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Editorial

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Neuroendocrine Tumours (NETs) of the Gastroenteropancreatic System (GEP) - Latest Updates

As an entity, neuroendocrine tumours have evolved a lot, over a century, and so has our understanding of it. Way back in 1907, S. Oberndofer noted multiple unusual tumours in the small bowel and proposed the term 'carcinoid', emphasizing their benign features.[1] They also came to be known as "APUDomas" (Amine Precursor Uptake and Decarboxylation Cells) in the eighties.[2]

Since then, after many breakthrough researches, our understanding of this tumour entity has improved. There remains a lot that still has to be unravelled.

In the most updated series of 29,664 patients with GEP-NETs reported to SEER (Surveillance,Epidemiology, and End Results) of National Cancer Institue(NCI), incidence of 3.65/1,00,000 individuals per year was reported.[3] The age adjusted incidence of GEP-NETs has increased steadily over the last four decades, with a 3.6 fold increase occurring between 1973 and 2007. The precise reason for this steep rise in incidence is unclear, but the expanding use of endoscopic and imaging studies is believed to play a role.

In the first WHO classification of GEP-NETs in 1980, the term carcinoid was used to describe all gastrointestinal neuroendocrine tumours, regardless of biologic behaviour and pathological characteristics.[4]

In 2000 and 2004, the WHO reclassified NET into well differentiated and poorly differentiated tumours.[5] Well differentiated tumours were subdivided into benign, of uncertain malignant potential or carcinoma. The scheme did not achieve widespread acceptance.

In 2010, WHO updated the classification and proposed the term 'Neuroendocrine Neoplasms' (NEN) for describing tumours originating from diffuse endocrine system in the body. NEN were divided into NET G1, NET G2, and NEC G3 (Neuroendocrine Carcinoma) based on cell proliferartion, hence taking into account MIB1 (Ki67) expression and counting mitoses/10 hpf.[6] (Table 1)

The ENETs (European Neuroendocrine Tumour Society) has proposed a pathological TNM staging of gastrointestinal neuroendocrine tumours, according to site.[8]

Current protocols require both a mitotic rate and Ki67 index to be counted separately in GEP NETs and to render the higher of the two as the final grade. It is exceedingly uncommon for the mitotic count to supersede Ki67 because it is seldom higher. Ki67 is expressed as percentage of positive cells over total tumour cells. Hot spots are selected and 500 to 2000 tumour cells are counted.[9]

Chromogranin and Synaptophysin, as neuroendocrine markers on immunohistochemistry has been helpful in clinching the diagnosis.

In the SEER database, the sites of primary GEP-NEN were rectum (17.7%), small intestine (17.3%), colon (10.1%), pancreas (7%), Stomach (6%), appendix (3.1%).[3] Experience at our institute at GCRI, shows predominant cases of neuroendocrine neoplasms presenting as metastasis of unknown origin in the liver. The primary sites in descending order were pancreas, followed by small intestine, stomach, esophagus, colon and appendix.

The WHO 2010 classification of NEN of GEP system has played an important role in predicting the biological behaviour of these tumours and judging the prognosis.

In our experience, survival is significantly better in the NET G1 and NET G2 subtypes, compared to NEC G3. Patients with NEC G3 have more chances of distant metastasis and higher mortality. These findings are in accordance with other studies.[10,11]

From the clinician's point of view, the new grading system is an important yardstick of patient survival and also a guiding tool on the treatment regimen to be followed. At present, G1 and G2 tumours are managed in a similar way. Therefore, the distinction between G1 and G2, while prognostic, does not have major therapeutic implications. A more important issue is the distinction between G2 and G3, as platinum based chemotherapy is generally recommended for G3 tumours. G3 tumours are responsive to combination chemotherapy. Low grade tumours are usually refractory to intensive systemic chemotherapy and are preferably treated with octreotide long-acting release .[12]

Hence, the need of the day, to combat these tumours is a multidisciplinary approach, with pathologists, radiologists, oncosurgeons, oncophysicians and nuclear medicine specialists joining hands.

Table 1: WHO 2010 Classification of Neuroendocrine Neoplasms

SN	Grade	Mitotic count	
1	NET G1	<2 mitoses/10 hpf	MIB 1≤ 2%
2	NET G2	2 to 20 mitoses/10 hpf	MIB 1 : 3-20 %
3	NEC G3	>20 mitoses/10 hpf	MIB1 > 20 %
4	MANEC(Mixed AdenoNeuroendocrine Carcinoma)	At least 30 % of either component should be identified	
5	Hyperplastic/preneoplastic lesions		

Table 2: Minimum Pathology Data Set: Information to be included in Pathology Reports on NETs (from Klimstra et al 2010)[7]

Resection of primary tumors
• Anatomic site of tumor
• Diagnosis (functional status need not be included in the pathology report)
• Size (3 dimensions)
• Presence of unusual histologic features (oncocytic, clear cell, gland-forming and other features)
• [OPTIONAL: immunohistochemical staining for general neuroendocrine markers]
• Chromogranin
• Synaptophysin
• Peptide hormones, if a specific clinical situation suggests that the correlation with a functional syndrome may be helpful
• Grade (specify grading system used)
• Mitotic rate (number of mitoses per 10 high-power fields of 2 mm2;count 50 high-power fields in the most mitotically active regions,count multiple regions)
• [OPTIONAL: Ki67 labeling index (count multiple regions with highest labeling density, report mean percentage; eyeballed estimate is adequate)]
• Presence of nonischemictumor necrosis
• Presence of other pathological components (eg, non-neuroendocrine components)
• Extent of invasion (use anatomic landmarks for the AJCC T staging of analogous carcinomas of the same anatomic sites)
• Presence of vascular invasion [OPTIONAL: perform immunohistochemical stains for endothelial markers if needed]
• Presence of perineural invasion
• Lymph node metastases
• Number of positive nodes
• Total number of nodes examined
• TNM staging (specify staging system used)
• Resection margins (positive/negative/close) [OPTIONAL: measure distance from margin if within 0.5 cm]
• Proliferative changes or other abnormalities in non-neoplastic neuroendocrine cells
Biopsy of primary tumors
• Anatomic site of tumor
• Diagnosis (functional status need not be included in the pathology report)
• Presence of unusual histologic features (oncocytic, clear cell, gland forming, and other features)
• [OPTIONAL: immunohistochemical staining for general neuroendocrine markers]
• Chromogranin
• Synaptophysin
• Peptide hormones, if a specific clinical situation suggests that the correlation with a functional syndrome may be helpful
• Grade (specify grading system used)
• Mitotic rate (number of mitoses per 10 high-power fields or 2 mm2, count up to 50 high-power fields)
• Ki67 labeling index, for biopsies in which a diagnosis of high-grade neuroendocrine carcinoma cannot be excluded (count multiple regions with highest labeling density, report mean percentage; eyeballed estimate is adequate)
• Presence of nonischemictumor necrosis
• Presence of other pathological components (eg, non-neuroendocrine components)

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"If you want to conquer the anxiety of life, live in the moment, live in the breath."

Amit Ray

Shri R. J. Kinarivala Research Oration Award

Dr Dilip Giri
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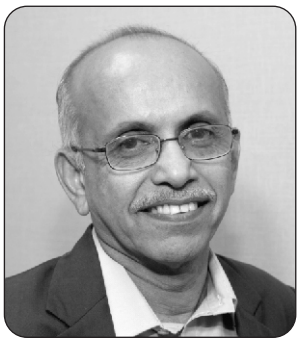
Trends in Clinical Breast Cancer Research: Opportunities and Challenges for a Surgical Pathologist

The practice of histopathology in breast cancer has evolved considerably since the time Joseph Bloodgood became the first surgical pathologist at Johns Hopkins Hospital in the era of the famous William Halstead, a pioneer in breast surgery. From those early days up until the early 1990s, pathologic assessment comprised essentially of gross and microscopic examination using conventional stains and light microscopy. In addition to confirming the diagnosis of breast cancer, the pathologist provided prognostic information based on morphologic characteristics of an individual tumor viz. tumor type, grade, size, nodal status etc. An oncologist then tailored treatment, saving aggressive regimen for histologically aggressive tumors that were more likely to metastasize over a short term. Surgical pathologists became able to predict response to a particular chemo-agent for the first time at the turn of 1990s when the use of immunohistochemistry (IHC) for Estrogen and Progesterone receptor (ER and PR) allowed identification of tumors most likely to benefit from hormonal agents such as Tamoxifen. This ability was further enhanced when IHC for HER2 gene overexpression enabled identification of tumors responsive to Herceptin or similar drugs.

These advances brought in to a pathologists armamentarium tests that until then were essentially only biochemical or molecular. In spite of these additions, the inadequacy of morphology as a diagnostic tool in enabling the delivery of individualized and precision therapy has always been felt. With the sequencing of the human genome around the year 2000, a major breakthrough occurred in molecular characterization of breast cancer. Researchers showed that breast cancer could be classified in to at least 5 distinct molecular classes mainly based on expression of genes for ER, PR, HER2 and cell proliferation. These classes were also shown to be prognostically relevant. Since suitable agents were already available to treat tumors expressing ER and HER2, research focus has turned to a group of tumors that are recalcitrant to treatment and also happen to lack ER, PR and HER2 (and designated as "Triple negative"). In the past several years an additional PCR based molecular test (Oncotype Dx) that assesses genes for ER, HER2 and proliferation and provides a numerical score is now being used to decide on use of adjuvant chemotherapy in patients with breast cancer. Current research is also addressed towards identifying major pathways involved in cancer cell growth under the rationale that once known, it will possible to therapeutically block these and thus cause arrest of tumor growth and metastases. PI3Kinase mediated pathway appears to play a major role in breast cancer and is being exploited in certain breast cancers which become resistant to treatment. Next generation sequencing techniques have greatly enhanced our ability to identify biologically relevant rare mutations. These advances in breast cancer research have direct implications in treatment and have once again raised questions about the validity and role of surgical pathology in the diagnosis and treatment of breast cancer. In Triple negative breast cancer surgical pathology has already elucidated the various tumor types of widely different prognosis that by molecular methods were regarded as a single entity.

