcomas.[9] A study of 30 cases showed CD117 reactivity in 87%, MPO, 97%; lysozyme, 93%; CD34, 47%; CD45, 84%; CD43, 97%; TdT, 37%; CD79a, 20%; CD20, 10%; CD3, 10%; and CD10, 1% .[10]

The correct diagnosis of myeloid sarcoma is important so appropriate therapy can be instituted. While the diagnosis is often thought of in patients with an established history of AML, MDS or a CMPD, in other patients the diagnosis is often missed. The differential diagnosis is lengthy and includes non-Hodgkin lymphoma (including precursor B- or Tcell, Burkitt, some peripheral NK/T-cell and diffuse large B-cell lymphomas), small round cell tumors (including neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma, peripheral neuroectodermal tumor and medulloblastoma), undifferentiated carcinoma or melanoma, malignant histiocytosis and malignant mastocytosis with atypical mast cells. Extramedullary localizations of chronic myeloproliferative diseases without blast crisis should also be differentiated from myeloid sarcoma. Immunohistochemistry may aid distinguish myeloid sarcoma from malignant lymphoma, however the coexpression of some T-cell markers and staining with TdT and CD 34 can cause difficulties in interpretation. Treatment is similar to that for AML, even in cases of isolated tumors with no blood or bone marrow involvement. [11] Radiotherapy has been proposed in association with chemotherapy for patients with massive tumors or for patients with spinal cord compression.

In patients with AML the progression of myeloid sarcoma has the same prognosis as the underlying leukemia. Patients with an AML associated with a t(8;21) and presenting myeloid sarcoma have a low rate of complete remission, and overall survival is poor.[12] This appears to be in contrast to the better prognosis generally seen in AML with t(8; 21). In patients with CMPD and MDS myeloid sarcoma defines a blastic transformation often associated with a short survival.

References:

1. Pileri SA, Ascani S, Cox MC, et al: Myeloid sarcoma: clinico-pathologic, phenotypic and cytogenetic analysis of 92 adult patients. Leukemia. 2007;21:340-350
2. Hancock JC, Prchal JT, Bennett JM, et al: Trilineageextramedullary myeloid cell tumor in myelodysplastic syndrome. Arch Pathol Lab Med. 1997;121:520-523
3. Imamura T, Matsuo S, Yoshihara T, et al: Granulocytic sarcoma presenting with severe adenopathy (cervical lymph nodes, tonsils, and adenoids) in a child with juvenile myelomonocytic leukemia and successful treatment with allogeneic bone marrow transplantation. Int J Hematol. 2004;80:186-189
4. Breccia M, Mandelli F, Petti MC, et al: Clinico-pathological characteristics of myeloid sarcoma at diagnosis and during follow-up: report of 12 cases from a single institution. Leuk Res 2004;28:116-1159
5. Borislav A, Wenle W, Yi Ning, et al: Myeloid sarcomas: a histologic, immunohistochemical, and cytogenetic study.Diagnostic Pathology 2007, 2:42 doi:10.1186/1746-1596-2-42
6. Running R.D, Bennett J, Matutes E, et al: Acute myeloid leukemia not otherwise categorised In: Jaffe E.S, Harris N.L, Stein H, and Vardiman J.W (eds). WHO Classification of Tumours; Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon 2001:pages 104-105
7. Audouin J, Comperat E, Le Tourneau A, Camilleri-Broet S, Adida C, Molina T, Diebold J: Myeloid sarcoma: clinical and morphologic criteria useful for diagnosis. Int J SurgPathol. 2003; 1:1 271-282
8. Zhang PJ, Barcos M, Stewart CC, Block AW, Sait S, Brooks JJ: Immunoreactivity of MIC2 (CD99) in acute myelogenous leukemia and related disease. Mod Pathol 2000; 13:452-458.
9. Chang CC, Eshoa C, Kampalath B, Shidham VB, Perkins S: Immunophenotypic profile of myeloid cells in granulocytic sarcoma by immunohistochemistry. Correlation with blast differentiation in bone marrow. Am J Clin Pathol 2000; 114:807-811
10. Chen J, Yanuck R, Abbondanzo S et al: c-Kit (CD117) Reactivity in Extramedullary Myeloid Tumor/Granulocytic Sarcoma. Archives of Pathology and Laboratory Medicine 2001; 125; 1448-1452
11. Byrd JC, Edenfield WJ, Shields DJ, Dawson NA: Extramedullary myeloid cell tumors in acute nonlymphocytic leukemia: a clinical review. J Clin Oncol. 1995; 13:1800-1816
12. Byrd JC, Weiss RB, Arthur DC, Lawrence D, Baer MR, Davey F, Trikha ES, Carroll AJ, Tantravahi R, Qumsiyeh M, Patil SR, Moore JO, Mayer RJ, Schiffer CA, Bloomfield CD: Extramedullary leukemia adversely affects hematologic complete remission rate and overall survival in patients with t(8;21)(q22;q22): Results from Cancer and Leukemia Group B 8461. J Clin Oncol 1997; 15:466-475

Summaries of Presentations at Clinical Meetings

01. Early metabolic response using 18f–18-f-fdg-pet can prognosticate better than conventional ct imaging in paediatric patients of Burkitt's Lymphoma

Patel Vishal
Nuclear Medicine

Summary

Burkitt's lymphoma is a rare and aggressive form of non-Hodgkin's lymphoma which is potentially curable if diagnosed and treated at an early stage. 18-F-FDG PET/CT has already been established as a better diagnostic modality than conventional imaging for staging, restaging and early detection of recurrence in various lymphomas. Even though a negative PET after completion of chemotherapy has an excellent prognostic value, it is not known whether PET scans done after 1 week of completion of first cycle of chemotherapy has any prognostic impact on therapeutic outcome. The aim of our study was to ascertain the value of early 18-F-FDG PET scan as compared to CT imaging in predicting therapeutic response in paediatric patients of Burkitt's lymphoma.

This was a prospective study involving 20 patients of paediatric age group. 18 males and 2 females (mean age 6.3 ± 2.73 years) with Burkitt's lymphoma who underwent a baseline(pre chemotherapy) whole body PET/CT evaluation followed by 18-F-FDG PET/CT scans at 8th day post therapy, interim and after completion of chemotherapy. Baseline scan, 8th day post chemotherapy scan and interim scan were performed in all the patients except 3 patients who died or lost to follow up during the course of the treatment. 5 patients were subjected to follow up scan assessment after completion of chemotherapy. Δ SUVmax and Δ Metabolic Index max were used as parameters for assessment of response evaluation on PET/CT scan and Δ Volume was used for response evaluation on CT scan.

As compared to the baseline, 8th day post treatment 18-F-FDG PET showed complete response(CR) in 3 of 20 patients, partial remission(PR) in 14 of 20 patients and progressive disease(PD) in 3 of 20 patients. In PR category, 9 of 14 patients showed CR while 1 of 14 patients showed stable disease (SD) and 4 showed PD on subsequent follow up scan. 2 of 3 patients who showed PD on early follow up scan remained in progression while 1 patient showed CR on subsequent follow up scan. Another pertinent observation is that metabolic response preceded morphological response in CR group. 2 of 3 patients showed persistent structural disease on CT scan who showed CR on early 18-F-FDG PET/CT scan. Δ Metabolic Index max was found out to be a better predictor of therapeutic response as compared to Δ SUVmax and Δ Volume. It also correlated with patients' final outcome (Bonferroni multiple comparison test- $P<0.001$).

18-F-FDG PET/CT can predict treatment response as early as on 8th day after chemotherapy. Metabolic response precedes morphologic recovery as compared to CT. Early responders remain disease free even at completion of chemotherapy. Quantitatively Δ Metabolic index max is the best predictor for patient's final therapeutic outcome.

02. Retrospective analysis of pt1/2N0 oral tongue carcinoma patients treated at GCRI

Gupta Sunnia
Radiotherapy

Summary

To study the outcomes of adjuvant radiotherapy in the form of local control, nodal control, DFS. The secondary aim was to identify the potential prognostic factors affecting the outcomes. This was retrospective analysis of all pT1-2N0 oral tongue cancer patients who were treated at GCRI from 2007 to 2011. All the patients had undergone surgery in the form of hemiglossectomy with neck dissection and were either treated with adjuvant radiotherapy or were kept on observation. The outcomes of adjuvant radiotherapy were analysed. 22 out of 34 (64%) patients kept on observation developed recurrence (10 local, 10 nodal and 2 at other sites like soft palate, angle of mouth). 33 out of 120 (27%) patients developed recurrence post RT (21 local, 9 nodal and 3 at other sites like PFS, RMT). Out of these 33 patients, 5 had received incomplete RT. Out of these 28 patients, 7 had DFS of 5yrs. No patient developed distant metastasis. Adjuvant radiotherapy improves disease free survival in pT1/2N0 oral tongue carcinoma patients.