This knowledge is extremely important in the management of this type of tumor. In balance, it appears that a surgical pathologist of the future will continue to play an important role in the diagnosis and treatment of breast cancer. By overcoming the various challenges that lay ahead the pathologist will exploit, as he has in the past, the various opportunities that exist to ultimately adapt the molecular tests to his microscope and thereby retain the primacy of surgical pathology in the diagnosis of breast cancer.

Dr. T.B. Patel Oration Award



Dr. M. R. Rajagopal
MD

Chairman of Pallium India, Director, W.H.O Collaborating Centre
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Palliative Medicine: Patient-Centered Care

In 1948, the World Health Organization (WHO) defined health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." But medical science never took it seriously. In fact, at times we openly said that practicing health care in its entirety was not practical . We conveniently shifted to disease-centered care.

Diseases are important, no doubt, but not at the exclusion of the well-being of the individual and his family. If adverse effects result in prolongation of life of pure misery, if the person feels that such life is worse than death and contemplates suicide, if the financial implications are such that family relationships break down, a home is lost and the children fall out of school, then we have medicine at its worst.

The last half century saw the worst of this phenomenon with the advent of advanced technology and imaging into medical science and the resultant skyrocketing of treatment costs. The patient gradually became an insignificant being in a maze of hospital corridors, surrounded by steel, concrete and plastic. The more medicine became disease-focused, symptoms got less attention, leave alone psychosocial issues. The situation became so bad as to make us wonder whether Alain Enthoven, the health economist was right when he said that "Increasing medical inputs at some point will become counterproductive and cause more harm than good"[2].

A welcome change was brought in by Dr. Cicely Saunders when she founded the global palliative care movement in the 1960s. What was then started as care of people dying with cancer, has today expanded to a branch of medical science moving towards integration into healthcare as a whole.

The essential elements of palliative care, each of which encroaches on another, are physical, social, psychological and spiritual. The foundation of this concept is that the human being should not be viewed just as a container of disease but should be viewed as a whole person who cannot be well unless the family is well. In palliative care, each of these elements is assessed and problems which threaten quality of life are identified. What can be improved is improved and for the rest, the patient is offered support without abandonment in the rest of his journey.

Recognizing the principles of palliative care are important from the onset of the disease, the WHO defined its definition of palliative care in 2002 as "Palliative care is an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial and spiritual"[3].

If added: Palliative care:

- provides relief from pain and other distressing symptoms;
- affirms life and regards dying as a normal process;
- intends neither to hasten or postpone death;
- integrates the psychological and spiritual aspects of patient care;
- offers a support system to help patients live as actively as possible until death;
- offers a support system to help the family cope during the patients illness and in their own bereavement;
- uses a team approach to address the needs of patients and their families, including bereavement counselling, if indicated;
- will enhance quality of life, and may also positively influence the course of illness;
- is applicable early in the course of illness, in conjunction with other therapies that are intended to prolong life, such as chemotherapy or radiation therapy, and includes those investigations needed to better understand and manage distressing clinical complications.

As it is clear from the above, all principles of palliative care are applicable in all diseases which decrease quality of life. Today, in the West, more and more hospitals are incorporating palliative care teams

in their emergency rooms and in intensive care units. Understandably, if the problem is not only physical but also psycho socio spiritual, then the solution also has to involve community. The palliative care system that has evolved in Kerala makes use of the social capital so that a nuclear family does not feel totally disconnected with the medical system; instead, the community around him comes forward to support the person and the family.

There are several barriers which come in the way of improving quality of life of the patient. While globally access to pain relief is seen as a human right, in our country regulatory and attitudinal problems prevent access to and use of opioids like morphine. The recent shift of medicine a service to "health care industry" ensured that profit was the key determinant of elements of care; quality of life and quality of life taking a back seat. Dying people, even in obvious futility of treatment, are subjected to traumatic intensive care. It is no wonder that the "Economist Intelligence Unit" in 2010, on studying "quality of death" found that of the 40 countries, India was the 40th – the worst place to die in – next to Uganda which had come as 39th.[4]

A few positive developments occurred in India recently. Thanks to the Government of India being willing to work with palliative care activists, in November 2012, the Ministry of Health and Family Welfare created a National Program for Palliative Care (NPPC)[5]. It makes provision for palliative care services to be delivered from the primary to the tertiary levels of care. In the same year in response to a Public Interest Litigation in the Supreme Court of India, the Medical Council of India and Indian Nursing Council agreed to incorporate palliative care in undergraduate curricula. The medical council accepted palliative medicine as a specialty and started a 3 year MD course in palliative medicine. And in February 2014, Indian Parliament amended the Narcotic Drugs and Psychotropic Substances (NDPS) Act, thus paving the way for improved access to morphine and other opioid medicines for pain relief.

To cap it all, in May 2014, the World Health Assembly (the decision making body of WHO) passed a resolution asking all member states to take steps to integrate palliative care into "all levels of healthcare"[6].

Patients can get humane health care only if policy is created and implemented, supported by professional education and access to essential drugs.

The GCRI, Ahmedabad is taking leadership not only in palliative care to patients, but also in starting training programs in palliative care for professionals.

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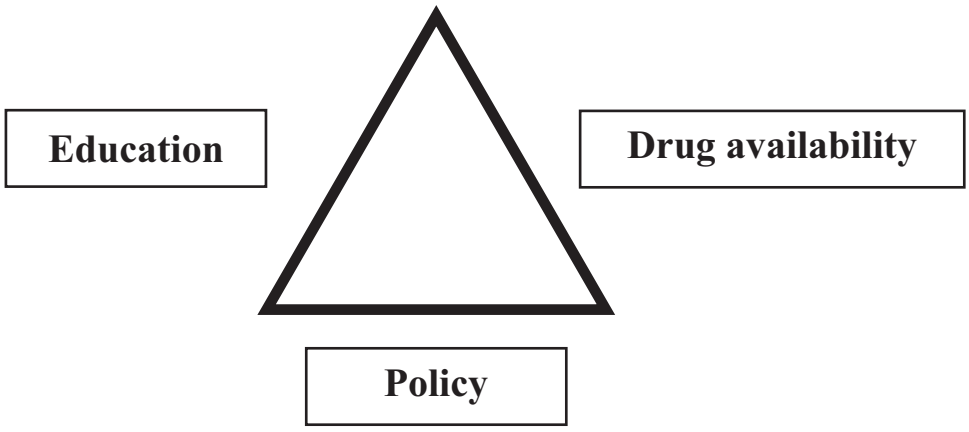

Clinical Significance of Glutathione S-transferase Enzymes in Acute Leukaemia Patients

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Summary

Leukemic cells produce higher amounts of reactive oxygen species (ROS) than non-leukemic cells as they are under a repeated state of oxidative blockade. Antioxidant defence system such as glutathione-S-transferase (GST) and glutathione reductase (GR) counteracting ROS induced oxidative stress. Therefore, the study was aimed to evaluate the role of antioxidant defence system in leukemogenesis. Twenty five untreated acute leukaemia patients and twenty five healthy individuals were enrolled. Plasma GST, GR and total thiol levels were measured using highly sensitive spectrophotometric methods. The prevalence of GSTM1 polymorphism was examined using polymerase chain reaction (PCR). Statistical analysis was done using SPSS version 13. Acute leukaemia patients showed significantly higher GST and GR activities ($p=0.052$ and $p=0.001$ respectively) whereas thiol levels were significantly lower ($p=0.001$) as compared to controls. Mean GR activity was found to be significantly higher ($p<0.05$) in acute myeloid leukaemia patients than acute lymphoid leukaemia patients. Correlation analysis revealed positive correlation between GST and GR, and negative correlation between GR and thiol levels. Receiver's Operating Characteristic (ROC) curve analysis revealed that GR activity and total thiol levels were highly efficient to discriminate between acute leukaemia patients and controls. 60% of acute leukaemia patients showed GSTM1 null genotype, whereas only 25% of controls demonstrated GSTM1 null genotype. In Conclusion, increased GST and GR activity, and decreased thiol levels were observed in acute leukemia. Moreover, GSTM1 null genotype was also associated with increased risk of acute leukemia patients. These results suggested significant role of GST, GR and thiol in acute leukemia patients.

Keywords: Acute leukemia, Glutathione Reductase, Glutathione S-transferase

Introduction

Leukaemia accounts for less than 3% of the worldwide cancer burden. The incidence rate in both sexes is 5 Age Standardised Ratio (ASR) and mortality rate is 3.8 ASR in both sexes. Likewise, in India, incidence of leukaemia is about 3.5% of all other cancer and incidence rate in both sexes is 3 ASR and mortality rate is 2.5 ASR in both sexes.[1] According to population based cancer registry of Ahmedabad, incidence of lymphoid leukaemia in urban area is 0.26 ASR in female and 0.35 ASR in male. However, in rural area, it is 0.32 ASR in female and 0.40 ASR in male. Whereas, incidence of myeloid leukaemia in urban area is 0.31 and 0.45 ASR in female and male, respectively. However, in rural area of Ahmadabad, it is 0.62 and 0.45 ASR in female and male, respectively.[2]

Leukaemia is categorized into four types including acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL) and chronic myeloid leukaemia (CML). The terms "myeloid" or "myelogenous" and "lymphoid", "lymphocytic" or "lymphoblastic" denote the cell types involved. In general, leukaemia is characterized by the uncontrolled accumulation of blood cells. However, the natural history and treatment for each type of each type are different.