03. Neo-adjuvant treatment in locally advanced vulvar cancer

Kumari Rashmi
Gynaecological Oncology

Summary

Present study aimed to evaluate tumor response, any significant adverse effect and ultimately disease resectibility after neo-adjuvant treatment in locally advanced vulvar cancer. It also reviewed the present status of the patient with respect to recurrence and disease free survival. Present study is a retrospective observational study. Ten patients were

assessed between January 2008 and December 2015 at the Department of Gynecologic Oncology, Gujarat Cancer and Research Institute, Ahmedabad. Participation in this study was limited to patients with clinical definition of locoregionally advanced vulvar cancer (LRAVC), involving midline structures (urethra, anorectum, upper half vagina) and would necessitate radical surgeries for adequate surgical cure. Between January 2008 and December 2015, 10 patients with locally advanced vulvar cancer were treated with neo-adjuvant treatment. The patients' median age was 59.5 years (range 40-72 years). Seven women had clinical FIGO stage II disease and 3 had stage IVA tumors. Biopsy report of two cases were of grade 1, 7 of grade 2, and 1 of grade 3. Four patients were clinically suspected for inguinal node metastasis, and 3 were subsequently subjected to histological evaluation, but all were negative for malignancy. One patient had large fixed node, unilaterally, which further developed fungating node, during the course of treatment. Six patients had disease with urethal involvement, with 1 having vaginal involvement of lower half as well and 4 had disease abutting anal margin. Different neo-adjuvant therapies were used for all patients, as data are sparse in this field to guide definitive neoadjuvant therapy. Three patients received only chemotherapy, 1 only radiotherapy, 3 received concurent chemoradiation, 1 had radiotherapy followed by chemotherapy, 1 had vice versa and 1 received chemotherapy followed by radiotherapy followed by chemotherapy. All patients had clinical objective response (3 CR and 7 PR). Out of ten patients, 7 had radical vulvectomy with bilateral inguino-femoral lymphadenectomy and 3 had only radical vulvectomy. Groin dissection was not performed in these 3 cases, because the inguinal nodes have already been treated during the chemoradiation phase. Stoma/urinary diversion was averted in all cases. Pathological responses included 3 cases with no residual tumor and 7 cases with residual sqaumous cell carcinoma. All patients had negative resection margins, adequate resection margins (≥ 8mm) was achieved in only 4 cases. Two patients received adjuvant post operative treatment, with radiation therapy, due to close resection margins. Only one patient had positive nodes in surgical specimen. Two out of ten patients had recurrence. One had local recurrence, three months after the completion of treatment, for which radical local excision was done. Another patient had systemic recurrence, to lungs, 3 months after the completion of treatment, for which palliative chemotherapy was given, but patient died after 6 months of recurrence. Rest all patients are alive without any evidence of disease. Two patients having 6 years of disease free survival and six out of ten patients have almost 1 year of disease free survival. Further follow up is being done. Individualized treatment is critically important. Neo-adjuvant therapy may represent a reliable and promising strategy. However, optimal delivery of multimodality therapy for advanced vulvar cancers are not clearly defined.

04. Evaluation of possum score in predicting morbidity and mortality following major oncosurgical procedures

Chakraborty Amit
Surgical Oncology

Summary

Aim of present study was to assess the accuracy of POSSUM in predicting mortality and morbidity in patients with different malignancies undergoing surgery at RCC at Ahmedabad. 100 patients each of head and neck, thoracic and gastrointestinal (GIT), and genitourinary (GUT) malignancy undergoing operative procedure at Gujarat cancer & research institute (GCRI) based at Ahmedabad were included in this study. Detailed standardized risk assessments and thorough documentation of postoperative courses were performed and POSSUM scores were calculated. Among all the three categories, surgery for Buccal Mucosa cancer in H&N group (20.6%), Colorectal surgery (12.6%) and Upper GI surgery (11%) in GIT group and radical hysterectomy (8.6%) & lower urinary tract surgery (9%) in GUT group was the most common surgical procedure. 23% patients in head and neck group, 34% in GIT and 31% in GUT group had morbidity. The 30 day mortality rate was 6% and 29.3% patients developed complications. POSSUM predicted morbidity for 38.07% and mortality for 8.99% patients. In all three groups for morbidity O: E ratio was >1 for low risk range (<10) and <1 for higher risk range (>30). Mortality is under predicted for head and neck cases in all risk while in GIT and GUT it is under-predicted for low risk range and over-predicted for high risk range. POSSUM failed to accurately predict morbidity and mortality. Modifications are needed prior to its application for comparative audit in oncosurgery in high volume centres. Presence of comorbidity and nutritional status along with technical competence of surgeon influence postoperative morbidity and these factors are not included in score. So inference of this study is to create a new model incorporating various other variables for better predictive equation.

05. Elastography and clinical uses

L. Gaurav
Radiology

Summary

Introduction to new type of imaging modality elastography and its clinical uses .

1. Elastography is supplementary to convention B

mode imaging
2. Its uses have been well established in breast and liver imaging
3. Better characterization of BIRAD III and IV categories
4. Evaluation of liver cirrhosis

06. Comparative evaluation of subclavian vein catheterisation using supraclavicular versus infraclavicular approach

Momin Atikahmed G.
Anasthesiology

Summary

Infraclavicular (IC) approach of subclavian vein (SCV) catheterisation is widely used as compared to supraclavicular (SC) approach. The aim of the study was to compare the ease of catheterisation of SCV using SC versus IC approach and also record the incidence of complications related to either approach, if any. In the study, 50 patients enrolled were randomly divided into two groups of 25 patients each. In Gp. SC Subclavian vein catheterisation was performed using SC approach and in Gp. IC catheterisation was performed using IC approach. Access time, success rate of cannulation, number of attempts to cannulate vein, ease of guidewire and catheter insertion and length of catheter inserted and any associated complications were recorded. SC approach offers distinct advantage that catheter is always guided downwards in contrast to IC approach where catheter guided upwards. Secondly it can be used intraoperatively and on mechanically ventilated patients duo to its cephalad access. Sometimes when IC approach fails, anesthetist awareness of this approach helps in placement of central venus catheter.

07. Antibody screening and identification

Kusumgar Rima
Blood Bank

Summary

Alloimmunization is one of the major concern in the management of patients who require repeated blood transfusion as a lifesaving treatment . Data about alloimmunization rate especially in oncology patient population who receive multiple transfusions is scarce. So, We undertook this study to determine prevalence and specificity of RBC alloantibodies in patients admitted in various clinical oncology specialities at a tertiary care hospital in Gujarat.Antibody screening was carried out in 9074 patients from May 2015 to April 2016.The data was compiled and statistically analysed. The overall incidence of RBC alloimmunization in oncology patients was 0.55% with more prevalence in Females(66%),with mean age group of 40 years. The most common alloantibody was Anti-D (14.9%) and Anti –E (14.9%) and remaining 70% was pan positivity with autocontrol negative with highest incidence in hemato-oncology patients followed by patients with gynecological malignancies. 30 % per cent of the total alloantibodies identified were against Rh antigens and remaining panpositive samples require further evaluation. These findings emphasize the role of extended antigen typing, and the importance of being able to provide Rh and Kell matched blood to reduce the complications of alloimmunisation due to blood transfusion.

Journal Club/Guest Lecture/ Review Lecture Presentations

(January 2016 to June 2016)

Sr. No.	Date	Presenter/ Department	Topic	Authors	Citation
1	23.01.16	Bhratri Bhushan Medical Oncology	Prospective Validation of a 21-Gene Expression Assay in Breast Cancer	JA Sparano, RJ Gray, DF Makower, KIPritchard, KS Albain	New England J Med, 2015; 373, no. 21
2	26.03.16	Kobawala Toral Division of Molecular Endocrinology	Anti-miRNA-221 sensitizes human colorectal carcinoma cells to radiation by upregulating PTEN	Qi Xue, Kai Sun, Hai-Jun Deng, Shang-Tong Lei, Jing-Qing Dong, Guo-Xin Li	World J Gastroenterol 2013 December 28; 19(48): 9307-9317
3	09.04.16	Sanghavi Priti Palliative Medicine	Why Palliative Care in Oncology practice? Early Palliative Care for patients with metastatic non-small cell lung cancer	Jennifer S Temel, Joseph A, et al	New England Journal of Medicine, 2010,363:8:733-742
4	23.04.16	Patel Rohini Community Oncology Department	Immunogenicity and HPV infection after one, two and three doses of quadrivalent HPV vaccine in girls in India: a multicentre prospective cohort study.	Rengaswamy Sankaranarayanan, Nirja Bhatla, Parimal Jivarajani, Geeta Joshi, et al.	The Lancet Oncology S1470-2045 1500414-3
5	14.05.16	Sankaye Smita Department of Pathology	The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System	Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA	Am J Surg Pathol 2016 Feb;40(2):244-252
6	28.05.16	Shah Kanisha Medicinal Chemistry & Pharmaco-genomics	Circulating Cell-Free Tumour DNA in the Management of Cancer	Glenn Francis and Sandra Stein	Int. J. Mol. Sci. 2015, 16, 14122-14142
7	11.06.16	Dange Avadhut Surgical Oncology	Role of prophylactic central compartment lymph node dissection in clinically N0 differentiated thyroid cancer patients: analysis of risk factors and review of modern trends	Giovanni Conzo Ernesto Tartaglia1, Nicola Avenia, Pier Giorgio Calò, Annamaria de Bellis, Katherine Esposito, Claudio Gambardella, Sergio Iorio, et al	World Journal of Surgical Oncology (2016) 14:149 DOI 10.1186/s12957-016-0879-4
8	25.06.16	Bharadwaj Bikram Gynaecological Oncology	Individualized Treatment of Patients With Early-Stage Epithelial Ovarian Cancer After Incomplete Initial Surgery	HuaTu, Ying Xiong,He Huang, et al	International Journal of Gynecological Cancer 2016 26(73- 81)