Acute leukaemia, including ALL and AML, develops at any age. It is the disease which progress rapidly without treatment that results into accumulation of immature, non-functional cells in the bone marrow and blood. The bone marrow often stops producing enough normal platelets, red cells and white cells. Anaemia, a deficiency of red cells, develops in virtually all people who have leukaemia. The lack of normal white cells impairs the body's ability to fight infection. A shortage of platelets results in bruising and easy bleeding.[3]

The aetiology of leukaemia is largely unknown, although it is considered to be multifactorial.[4] The involvement of certain environmental carcinogen exposure has been linked to leukaemia, such as contact with industrial chemicals like benzene,[5] household chemicals [6-7] and chemotherapeutic drug, such as topoisomerase inhibitors[8] and ionizing radiation.[9] In addition, leukaemogenesis is considered to be affected by interaction between genes and environment.[10] Particularly, it was reported that potential carcinogens from the environment or their metabolites, such as dipyrone[6] and permethrin[7] causes DNA damage that leads to initiation and progression of leukaemia.[10] One mechanism which can result in DNA damage is mediated by reactive species generated either by environmental carcinogens or endogenously as result of oxidative metabolism.[11]

The glutathione S-transferase (GST) super family of enzymes catalyzes the conjugation of xenobiotics and endogenous substances with glutathione, and thereby plays a significant role in inactivation and occasionally the activation of many drugs and xenobiotics,[12-13] Polymorphisms in GST gene leads to significant alteration in the metabolism of many substrates, including carcinogens and

chemotherapeutic agents.[13,14] As a result, these polymorphisms have been suggested to play role in the susceptibility to various cancers like breast, lung, colorectal in chemotherapy induced leukaemia etc.[13, 15,18] GSTM1 is one of the gene that encodes the mu class of enzymes which is located on 1p 13.3. It is involved in the detoxification of polycyclic aromatic hydrocarbons and other mutagens. Individuals with genotype GSTM1 null have been found to be more susceptible to DNA damage caused by these agents.[19] The frequency of GSTM1 null genotype ranges from 23% to 48% in African population, 39% to 62% in European population, 33% to 63% in Asian population 20 and 22.4% in South Indian population.[13,21]

Various investigators have reported alternations in GSTs and its associated enzymes and increased frequency of GSTM1 null genotypes in leukaemia patients.[22-24] However, till date no study has been reported to evaluate the detoxification enzymes in leukemic patients from Gujarati population. Therefore, major aim of the present study was to analyse the detoxification enzyme levels and activity prevalence of GSTM1 polymorphism in acute leukaemia patients.

Materials and Methods

Subjects: The study was approved by the Institutional Review Board (IRB) and ethics committee of The Gujarat Cancer and Research Institute, Ahmedabad, India. Twenty five untreated acute leukaemia patients and twenty five age and sex matched healthy individuals were enrolled. The patients were 16(64%) men and 9(34%) women with the age range from 16 to 72 years (median age :38 years). The health individuals were 17 (68%) men and 8 (32%) women with age range from 19 to 54 years (median age: 38 years). (Table 1). Further, there were patients were classified into two groups (i) acute myeloid leukaemia (n=12) (ii) acute lymphoid leukaemia (n=13).

Sample collection and methods: Blood samples from the subjects were collected in heparinized EDTA containing vaccutes. Plasma and leukocytes were separated from the blood samples. DNA samples were isolated from leukocytes by DNA isolation kit(Quiagen, USA). Plasma GST, GR and total thiol levels were assayed by spectrophotometric methods.[25-27] The GSTM1 polymorphisms were analyzed using polymerase chain reaction (PCR) using PCR amplification kit obtained from fermentas. GSTM1 (forward) 5-GAA CTC CCT GAA AAG CTA AAG C-3 and GSTM1 (reverse) 5-GTT GGG CTC AAA TAT ACG GTG G-3 primers were used for amplification of GSTM1 gene. The β-Globin gene was used as house keeping gene or internal control.

PCR was performed in a 25 µl reaction containing 100ng of genomic DNA, 200µM dNTPs, 10XPCR buffer of 1.5 µM $MgCl_2$, 1U Taq polymerase (fermentas) and 25ng of each pairs of primers. The standard PCR condition for GSTM1 was as follows. Initial denaturation at 94°C for 5 minutes was followed by 30 cycles of 1 minute at 94°C, 1 minute at 64°C, 1 minute at 72°C and final extension for 10 minutes at 72°C. The PCR products were analyzed on 1.5% agarose gel containing ethidium bromide. The absence of 215 bp product for GSTM1 in presence of 268 bp product of β-globin gene indicates null genotypes.

Statistical Analysis

Data were analyzed using the SPSS statistical software version 13; SPSS, Inc., Chicago, IL. Student-independent 't' test was performed to compare enzyme activities between healthy individuals and patients. Receiver's operating characteristic (ROC) curves were constructed to evaluate discriminatory efficacy of the enzyme levels between healthy individuals and acute leukaemia patients. Pearson's correlation analysis was also carried out to check correlation between plasma GST, GR activities and total thiol levels. The odds ratio was calculated to find out the risk of leukaemia associated with GSTM1 polymorphism. The Chi square test was also performed to check the difference in distribution of GSTM1 null genotype between controls and patients. Data were considered statistically significant when p values were 0.05 or less.

Results

Comparison of detoxification enzymes in healthy individuals and acute leukaemia patients

Figure 1 shows mean plasma GST, GR activity and total thiol levels in healthy individuals and acute leukaemia patients. The mean plasma GST and GR activities ($p=0.05$ and $p=0.01$, respectively) were significantly higher in acute leukaemia patients as compared to the healthy individuals. However, total thiol levels were significantly decreased in patients compared to control ($p<0.0001$).

Receiver's Operating Characteristic (ROC) curve analysis in the subjects

In the present study, ROC curve was plotted for plasma GST, GR activities and total thiol levels to evaluate their efficacy to discriminate between leukaemia patients and controls as depicted in Figure 2. ROC curve analysis revealed that GST, GR activitiy and thiols levels could significantly distinguish between acute leukaemia patients and healthy individuals. The area under the curves for GST, GR and total thiols were 0.629, 0.915 and 0.961, respectively.

Table 1: Clinical details of the subjects

Characteristics	No. of Subjects (%)
Healthy Individuals (n = 25)	
Sex	
Male	17(68%)
Female	8(32%)
Age(Years)	
Median (Range)	38(19-54)
Tobacco habits	
With habit (WHT)	9(36%)
No habit (NHT)	16(64%)
Acute Leukaemia Patients (n = 25)	
Sex	
Male	16(64%)
Female	9(36%)
Age(Years)	
Median (Range)	38(16-72)
Tobacco habits	
With habit (WHT)	5(20%)
No habit (NHT)	20(80%)
Type of acute leukaemia	
ALL	13(52%)
AML	12(48%)

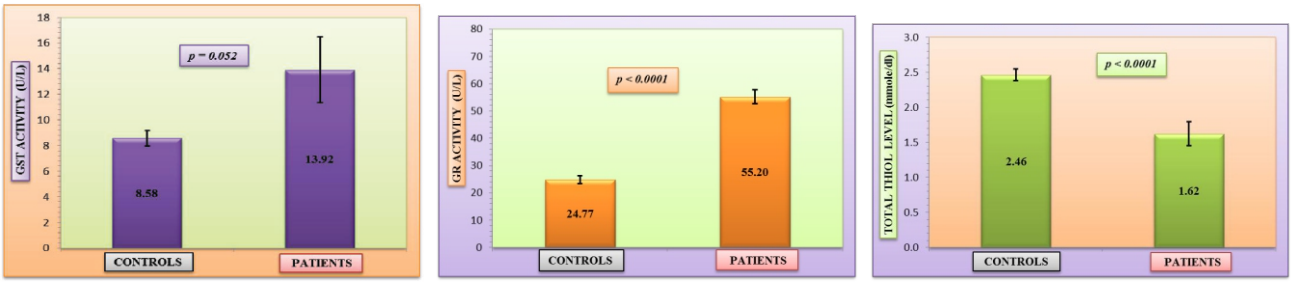


Figure 1: Mean GST, GR and thiol levels in healthy Individuals and acute leukaemia patients

Table 2: Mean GST, GR and total Thiol levels in healthy individuals, ALL and AML patients

Subjects	GST (U/L) (Mean ± S.E.)	GR (U/L) (Mean ± S.E.)	THIOL (mmole/dl) (Mean ± S.E.)
Controls	8.58 ± 0.61	24.77 ± 1.47	2.46 ± 0.08
ALL	10.37 ± 1.42	43.93 ± 5.01*	1.62 ± 0.17**
AML	17.76 ± 4.98	67.42 ± 9.59**#	1.64 ± 0.10**

* p<0.05, **p<0.001 compared with controls
# p<0.05 compared with ALL

Comparison of detoxification enzymes with various clinicopathological parameters of the leukaemia patients

Table 2 shows mean GST, GR and total thiol levels in healthy individuals, ALL and AML patients. Mean GR activity was significantly higher (p=0.003) and total thiol levels were significantly lower (p<0.0001) in ALL patients as compared to healthy individuals. AML patients showed higher GST and GR activities as compared to healthy individuals. Total thiol levels were significantly decreased in AML patients as compared to controls (p<0.0001). The comparison between AML and ALL shows that mean GR activity was significantly higher in AML patients as compared to ALL (p=0.022). However, mean GST activity and total thiol level were comparable between AML and ALL.

Comparison of plasma GST, GR activities and total thiol levels with Blast cells of patients was performed. The results are as depicted in Table 3. Mean Blast cells (79.29%) was considered as cut-off value. Based on this cut-off, patients were grouped into two categories having <79.29 and > 79.29 blast cells count. No significant difference was observed in plasma GST, GR activities and total thiol levels (p=0.716, p=0.264 and p=0.785 respectively) between two group of patients.

Comparison of mean GST, GR activities and total thiol levels with WBC count of patients was performed considering mean WBC count ($54.39x10^3$/cmm) as a cut-off value (Table 3). Based on this grouping of patients was done into two categories having $<54.39x10^3$/cmm and $> 54.39x10^3$/cmm WBC count. No significant difference was found in mean GST activity and total thiol levels (p=0.691 and p=0.075, respectively) between two groups. Plasma GR activity was significantly higher (p < 0.0001) in patients having $> 54.39X10^3$/cmm WBC count as compared to patients group having $< 54.39X10^3$/cmm WBC count.