Case Presentations for Morbidity, Mortality at Clinical Meetings

(January 2016 to June 2016)

Sr. No	Date	Presenter/Department	Case Discussion
1	23.01.2016	Chavda Naimish Anesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
2	13.02.2016	Cherian Roy Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
3	26.03.2016	Surekha Shaboo Anesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
4	23.04.2016	Cherian Roy Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
5	14.05.2016	Cherian Roy Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
6	25.06.2016	Cherian Roy Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments

Presentations at the Clinical Meetings

(January 2016 to June 2016)

Sr. No.	Date	Speaker/Department	Title
1	09.01.2016	Patel Vishal Nuclear Medicine	Early metabolic response using 18f–18-f-fdg-pet can prognosticate better than conventional ct imaging in paediatric patients of Burkitt's Lymphoma
2	23.01.2016	Gupta Sunnia Radiotherapy	Retrospective analysis of pt1/2N0 oral tongue carcinoma patients treated at GCRI
3	13.02.2016	Kumari Rashmi Gynaecological Oncology	Neo-adjuvant treatment in locally advanced vulvar cancer
4	12.03.2016	Chakraborty Amit Surgical Oncology	Evaluation of possum score in predicting morbidity and mortality following major oncosurgical procedures
5	26.03.2015	L. Gaurav Radiology	Elastography and clinical uses
6	28.05.2015	Momin Atikahmed G. Anasthesiology	Comparative evaluation of subclavian vein catheterisation using supraclavicular versus infraclavicular approach
7	25.06.2016	Kusumgar Rima Blood Bank	Antibody screening and identification

About the Journal and Instructions to Author

Gujarat Cancer Society Research Journal is a biannually (April and October), ISSN 2320-1150, peer-reviewed journal published by the Gujarat Cancer Society. The journal is indexed with Directory of Open Access Journals (DOAJ), Googie Scholar, Index Coperinicus, Journals Master List. The journal's full text is available online at http://www.gcriindia.org

The Editorial Procss

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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The following documents are required for each submission: (Font: Times New Roman)

- Title Page (Font size: 12)
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- Summary and Keywords (Font size: 9)
- Text (Introduction, Aims and Objectives, Materials and Methods, Results and Analysis, Discussion with Conclusions; Font size: 12).
- Tables (separate page, Number Arabic numerals (e.g. 1,2,3) as it comes in results) (Font size: 12)
- Figures and Illustration (separate page, JPEG format, Number Arabic numerals (e.g. 1, 2,3) as in results, if photographs of persons are used, the subjects or patients must not be identifiable).
- Legends to Figures and Illustration: Present the legends for illustrations separate page using double-spacing, with Arabic numerals corresponding to the Illustrations. (Font size: 12)
- 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 and parenthesis; Font size: 12).
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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.

Abbreviations of units should conform to those shown below:

Decilitre	dl	Kilogram	kg
Milligram	mg	Hours	h
Micrometer	mm	Minutes	min
Molar	mol/L	Mililitre	ml
Percent	%		

Title Page

The title page should include

1. Type of manuscript (article/case report)
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Language and grammar

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- Numerals from 1 to 10 spelt out
- Numerals at the beginning of the sentence spelt out

Summary and Keywords: Summary no more than **250 (150 for Case Report)** words. 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 and 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. Three to eight keywords must be included below the summary.

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/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.

Identify precisely all drugs and chemicals used, including their generic names, their manufacturer's name, city and country in parenthesis, doses, and routes of administration.

Results: Present your results in a logical sequence in the text, Tables, and Illustrations. Do not repeat in the text all the data in the Tables or Illustrations. Emphasize or summaries only important observations. Specify the statistical methods used to analyze the data. Restrict Tables and Illustrations to those needed to explain the argument of the paper and to assess its support. Where possible, use Graphs as an alternative to Tables with many entries. Do not duplicate data in Graphs and Tables.

Discussion: Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or the Results section. Include in the Discussion section the implications of the findings and their limitations, including the implications for future research. Relate the observations to other relevant studies.

Tables: Print each Table double-spaced on a separate sheet. Number Tables consecutively in Arabic numerals (e.g. 1, 2, 3) in the order of their first citation in the text and supply a brief title, which should be shown at the top of each table.

Illustrations (Figures) and Legends for Illustrations: All Illustrations must be submitted in JPEG finished format form that is ready for reproduction. Figures should be numbered consecutively in Arabic numerals (e.g. Figure 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.

Acknowledgements: State contributions that need to be acknowledged.

References
A list of all the references cited in the text should be given at the end of the manuscript and should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in the text by Arabic numerals in superscript. Omit month and issue number. List all authors, but if the number is six or more, list first three followed by et al. The references should be cited according to the Vancouver agreement. Authors must check and ensure the accuracy of all references cited. Abbreviations of titles of medical periodicals should conform to the latest edition of Index Medicus. Some examples are shown below:

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: Up To Date 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.

Referees
Generally, submitted manuscripts are sent to one experienced referee from our panel. The contributor's may submit names of two qualified reviewers who have had experience in the subject of the submitted manuscript, but not associated with the same institution(s) as contributors nor have published manuscripts with the contributors in the past 10 years.

Shri Hiralal Bhagwati Interventional Therapy Center

Interventional Therapy Center (IVTC) is a state of the art center for diagnostic and therapeutic endoscopy. It was established with the aim of providing a comprehensive center for modern and advanced techniques and new directions in endoscopy procedures. It is the only center of its kind in the campus of civil hospital, Ahmedabad, and since then, it continues to provide comprehensive endoscopy care not only to the patients of Gujarat Cancer Research Institute (GCRI), but also those of entire civil hospital campus.

Foundation and formation

IVTC was established in December 1993 after relentless efforts by the then director, Dr. Devendra Patel and developed during the initial years by Dr. Yatin Patel, who was associated with the department till recently.

IVTC is situated on 2nd floor in room no 251 in the main building of GCRI, and occupies around 3000 square feet area. It comprises of a large waiting area and recovery room of 6 beds. Both the waiting area and the recovery rooms occupy about 1000 sq ft each. There are two operation theatres: One (280 sqft) for gastroscopy & advanced procedures like ERCP which is equipped with Image Intensifying Television (IITV). The other one (340 sq ft) is dedicated to colonoscopy and bronchoscopy. Apart from this, there is a large storage room, separate area for manual cleaning and separate rooms for doctor, nursing staff and ward servants. For the easy and smooth working of the center, policies are developed into a procedural manual for patient reception, preparation, endoscopy procedure, recovery stage and discharge from center with proper advice.

Equipment and facility

At present, IVTC has two video- gastro scopes, one colonoscope and one side view endoscope (Olympus® endoscopy, Q150 series) along with two video processors (CV-150). Apart from this, the department is equipped with Image Intensifying Television (IITV – Surgico® 100R-HF), ERBE® electrosurgical unit (300s) and Dräger® anesthesia trolley. In the center, we use standard endoscopic accessories and provide computer generated report of the procedure.

The Team

IVTC works under the department of surgical oncology, and since its inception it has been managed by a team of medical gastroenterologists, pulmonologists, surgical-oncologists and anesthetists. At present, there are two full time gastroenterologists and one part time gastroenterologist. On the pulmonary side, we have a team of two pulmonologist who give part time service, daily. There is an experienced surgical back up and anesthesia team to provide comprehensive care. IVTC also has four experienced nursing staff, one OT technician and four ward servants.