Correlation between GST, GR and thiol levels in acute leukaemia patients

Pearson's correlation analysis was performed to find correlation between GR, GST and total thiol levels of leukaemia patients (Table 4). There was a positive correlation between GR and GST activity ($r^2 = 0.150$, $r^2 = 0.297$). Whereas, negative correlation was observed between GR activity and total thiol level ($r^2 = 0.067$, $r^2 = -0.418$).

GSTM1 gene polymorphism in healthy individuals and acute leukaemia patients

Representative patterns of GSTM1 gene expression in leukaemia patients are documented in Figure 3. The band obtained at the 265 base pairs (bp) was ß goblin gene which was used as the internal control (housekeeping gene). The band of 220 bp was for GSTM1 gene. The individuals in which there was presence of 220 bp and 265 bp band which corresponds to GSTM1 gene and ß globin gene respectively were taken as individuals with GSTM1 not null genotype, while the absence of the 220 bp with presence of 265 bp were taken as individuals with GSTM1 null genotype. Table 5 represents genotype frequency of GSTM1 gene in patients and healthy individuals. It was observed that 25% controls and 60% acute leukaemia patients represent GSTM1 null genotype. There was significant difference in the distribution of GSTM1 genotype between controls and patients ($\chi^2 = 3.683$, p = 0.055). Further, odd ratio analysis also suggests that risk of leukaemia was higher in individual harbouring GSTM1 null genotype.

Discussion

Leukaemia originates from hematopoietic stem cells that lose the capacity to differentiate normally in mature blood cells at different stages of their maturation and differentiation.[28] The origin of acute leukaemia may be explained by a combination of genetic susceptibility factors and environmental exposure. At higher concentrations, ROS can be important mediators for the damage of biomolecules such as DNA, proteins, and lipids, leading to cellular dysfunction and cell death. Accumulation of such molecules causes toxious effects on individuals, resulting in diseases such as acute leukemia.[29]

There is increasing evidence that predisposition to acute leukaemia is associated with interaction of DNA in the haematopoietic precursor cells with the ROS species generated from exposure to exogenous chemicals such as benzene, chemotherapeutic agents and also from endogenous metabolites.[30,31] Therefore, an imbalance between production of free radicals and reactive metabolites, so-called oxidants or ROS, and their elimination by protective mechanisms, referred to as an antioxidants leads to oxidative stress. Therefore, antioxidants involved in the metabolism of these carcinogens have received a reasonable level of attention.

GSTs are important enzymes in the detoxification of wide range of reactive oxygen species. It is widely accepted that alterations in GSTs play role in the process associated with aetiology of cancer. In present study, higher GST and GR activities in acute leukaemia patients was observed as compared to healthy individuals. Mean total thiol levels were lower in acute leukaemia patients as compared to healthy individuals. In previous studies, it was reported that GST activity were higher and total thiol levels were lower in various malignancies.[28,32-34]

Table 3: Mean GST, GR activities and total thiol levels with Blast cells and WBC count of acute leukaemia patients

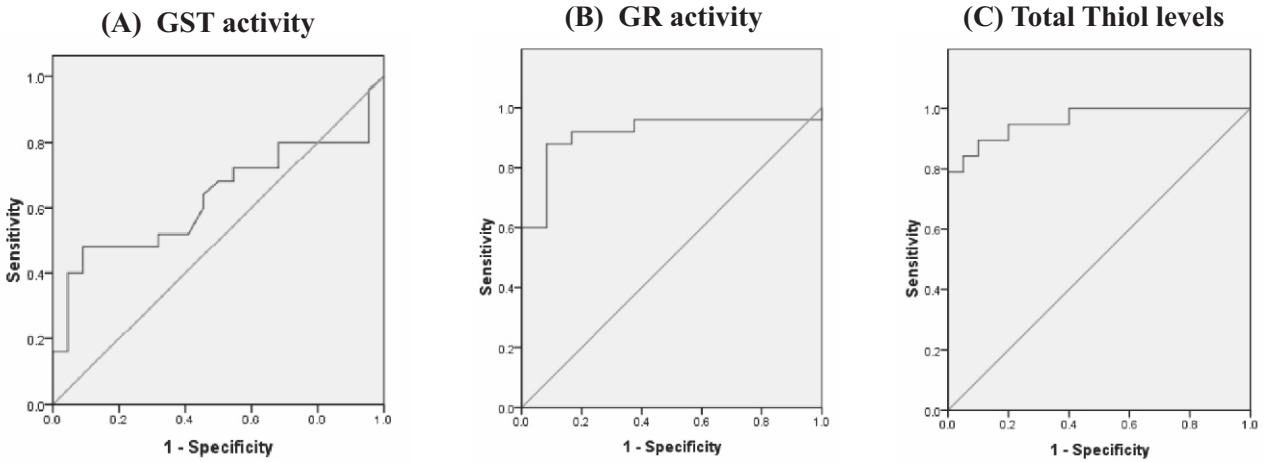
	GST (U/L) (Mean±S.E.)	GR (U/L) (Mean±S.E.)	THIOL (mmole/dl) (Mean±S.E.)
< 79.29	12.40 ± 3.18	64.67 ± 9.03	1.62 ± 0.15
>79.29	14.5 ± 4.36	50.67 ± 8.14	1.68 ± 0.15
***p* value**	0.716	0.264	0.785
WBC (x 10^3/cmm)			
< 54.39	15.13 ± 3.8	43 ± 4.55	1.75 ± 0.10
> 54.39	12.68 ± 2.69	85.17 ± 11.27	1.37 ± 0.20
p value	0.691	< 0.0001	0.075

Table 4: Correlation between plasma GR, GST activities and total thiol levels of acute leukaemia patients

		GST	GR	THIOL
GST	r^2	1	0.297	0.078
	Significance (p value)		0.150	0.743
GR	r^2	0.297	1	-0.418
	Significance (p value)	0.150		0.067

Table 5. Frequency distribution of GSTM1 genotypes in controls and acute leukaemia patients

GSTM1genotype	Controls N(%)	Patients N(%)	χ2	Odds Ratio	95% CI	p value
Not Null	15(75%)	8(40%)	3.683			
Null	5(25%)	12(60%)		4.5	1.2-17.4	0.029
Total	20(100%)	20(100%)	**p = 0.055**			



Parameters	Area under curve	Significance	95% CI Lower	95% CI Upper
(A) GST	0.629	0.130	0.465	0.793
(B) GR	0.915	0.0001	0.819	1.000
(C) Total thiol	0.961	0.0001	0.000	1.000

Figure 2: ROC curve for GST, GR and total thiol levels between subjects

Previous reports have shown that glutathione S-transferase genotype was also significantly associated with acute leukaemia patients.[35-37] In the present study, comparison of detoxification enzymes was done in subtypes of acute leukaemia patients and healthy individuals. Both AML and ALL patients showed increased mean GST and GR activities and decreased total thiol levels as compared to healthy individuals. But mean GR activity was significantly higher in AML patients as compared to ALL.

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 gene. In current study, GSTM1 null genotype was observed in 60% of acute leukaemia patients as compared to 25% in healthy individuals (χ^2= .683, p = 0.055). In previous studies (Table 6), it was observed that polymorphism in GSTM1 was significantly associated with higher risk of acute leukaemia patients.

In conclusion, increased GST and GR activity and decreased total thiol levels were observed in acute leukaemia. Moreover, correlation analysis showed that alterations in GST enzymes were significantly associated with changes in GR activity and total thiol levels in acute leukaemia patients. Furthermore, GSTM1 null genotypes were also associated with risk of acute leukaemia. These results suggest a significant role of GST and GR enzymes in leukaemia patients.

Table 6: Significance of glutathione S-transferase genotype in leukaemia patients

References	Inferences
Suneeth et al,2008[13]	It was reported significant increased risk for ALL with GSTM1 null genotype in South Indian Population
Ma et al, 2013[22]	The GSTM1 null genotype is significantly associated with increased risk of childhood acute leukaemia in Chinese.
Huang et al 2013[38]	The meta-analysis indicated that the GSTP1 A1578G polymorphism was not associated with the risk of childhood ALL.
Franca et al 2012[39]	GSTM1 and GSTT1 homozygous deletions have opposite correlation with relapse, the former being protective and the latter unfavourable in specific subsets of acute lymphoblastic leukaemia patients
Lordelo et al, 2012[40]	A significant association with risk of developing CML was found for GSTM1 non-null (OR = 1.649; 95%CI = 1.05-2.6) genotypes, while GSTM1 null (OR = 0.606; 95%CI = 0.21-0.77) genotypes significantly decreased risk of CML.
Bhat et al, 2012[41]	The presence of GSTT1 genotype may have protective role against the CML. It was found a statistically significant (OD = 3.09, 95% CI: 1.122-8.528; P value = 0.0472) thus individuals carrying null genotypes of both GSTM1 and GSTT1 genes were at elevated risk of CML
Borst et al 2012[42]	The combined gene dose of GSTM1 and GSTT1 may influence outcome in childhood ALL.
Ouerhani et al, 2011[43]	The comparison of leukaemia subgroups according to GSTM1 and GSTT1 genotypes, suggests that leukemogenesis of different leukaemia subgroups is very distinct.
Ozten et al, 2012[44]	The results indicated an association between the GSTT1(-) genotype, either alone or in combination with GSTM1(-) genotype, and risk of CML, suggesting a possible interaction between GSTM1 and GSTT1.
Das et al, 2009[33]	The statistically significant increased risk of AML was observed with GSTM1 null genotype while borderline significance was seen with GSTT1 null genotypes.
Lima et al,2009[45]	Different thresholds of chemical exposure relative to distinct GSTM1 and GSTT1 genotypes may determine whether AML or neutropenia manifests in benzene exposed individuals from South Eastern Brazil.
Suneetha et al 2008[13]	Combined analysis of GSTM1 and GSTP1 showed significant higher risk of ALL associated with the GSTM1 (null/null) and GSTP1 [(Ile/Val)/ (Val/Val)] genotype (OR=2.78: 95%CI=1.16-6.69).

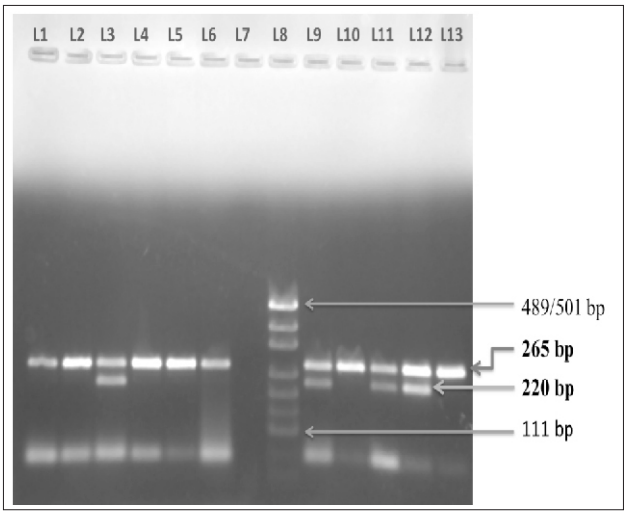


Figure 3: Representative Agarose gel for GSTM1 PCR amplifications All the lanes show a constitutive band of β-globin house keeping gene (265bp) Lanes 3, 9, 11, 12 show an additional band (220bp) of the GSTM1 gene Lanes 1, 2, 4, 5, 6, 10, 13 show only 265bp band which indicates loss of GSTM1 gene in these subjects Lane 8: PUC18/MSP1 digest DNA ladder

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A Study of Her-2/Neu SNP in Breast Cancer Patients

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Summary

The single nucleotide polymorphism (SNP) in the human Her-2 was identified in the transmembrane coding region of the gene at codon 655, encoding either isoleucine (Ile, ATC) or valine, this missense variant in the transmembrane region of the Her-2/neu gene may influence breast cancer risk. To evaluate the role of Her-2/neu gene Ile655Val SNP by evaluating SNP in transmembrane coding region at codon 655Her-2/neu gene, encoding either isoleucine or valine (Ile655Val). In this retrospective study, 127 untreated breast cancer patients were included in the study. Her-2/ neu SNP was evaluated by PCR followed by enzyme digestion. The incidence of Ile/Ile genotype and Val/Val genotype was observed in 20% (25/127) patients each, and Val/Ile genotype heterozygous genotype in 60% (77/127) of patients with a higher incidence of Val/Val genotype in patients with LN positivity and lower incidence in patients with luminal A subtype. Her Ile655Val polymorphism especially in homozygous form might play a role in etiology of breast carcinoma.

Introduction

An important oncogene over expressed in breast cancer routinely used for therapeutic implication is Her-2/neu, a transmembrane tyrosine kinase. The importance of this amplification in the pathogenesis and progression of human breast cancer was shown by Slamon et al (1987).[1] As Her-2/neu has an important role in prognosis after diagnosis of breast cancer, the gene encoding it is a natural target for investigation regarding polymorphisms that might indicate resistance or susceptibility for breast cancer development and also for disease outcome. SNP genotyping have uncovered a variety of cancer predisposition syndromes based on single and multiple gene variants.[2] Experimental studies of rats show that a single missense point mutation (Val664Glu) in the transmembrane domain of the neu proto-oncogene (HER-2 human homologue) that greatly increases its activity and cell transformation properties.[3,4]

The single nucleotide polymorphism (SNP) in the human Her-2 was identified in the transmembrane coding region of the gene at codon 655, encoding either isoleucine (Ile) or valine (Val).[5] A study by Xie et al suggested that a germ-line missense variant in the transmembrane region of the Her-2/neu gene may influence breast cancer risk.[6] However none of the studies have determined if this polymorphism affects the ability of Her-2/neu to transform cells, and/or affect its tyrosine kinase activity. The relationship between the Val allele and breast cancer suggests that this polymorphism may be functionally important.[7]

The actual relationship between the Her-2 Ile655Val variant and Her-2 somatic activation is yet to be explored. Hence the present study explored the incidence and clinical significance of Her-2/neu gene Ile655Val SNP by evaluating SNP in transmembrane coding region at codon 655 Her-2/neu gene, encoding either isoleucine or valine. Additionally, we correlated it with Her-2/neu protein expression, clinicopathological parameters, estrogen receptor (ER), progesterone receptor (PR) status, disease recurrence and metastases and disease free and overall survival, treatment offered and molecular subtypes.

Materials and Methods

Patients

In this retrospective study, 127 untreated early and advance stage breast cancer patients who had been diagnosed and treated at the Gujarat Cancer and Research Institute between the years 1999 to 2005 with a minimum follow-up period of 5 years were included. Informed consent was obtained from all patients enrolled in the study. The treatment of the patients was decided by the clinicians of the institute. The primary treatment offered to all patients was surgery followed by adjuvant chemotherapy and /or radiotherapy (RT) and /or endocrine therapy. Surgical procedures were performed by oncosurgeons. Chemotherapy was instituted by the medical oncologists. The main treatment included CMF (cyclophosphamide + methotrexate + 5-fluorouracil), CMF + Tamoxifen (TMX), CMF + RT, CMF + TMX + RT and FAC (5-fluorouracil + adriamycin + cyclophosphamide), FAC + TMX, FAC + RT, FAC + TMX + RT, and OTHERS (FAC+CMF+TMX+RT, RT+TAXOL, EMSET+5FU+TMX). The histological assessments were done independently by histopathologist. Disease staging was done according to UICC TNM classification. Disease status was assessed by clinical examination, radiological and biochemical investigations. The disease status was correlated with the treatment offered.

Methods

For evaluation of Her-2/neu SNP polymorphism DNA was extracted from formalin fixed paraffin embedded (FFPE) tissues using QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's instruction. Briefly 15-20, 7 microns sections were taken in a microcentrifuge tube and paraffin was removed with 2 washes of xylene and

ethanol each, with 15 minutes of incubations followed by centrifugation. To the pellet 30 µl of proteinase K and 200 µl of ATL buffer were added and incubated at 57°C until the tissue was completely lysed. Then 200 µl of buffer ATL and ethanol were added mixed, centrifuged and the pellet was transferred to spin column. The lysate was washed with buffers (AW1 and AW2) and finally elute buffer ATE was added and DNA was obtained. The extracted DNA was stored at -20°C quantitated on spectrophotometer and optical density (OD) was taken at 260 nm and 280 nm to evaluate ratio and DNA concentration.

For PCR reaction briefly, approximately 5µg of DNA was added to PCR mix containing 5 µl of 10X PCR buffer, 10 µl of 5X Q solution, 2 µl $MgCl_2$ (2.5 mM final concentration), 1.5 µl dNTP (300 µM each final concentration) and 0.5 µl Taq DNA polymerase of PCR Core kit (Qiagen, Germany) and 5 µl (1.5 µM final concentration) each of forward (5'-agagcgccagccctctgacgtccat-3') as well as reverse primers (5'-tccgtttcctgcagcagtctccgca-3') to make a final volume of 50 µl. PCR amplification for Her-2neu SNP was carried out in Mastercycler gradient (Eppendorf, Germany) with conditions consisting of initial denaturation step at 95°C for 2 minutes followed by 40 cycles each of denaturation at 95°C for 30 seconds, annealing at 72°C for one minute and extension at 72°C for one minute and final extension step at 72°C for 10 minutes.

The PCR products were then subjected to enzyme digestion. Twenty five µl of amplified products were digested with 7 units of BsmaI enzyme (New England Biolabs, USA) and incubated overnight at 37°C. Undigested and digested products were loaded on 2% agarose gel with ethidium bromide was visualized on UV transilluminator and image was captured on Gel Documentation System (Alpha Innotech Corporation, USA). β-Actin served as internal control and exhibited amplified product of 350 base pair (bp) on 2% agarose gel.

Further Her-2/neu protein expression was evaluated by immunohistochemistry. Her-2/neu staining was done using CB 11 clone which showed membranous as well as cytoplasmic staining and was termed as membranous Her2/neu internal domain (ID) and cytoplasmic Her-2/neu ID respectively. Pure membranous staining was observed using SP3 clone and was termed as membranous Her-2/neu external domain (ED).

Also ER and PR status was evaluated to categorize patients into molecular subtypes, as luminal A (ER+, PR+, Her-2/neu-), luminal B (ER+,PR+, Her-2/neu+), Her-2+ (ER-, PR-, Her-2/neu+) and triple negative (ER-, PR-, Her-2/neu-). The study was approved by the institutional scientific and ethical committees.

Statistical analysis

The data was statistically analyzed using the SPSS statistical software, version 15. Two tailed $\chi 2$ test was used to assess the association between two parameters. Correlation between two parameters was calculated using Pearson's correlation coefficient (r) method. Univariate and multivariate survival analysis for disease free survival (DFS) and overall survival (OS) was done by Kaplan-Meier method and Cox-Forward Stepwise Regression method, respectively. P values ≤ 0.05 was considered significant

Results

The single nucleotide polymorphism of the Her-2/neu gene had been studied at codon 655 (Ile655Val) in 127 breast cancer patients. Three different genotype frequencies were observed. Ile/Ile homozygous genotype was observed at 148 bp (Figure 1a), Val/Val homozygous genotype was observed at 116 and 32 bp (Figure 1b) and Val/Ile heterozygous genotype was observed at 116 and 32 and 148 bp (Figure 1c).