Procedures

The IVTC department is a high volume center for endoscopy services and performs advanced endoscopy like ERCP & Pleuroscopy. We perform about 25-30 procedures daily, that includes diagnostic Upper GI & Lower GI procedures and bronchoscopy which constitute major bulk of procedures. We also perform entire range of therapeutic Upper & Lower GI procedures like endoprosthesis placement, placement of PEG, along with advanced endoscopy procedures like ERCP and pleuroscopy. At IVTC, we perform procedure not only related to malignancy but also provide complete endoscopy care for benign diseases like dilatation for caustic injury strictures of esophagus, Endoscopic Variceal Ligation, sclerotherapy, balloon dilatation and polypectomy. In last 5 years, IVTC has catered to around 5000 patient per year. It has conducted around 6759 procedure in period from April 2015 to March 2016 which includes more than 4000 diagnostic endoscopy and 1500 therapeutic endoscopy as well as more than 400 advanced endoscopy procedure including ERCP. Very few centers in India carry out this magnitude of diagnostic or therapeutic procedures, making IVTC one of the high volume centers.

At IVTC, around 25-30 patients are seen daily requiring super specialty consultations as part of complete care in cancer patient.

Training, education and academic activities:

Department faculties regularly attend and participate in conferences and workshops to remain updated and present various papers in relevant conferences. In the past, IVTC has conducted many workshops and conferences which were attended by a large number of national delegates. The department has also been imparting training program for those other than surgical oncology residents, interested in learning endoscopy.

Future plan and scope

There is proposal for purchase of latest endoscopy system with image enhancing technology along with endoscopic ultrasound (EUS) which is now essential for any gastro-oncology care. The department with its original concept in proposed design at new GCRI building will be state of art center and will be at par with best gastro-oncology institute in world.

GC&RI - Update

Kothari Kiran C.
MS, DNB, PGDHHM, FMAS, FCLS, FIAGES
Dy. Director & Professor, Department of Surgical Oncology
The Gujarat Cancer & Research Institute (GCRI), Ahmedabad

Minimal Invasive Surgery Department was inaugurated by the then health minister Shri I. K. Jadeja on 16-9- 2003 and well supported by then Director Dr. Pankaj M Shah. Laparoscopic HD camera, ultrasonic coagulator and dissector and other instruments were procured to start this department. At GCRI from September 2003 onwards advanced laparoscopic procedures and diagnostic Laparoscopies are done quite routinely in this departments which includes diagnostic laparoscopy, minimally invasive esophagectomy, lap sssisted gastrectomy, hemicolectomy, anterior resection, low anterior resection, APR, lap nephrectomy, adrenalectomy, lung lobectomy and pneumonectomy, lap assisted mediastinal mass excision, etc. We are in a process of procuring another HD and 3D laparoscopic systems, vessel sealer equipment, and Cusa, etc. I sincerely desire that we will be able to start Robotic surgery when we establish in NEW GCRI building.

The Gujarat Cancer & Research Institute (GCRI) is now offered the status of State Cancer Institute (SCI) by Govt. of India. There can be only one SCI in each state. So GCRI has grown from RCC to SCI. There are many benefits to this. All other tertiary cancer centers (TCC) may come under GCRI or GCRI may be asked to extend help for establishing such new TCCs; viz. Vadodara, Bhavnagar etc. GCRI is already giving guidance and suggestions to Rajkot TCC. We are already managing Siddhpur cancer centre, Radiotherapy and Chemotherapy services are running quite well.

Long awaited dream of having NEW GCRI is coming true. Our new GCRI building will be built in two phases, phase 1 and 2. This is only possible by tremendous help from Government of Gujarat (GoG). In fact GoG is building NEW GCRI with future plans of our need. Phase 1 will have phases 1-A, 1-B and 1-C. Phase 1-A & 1-B is almost ready and will be functional in early 2017, whereas construction for 1-C will also begin in 2017 and probably over in another two years. Complete phase-1 of GCRI will have out-patient department, 600 indoor beds, pathology lab, radiology department, nineteen modern modular operation theatres, state of the art auditorium (Cama hall) apart from blood bank, administrative block, double basement parking and two bunkers for latest radiotherapy machines in basement. Phase-2 will have research, radiotherapy, bone marrow transplantation (BMT) departments apart from another 400 indoor beds, students hostel, etc. This will make GCRI 1000 bedded Cancer Institute, the largest in our country. We extend our gratitude towards our Chairman, Shri Pankajbhai R. Patel for giving us noteworthy guidance, suggestions and help.