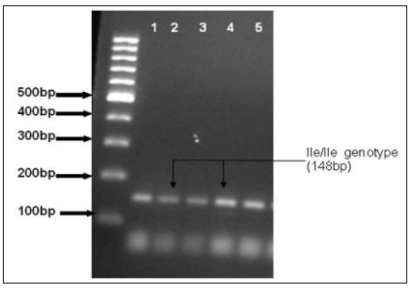

Figure 1a: Representative gel image of Her-2/neu SNP Lane 1,3,5-Undigested products of Her-2/neu at 148 bp Lane 2,4 - Ile/Ile genotype at 148 bp

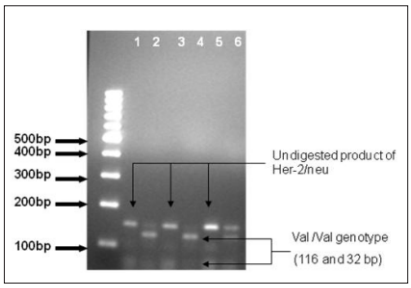

Figure 1b: Representative gel image of Her-2/neu SNP Lane 1,3,5-Undigested products of Her-2/neu at 148 bp Lane 2,4 - Val/Val genotype at 116 and 32 bp

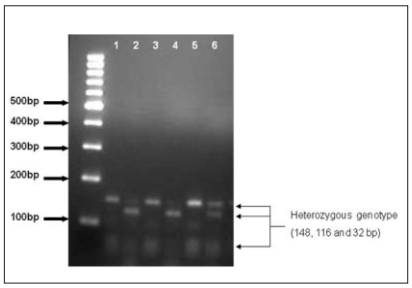

Figure 1c: Representative gel image of Her-2/neu SNP Lane 1,3,5 Undigested products of Her-2/neu at 148 bp Lane 4,6 - Val/Ile heterozygous genotype at 148 bp, 116 and 32 bp

Incidence of Her-2/neu gene Ile655Val SNP

The incidence of Ile/Ile genotype and Val/Val genotype was observed in 20% (25/127) patients each, and Val/Ile genotype heterozygous genotype in 60% (77/127) of patients (Table 1).

Correlation of Her-2/neu gene Ile655Val SNP with clinical parameters

Ile/Ile genotype, Val/Ile heterozygous genotype and Val/Val genotype were correlated with clinical parameters as age and menopausal status, wherein no significant correlation was observed as similar incidence of genotype frequencies was observed between subgroups of age and menopausal status (data not shown).

Correlation of Her-2/neu gene Ile655Val SNP with pathological parameters

In correlation of genotype frequency with pathological parameters the following correlation was observed.

Ile/Ile genotype:

A trend of higher incidence of Ile/Ile genotype was observed in early stage patients (22%, 17/78) as compared to advance stage patients (16%, 8/44). Further an increasing incidence of Ile/Ile genotype was observed with advancement of HG of the tumor {HG I (0%, 0/4), HG II (16%, 14/88) and HG III (31%, 11/35)} and with an increase in BR score {(low (0%, 0/5), intermediate (10%, 4/39) and high (22%, 6/27)} of the tumors. The incidence of Ile/Ile genotype was similar in NG II (22%, 5/23) and NG III tumors (20%, 2/10) which was higher than NG I tumors (0%, 0/2). With histological type, Ile/Ile genotype was observed in 33% (2/6) of patients with lobular carcinoma and 20% (22/111) of patients with IDC where as none of the patients with medullary carcinoma had Ile/Ile genotype. The incidence of Ile/Ile genotype was similar in patients with subgroups of disease stage, lymph node (LN) status, tumor size, lymphatic permeation (LP), vascular permeation (VP), ER and PR status. With metastatic site, higher incidence of Ile/Ile genotype was observed in patients with lung metastasis (29%, 2/7) as compared to other metastatic site.

Val/Ile heterozygous genotype:

A decreasing incidence of Val/Ile heterozygous genotype was observed with advancement of HG of the tumor {HG I (75%, 3/4), HG II (64%, 56/88) and HG III (51%, 18/35)} and NG of the tumor {NG I (100%, 2/2), NG II (65%, 15/23) and NG III (40%, 4/10)}. A higher incidence of Val/Ile heterozygous genotype was observed in T1 tumors (76%, 13/17) followed by T2 (62%, 35/57) and T4 tumors (62%, 5/8) followed by T3 tumors (53%, 24/45). The incidence of Val/Ile genotype was found to be similar in patients within subgroup of disease stage, LN status, BR score, LP, VP, ER and PR status. With metastatic site, higher incidence of Val/Ile heterozygous genotype was observed in patients with local recurrence (86%, 6/7) and multiple metastasis (82%, 10/12) as compared to other metastatic site.

Val/Val genotype:

A higher incidence of Val/Val genotype was observed in patients with LN positivity (24%, 18/75) than patients with LN negativity (14%, 7/52) and in patients with stage IV disease (50%, 1/2) as compared to stage III (25%, 12/47), stage II (16%, 11/68) and stage I (10%, 1/10) disease and in low BR score tumors (40%, 2/5) than intermediate BR score tumors (21%, 6/39) and high BR score tumors (19%, 5/27). The incidence of Val/Val genotype was similar in T3 tumors (25%, 11/45), T4 tumors (25%, 2/8) and T2 tumors (19%, 11/57) which was higher than T1 tumors (6%, 1/17). With histological type similar incidence of Val/Val genotype was observed in patients with medullary carcinoma (25%, 1/4) and patients with IDC (20%, 21/111), whereas none of the patients with lobular carcinoma had Val/Val genotype. Only one patient had phylloid tumor and expressed Val/Val genotype. The incidence of Val/Val genotype was similar in patients among groups of LP and VP. The incidence of Val/Val genotype was high in ER positive tumors (29%, 10/34) than ER negative tumors (16%, 15/93) and PR positive tumors (30%, 9/30) than PR negative tumors (17%, 16/97). With metastatic site higher incidence of Val/Val genotype was observed in patients with liver metastasis (50%, 4/8) than other metastatic site (Table 2).

Incidence according to molecular subtypes:

When correlated with molecular subtypes similar incidence of Ile/Ile genotype and Val/Ile heterozygous genotype among all molecular subtypes. In relation to Val/Val genotype, similar incidence was observed in patients with triple

Table 1: Incidence of genotype frequency of Her-2/neu in patients with breast cancer

Genotype	Incidence N(%)	Base pair
Ile/Ile	25(20)	148
Val/Ile heterozygous	77(60)	148 & 116 & 32
Val/Val	25(20)	116 & 32

Table 2: Correlation of genotype frequency with pathological parameters

Parameters		Ile/Ile N(%)	Val/Ile N(%)	Val/Val N(%)	χ^2	r	P
Tumor size	N (%)						
T1	17 (13)	03(18)	13(76)	01(06)	3.78	-0.13	0.70
T2	57 (45)	11(19)	35(62)	11(19)			
T3	45 (35)	10(22)	24(53)	11(25)			
T4	08 (06)	01(13)	05(62)	2(25)			
Lymph node							
Negative	52 (41)	12(23)	33(63)	07(14)	2.36	-0.09	0.30
Positive	75 (59)	13(17)	44(59)	18(24)			
Stage							
I	10 (08)	02(20)	07(70)	01(10)	3.68	-0.12	0.71
II	68 (54)	15(22)	42(62)	11(16)			
III	47 (37)	08(17)	27(58)	12(25)			
IV	02 (02)	00(00)	01(50)	01(50)			
Stage							
Early Stage	78 (61)	17(22)	49(63)	12(15)	2.51	-0.10	0.25
Advance Stage	44 (39)	08(16)	28(57)	13(27)			
Histology							
IDC	111 (87)	22(20)	67(60)	22(20)	12.47	12.47	0.40
Lobular	06 (05)	02(33)	04(67)	00(00)			
Phylloid	01 (01)	00(00)	00(00)	01(04)			
Mucinious	01 (01)	00(00)	01(100)	00(00)			
Medullary	04 (03)	00(00)	03(75)	01(25)			
IDC+Lobular	03 (02)	01(33)	02(67)	00(00)			
Comedo+DCIS	01 (01)	00(00)	00(00)	01(100)			
Histological Grade							
I	04 (03)	00(00)	03(75)	01(25)	4.83	-0.05	0.30
II	88 (69)	14(16)	56(64)	18(21)			
III	35 (28)	11(31)	18(51)	06(17)			
Nuclear Grade							
I	02 (06)	00(00)	02(100)	00(00)	4.69	-0.34	0.32
II	23 (66)	05(22)	15 (65)	03(13)			
III	10 (29)	02(20)	04(40)	04(20)			
BR Score							
Low (BR3- BR 5)	05 (07)	00(00)	03(60)	02(40)	3.58	+0.11	0.46
Intermediate(BR6-BR7)	39 (55)	04(10)	27(69)	08(21)			
High(BR8-BR9)	27 (38)	06(22)	16(59)	05(19)			
Lymphatic Permeation							
Negative	29 (34)	04(14)	19(65)	06(21)	0.07	-0.02	0.96
Positive	57 (66)	07(12)	39(69)	11(19)			
Vascular Permeation							
Negative	76 (89)	10(13)	52(68)	14(19)	0.09	-0.02	0.95
Positive	09 (11)	01(11)	06(67)	02(22)			
Estrogen Receptor							
Negative	93 (73)	20(22)	58(62)	15(16)	2.98	-0.11	0.22
Positive	34 (27)	05(15)	19(56)	10(29)			
Progesterone Receptor							
Negative	97 (76)	20(21)	61(63)	16(17)	2.65	-0.12	0.16
Positive	30 (24)	05(17)	16(53)	09(30)			
Metastatic site							
Bone	22 (34)	03(14)	15(68)	41(08)	22.76	0.14	0.12
Lung	07 (11)	02(29)	03(42)	02(29)			
Liver	08 (13)	01(13)	03(38)	04(50)			
Brain	05 (08)	01(20)	03(60)	01(20)			
Local Recurrence	07 (12)	01(14)	07(86)	00(00)			
Ovary	01 (02)	01(100)	00(00)	00(00)			
Multiple Metastasis	12 (20)	01(09)	10(82)	01(09)			

Table 3: Incidence of genotype frequency according to molecular subtypes

Molecular subtype	N(%)	Ile/Ile N(%)	Val/Ile N(%)	Val/Val N(%)	χ^2	r	p
Luminal A	20 (16)	03(15)	09(45)	08(40)	6.94	0.02	0.78
Luminal B	17 (13)	04(23)	11(65)	02(12)			
Her2-Positive	34 (27)	08(23)	21(62)	05(15)			
Triple Negative	56(44)	10(18)	36(64)	10(18)			

Table 4: Correlation of genotype frequency with Her-2/neu protein expression in total patients and in patients with Luminal A, B and Her-2 positive subtypes.

	Total patients				Patients with Luminal A, B and Her2 positive subtypes			
Breast Carcinoma	N(%)	Ile/Ile N(%)	Val/Ile N(%)	Val/Val N(%)	N(%)	Ile/Ile N(%)	Val/Ile N(%)	Val/Val N(%)
Membranous Her-2/neu ID								
Negative	78(61)	14(18)	45(58)	19(24)	22(31)	04(18)	09(41)	09(41)
Positive	49(39)	11(22)	32(65)	06(12)	49(69)	11(22)	32(65)	06(12)
		χ^2=2.84, r=+0.12, p=0.17				χ^2=7.60, r=+0.30, p=0.02		
Cytoplasmic Her-2/neu ID								
Negative	106 (84)	20(19)	64(60)	22(21)	50(70)	10(20)	28(56)	12(24)
Positive	21(16)	05(24)	13(62)	03(14)	21(30)	05(24)	13(62)	03(14)
		χ^2=0.59, r=+0.03, p=0.67				χ^2=0.85, r=+0.08,p=0.65		
Negative	102 (80)	19(18)	61(60)	22(22)	46(65)	09(20)	25(54)	12(26)
Positive	25 (20)	06(24)	16(64)	03(12)	25(35)	06(24)	16(64)	03(12)
		χ^2=1.28, r=+0.06, p=0.44				χ^2=1.93, r=+0.14,p=0.38		

Table 5: Disease Free Survival and Overall Survival of genotype frequency

	Disease Free Survival			Overall Survival		
Genotype	N(%)	Remission N(%)	Relapse N(%)	N(%)	Alive N(%)	Dead N(%)
Ile/Ile	24 (21)	12(50)	12(50)	25 (19)	18(72)	07(28)
Val/Ile	68 (60)	30(44)	38(56)	75 (59)	60(80)	15(20)
Val/Val	22 (19)	11(50)	11(50)	25 (19)	18(72)	07(28)
		(Log rank = 0.55, df=2, p=0.75)			(Log rank = 2.26, df =2, p=0.32)	

negative (18%, 10/56), Her-2 positive (15%, 5/34) and luminal B (12%, 2/17) subtypes which was lower as compared to patients with luminal A (40%, 8/20) subtype (Table 3).

Correlation of genotype frequency with membranous Her-2/neu (ID), cytoplasmic Her-2/neu (ID, truncated Her-2/neu) and membranous Her-2/neu (ED) expression:

The genotype frequency when correlated with Her-2/neu protein expression, in total patients the incidence of Val/Val genotype was low in patients who expressed membranous Her-2/neu ID (12%, 6/49), cytoplasmic Her-2/ID (14%, 3/21), and membranous Her-2/neu ED (12%, 3/25) as compared to the patients who did not express membranous Her-2/neu ID (24%, 19/78), cytoplasmic Her-2/neu ID (21%, 22/106) and membranous Her-2/neu ED (22%, 22/102) respectively The incidence of Ile/Ile genotype and Val/Ile heterozygous genotype was similar in patients with and without expression of membranous Her-2/neu ID, cytoplasmic Her-2/neu ID and membranous Her-2/neu ED (Table 4).

In patients with Luminal A, luminal B and Her-2 positive subtypes, the incidence of Val/Val genotype was significantly low in patients who expressed membranous Her-2/neu ID (12%, 6/49), as compared to the patients who did not express membranous Her-2/neu ID (41%, 9/22; χ^2=7.60, r=+0.30, P=0.02). The incidence of Val/Val genotype

was similar in patients with and without expression of cytoplasmic Her-2/neu ID and membranous Her-2/neu ED. Similar incidence of Ile/Ile genotype was observed in patients with and without expression of membranous Her-2/neu ID, cytoplasmic Her-2/neu ID and membranous Her-2/neu ED. A trend of high expression of Val/Ile heterozygous genotype was also noted in patients with expression of membranous Her-2/neu ID (65%, 32/49) than without (41%, 9/22) expression of membranous Her-2/neu ID and was found to be similar in patients and without expression of cytoplasmic Her-2/neu ID and membranous Her-2/neu ED (Table 4).

Univariate Survival analysis:

In total patients, genotype frequency showed no correlation with DFS and OS as similar incidence of disease relapse and death was observed in patients who expressed Ile/Ile genotype, Val/Ile heterozygous genotype and Val/Val genotype (Table 5).

Multivariate analysis with Her-2/neu gene Ile655Val SNP and conventional parameters:

Cox regression Forward Likehood ratio multivariate analysis was performed with inclusion of Her-2/neu gene Ile655Val SNP and conventional parameters (age, menopausal status, tumor size, LN status, disease stage, HG, lymphatic permeation, vascular permeation, BR score, and ER and PR). None of the parameters entered the equation for disease free survival or overall survival in total patients and in patients with Luminal A, B and Her-2 positive subtypes.

Discussion

The single nucleotide polymorphism (SNP) in the human Her-2/neu gene was identified in the transmembrane coding region of the gene at codon 655, encoding either isoleucine (Ile) or Valine (Val).[8] Changing the existing isoleucine (Ile ATC) to Valine (Val GTC) at codon 655 suggests an increased dimerisation, autophosphorylation of Her-2 and tyrosine kinase activity which may cause the transformation of cells.[9] Some of the reports have revealed presence of development of Ile655Val polymorphism is associated with increased risk of breast cancer risk.[6,10-13] On the other hand others have shown that this correlation is controversial.[14-19] One reason for these contradictory results might be the sustained differences in genetic polymorphism in Her-2/neu codon 655 between ethnic groups. In a study by Papadopoulou E et al Val/Val or Val/Ile genotype was associated increased breast cancer risk than Ile/Ile in Christian population and no such significant association was observed in Muslim population of Thrace.[20] This inconsistent association between SNP and breast cancer risk across these two different ethnic groups and supported that polymorphism varies according to racial decent.

In the present study, SNP of Her-2/neu gene had been studied at codon 655 in FFPE extracted DNA of 127 breast cancer patients. Of these 127 patients, 20% (25/127) patients showed expression of Val/Val and Ile/Ile genotype each and 60% (75/127) of the patients showed Val/Ile heterozygous genotype. The frequency of the incidence of Val/Ile heterozygous genotype was higher in breast cancer patients in the present study. However, majority of the studies have observed higher frequency of Ile/Ile genotype than Val/Ile and Val/Val genotypes. Cox. et al in a series of 1271 breast cancer patients observed Ile/Ile genotype in 60%, followed by Val/Ile heterozygous genotype in 35.5%, and Val/Val genotype in 4.5%.[10] Similarly Nelson et al demonstrated higher incidence of Ile/Ile genotype in 58.2% followed Val/Ile heterozygous genotype in 36% and Val/Val genotype in 5% of the patients.[21] Zubor et al in 47 Caucasian population observed higher frequency of Ile/Ile genotype (47%) and Val/Ile heterozygous genotype in (47%) than Val/Val genotype in (6%).[22] Kallel et al observed Ile/Ile genotype in 87%, Val/Ile heterozygous genotype in 9% and Val/Val genotype expression in 3% in patients of Tunisia.[19] In these studies Ile/Ile genotype was observed in range of 47% to 80%, Val/Ile genotype was 9% to 47% and Val/Val genotype was 3% to 6%. Compared to these studies a higher incidence of Val/Val and Val/Ile genoytpes and lower incidence of Ile/Ile genotype was observed in the present study. The observed difference could be of geographic and ethinic variations. Further, there was no data on Her-2/neu SNP available from India. Majority of the studies have determined Her-2/neu protein by IHC and gene by FISH. However, meta analysis of 22 studies by Yanlei et al indicated a modest association between the Her-2/neu Ile polymorphism and Asian population, suggested that difference in genetic background, environment do not influence the Her-2/neu Ile655Val polymorphism and breast cancer susceptibility.[23] Contrary to that study of Tao et al indicated that SNP at Her-2 codon 655 could be considered as a susceptibility biomarker for Asian women age 45 years or younger.[24]

In the present study, the uniform distribution was observed in genotype frequencies when correlated with clinical parameters such as age and menopausal status. Whereas few studies demonstrated that women with age less than 40 years with homozygosity for Val/Val genotype had an increased risk of early onset of breast cancer.[6,25] Contrary to that another study indicated that SNP at Her2 codon 655 could be considered as a susceptibility biomarker for breast cancer for Asian women of age 45 years or younger.[24] In postmenopausal women with age > 45 years Xie et al

and Cowdin et al demonstrated that Ile655Val variant confers a modest increase in breast cancer risk among women for all stages of disease.[6,26] Moreover, women with germline Val genotypes are more likely to develop localized disease and less likely or slower to progress to high stage breast cancer than women with Ile genotype. Unlike our study observed increasing incidence of Val/Val genotype with disease advancement. Higher frequency of heterozygotes for Val allele was observed among premenopausal breast cancer patients and patients with, positive for HER2/neu status and advanced stage in a study by Surekha et al. [27] A higher frequency of Val allele was demonstrated by Wu Ch et al in cases having nodal metastasis and tumor recurrence.[28] In a study on breast cancer, invasive carcinomas, low differentiation tumors, advanced stage, positive lymph nodes, high number of lymph nodes and Her-2 /neu overexpression were more frequent in patients with allele Val than those with allele Ile.[20] The association of Val/Val Her-2/neu genotype with clinicopathological characteristics and Her-2/neu expression indicated its possible implication on more aggressive phenotype.

Interestingly all patients with triple negative subtype, exhibited Her-2/neu gene expression with an incidence of 64% of Val/Ile and 18% each of Val/Val and Ile/Ile gentoypes. Among molecular subtypes luminal A had higher frequency of Val/Val genotype (40%) and lower frequency of Val/Ile genotype than luminal B, Her-2 positive and triple negative subtypes.