We have stared region-wise specialty services in surgical oncology from July 2016. Three major services are started viz. 1) Head and Neck, Breast, 2) Thorax & Miscellaneous and 3) GI and HPB. These services are working very effectively and smoothly. There is lot of scope of further improvising and modernizing these services by procuring latest equipments (e.g. gamma probe for sentinel lymph node mapping of breast cancer) and we are working on it. We will start tumor board activity in each of these specialties from January 2017. Other super specialties are already working for long time viz., gynec-oncology, uro-oncology, ortho-oncology, pediatric oncology, neuro-oncology and plastic and reconstructive services.

We are happy to announce that GCRI has started MCI approved M.Ch Gynec Oncology course from 2016 and one student has already joined.

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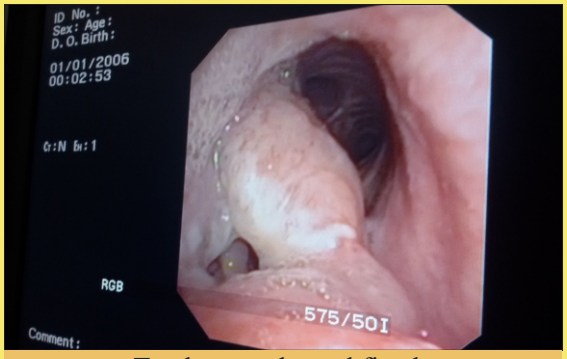
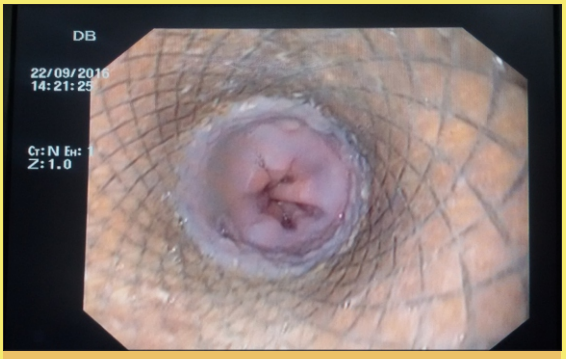
Interventional Therapy Centre - IVTC

Operation Theatre No.1 with IITV, Electrosurgical unit & Anesthesia trolley.

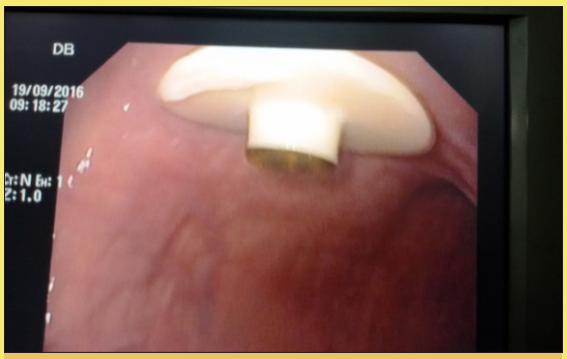
Operation Theatre No. 2



Tracheoesophageal fistula (Bronchoscopic View)

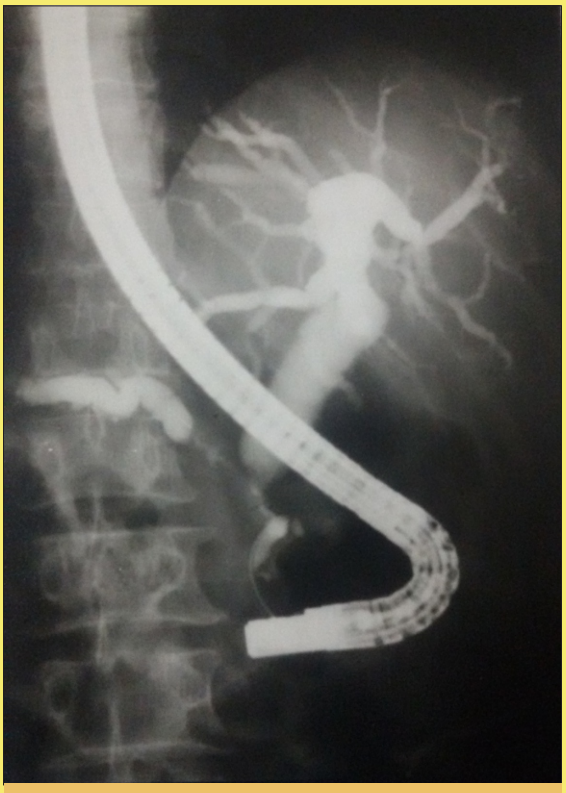


Esophageal SEMS (Metal stent)



Gastroscopic view of PEG tube

Recovery room



ERCP image