The incidence of Val/Val genotype was found to be low in patients with expression of membranous Her-2/neu ID, cytoplasmic Her-2/ID and membranous Her-2/neu ED positive group. When the correlation was evaluated in luminal A, luminal B and Her-2 positive subtype the incidence of Val/Val genotype was significantly low in patients with expression of membranous Her-2/neu ID than without expression of membranous Her-2/neu ID. The observation of lower incidence of Val/Val genotype in Her-2/neu protein negative tumors remained unexplained. It has been hypothesized that germ-line variant initially increases cellular proliferation while subsequently decreasing the likehood that Her-2 gene will undergo amplification or protein overexpression.[26] However, over expression of Her-2/neu in large number of cases indicates that activation of this gene is an important step in breast carcinogenesis.

With disease status, breast cancer patients with Ile/Ile, Val/Ile and Val/Val genotypes showed similar incidence of relapse and death. However, Val allele expression in oral squamous cell carcinoma was associated with increased risk of tumor recurrence and with poorer recurrence free survival of patients.[28] Also, a study suggested that TNF-alpha remained as an independent prognostic factor of worse overall survival however in combination with Val/Val genotype predicted a worse prognosis than high TNF-alpha alone.[29]

The results suggest that Her Ile655Val polymorphism especially in homozygous form might play a role in etiology of breast carcinoma. However, to evaluate incidence of SNP in patients with breast carcinoma it needs to be confirmed in larger women cohort.

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"Yoga is not a religion. It is a science, science of well-being, science of youthfulness, science of integrating body, mind and soul."
Amit Ray,

Stress and Doctors- Embrace Meditation as Medicine

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Few months ago, I attended a medical conference where one of the India's leading meditation gurus gave a talk on how meditation can positively help doctors relieve their stress and also improve doctor patient relationships. The research is becoming overwhelming that meditation is the most powerful moment to moment stress reducing, burnout preventing technique for physicians.

I also want to share 'an 80-year-old's letter to a doctor'.1 "I think I have been visiting you now for more number of years than I can remember. My age is to be blamed for my poor memory. At 80, I can hardly remember much. Yet, what I can never forget is your pleasing smile each time you saw me enter your clinic. And the patience with which you listened to my never-ending health woes... and the brilliance with which you diagnosed the problem and treated it - I'm eternally grateful. As I was sipping my morning coffee today, I happened to come across an article in a health magazine which said that according to a survey, nearly 78 percent of the doctors become patients themselves. I was astonished and worried at the same time, for I would never want my favorite family doctor to fall sick! The article said that since the job of a doctor demands them to be available all the time, they are hardly able to get sufficient rest and are also quite stressed out. I do realize doctor that considering your extensive experience in the profession, I'm in no position to give you any word of advice. Yet because you have always been as kind to me, not just as a family doctor but as a friend and guide all through these years, I would just request you to pay heed to this. Meditation is a very simple yet extremely effective technique, so I hear from a few friends - some of whom are doctors who practice meditation themselves. They shared varying benefits of practicing meditation. This very young-at-heart lady has taken the liberty of demanding your attention for a little while longer."

Dr. Devi Shetty, a famous cardiac surgeon in Bangalore also said in one of his interviews that while a doctor's body needs to endure long hours of standing to perform an operation, his mind has to be equally or more fit to endure the stressful long hours. He also said that a surgeon getting into pressure and anxiety does not help the patient and so meditation helps keep the mind calm and relaxed so that we are able to support the patient in turn.

One senior surgeon once told me that regularly meditating has enhanced the loving and caring side of her personality which has immensely helped her as a doctor. She feels she has become much more humane in dealing with her patients. I think meditation can only add more to what you are already gifted with.

Meditation is slowly expanding beyond its fringe following, appealing to a wider audience, even in the data-driven medical world. More doctors are prescribing meditation to help treat anxiety and depression, lower blood pressure and manage pain, according to a recent study by the Harvard Medical School. It's one of several studies showing that meditation can actually alter how the brain works. The trend has gained a foothold especially among health professionals, some of whom practice meditation themselves to cope with the demands of their stressful occupations. Ever so gradually, they've moved from practicing the technique to preaching it. For a long time, doctors who meditated were quiet about it, said Dr. Selma Sroka, medical director of Hennepin County Medical Center's Alternative Medicine Clinic. "It wasn't professional. It wasn't medical to talk about it," she said. "I think things are getting more open."[2]

The body's stress response, also known as "fight or flight," is aggravated by emotional or physical stress, she explained. The opposite of that reaction is the body's relaxation response. Meditation triggers that response. "Any chronic illness can be benefited from emptying one's mind and not thinking, and breathing more deeply," Sroka said. "That's all part of meditation."[2]

Job of a doctor is extremely demanding so learning meditation can give you new lease on life. I read that meditation helps give a positive mental outlook. I think this would be particularly important in a profession like ours where we keep listening to so many patients sharing their challenges. Meditation can really keep us positive, balanced and unshaken even while we offer them solace. Some reports say that meditation can instantly charge you up. Being on our toes all the time is not very easy and there may be times when we feel stressed out and pulled down on energy. Maybe we can try meditating then. Meditation can really help increase our patience. We all know that the virtue of patience is the most relevant in the field of medicine. And this is where it can help doctors deal with so many different kinds of patients each day, each moment in an effective way. Regular practice of meditation helps improve time management and focus so that we are able to give your 100 percent to what we are doing. Meditation helps improve clarity of mind

and makes you more intuitive.[1-3]

Quieting the mind in our loud and chaotic world is a skill that can be difficult to master.

How can docs meditate?

- All you need to do is just sit comfortably in a quiet corner, close your eyes and be with yourself. You may start with 10 minutes and gradually increase it to 20 as you get comfortable.
- See what time suits you best for meditation. You may do it while going to work for 10 minutes (if you are not driving). Before a surgery, meditation can be the best thing to do. It instantly calms the mind and sharpens focus.
- Once you start experiencing its benefits, you may want to encourage at least one doctor or nurse on your team every week to follow suit.

Instead of going on medication, take a different track: meditation.

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Management of Chronic Refractory Pain in a Case of Brachial Plexus Avulsion Injury - A Case Report

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Summary

Avulsion of one or more cervical roots of the brachial plexus causes severe, disabling pain. Various medical and surgical treatment modalities have been used to relieve such pain. Most of them offer only limited benefits. Radiofrequency thermo coagulation (RFTC) of the dorsal root entry zone (DREZ) of spinal cord is one of the most effective surgical treatments for these patients. A case of chronic refractory neuropathic pain following brachial plexus injury is described here. RFTC of cervical (C5–T1) DREZ was carried out with good pain relief.

Keywords: Brachial plexus avulsion, Dorsal root entry zone (DREZ), Neuropathic Pain, Deafferentation pain

Introduction

Brachial plexus injury is frequently associated with avulsion of the dorsal or ventral root, or both, from their associated entry zones into the spinal cord. This leads to the production of deafferentation pain in up to 20-30% of patients.[1] This pain is resistant to several pain control procedures including analgesic drugs like narcotics, antidepressants and anticonvulsants, cervical sympathetic ganglion blockade, trans electrical nerve stimulation amputation, sympathectomy, cordotomy etc. Radiofrequency thermo coagulation (RFTC) of dorsal root entry zone (DREZ) of the avulsed roots was first described by Nashold et al in 1976 as a treatment for this type of pain.[2]

Case report

A 42 years old male patient with history of fall from motor cycle resulting in right brachial plexus injury, for which he had undergone plexus repair six months back came to our hospital. Since that time, patient had paralysis with loss of sensation and severe crushing and burning type pain in the right arm. On examination right upper limb power grade was 0/5, sensory loss from C5 – T1 and reflexes were absent. MRI was suggestive of contusion in the right supraclavicular area. Pain persisted in right arm and was referred for pain management. He was treated with oral analgesic drugs like non steroidal anti-inflammatory drugs (NSAIDs) tramadol hydrochloride, anticonvulsants like pregabalin, gabapentin, antidepressants like amitryptalin with partial pain relief. Intravenous 2% lignocaine hydrochloride infusion at the rate of 5 mg/kg and epidural ketamine hydrochloride with bupivacaine hydrochloride were effective for short term pain relief. Pulsed Radiofrequency (RF) lesioning of stellate ganglion (SG) done under image intensifier television (IITV) guidance. Pain relief was only 10-15% followed by pulling sensation of muscles which was treated by tablet baclofen 10mg once a day and diclofenac gel local application. None of this treatment gave satisfactory pain relief. Then he was subjected to temporary spinal cord stimulation (SCS) implantation at private hospital. Pain relief was 30% only. Ultimately RFTC DREZ was planned for this refractory pain. After C5 – T1 laminectomy DREZ lesions were spaced at interval of 2 mm for 15 seconds and temperature 800C, about 50 in numbers using thermo coagulation radio frequency generator. (RFG-3CF graphics RF lesion generator system, Radionics, INC, Burlington, MA) Operative findings showed atrophic nerve roots on right side.

Post operatively immediate pain relief was 100%. After surgery patient developed pad like feeling and heaviness in right lower limb within 48 hours, however he was able to walk, for which he was treated with drugs pregabalin and vitamin B_{12}. At 6 months and 2 years follow up patient had complete pain relief and right lower limb problems gradually abated.

Discussion

Significant numbers of patients experience intractable pain after brachial plexus root avulsion. Medications and surgical procedures are often not successful. Central diafferentation pain that persists and becomes intractable among patients with traumatic cervical avulsion root has been difficult to treat.[3]

In acute phase $NSAID_s$ are useful however gabapentin and pregabalin may be considered the first line of drugs for the treatment of central pain due to their consistent efficacy, safety and minimal potential for drug-drug interactions and tricyclic antidepressants a second line of treatment and third line of treatment include opioids and tramadol. In many cases treatment is insufficient, associated with range of side effects and often combination therapy is used.[4]

In neuropathic pain intravenous lignocaine produces dose dependent suppression of allodynia without blocking nerve conduction, reduces neuropathic pain behavior by increasing the threshold for mechanical allodynia and reduces the discharge rate of injured nociceptive fibers. One retrospective study provides evidence that intravenous lidocaine administered in an escalating dose to 5 mg/L under carefully monitored conditions is safe and may decrease many signs and symptoms of severe complex regional pain syndrome.[5]

Ketamine has an analgesic action both centrally and peripherally. In situations where standard analgesic options have failed, ketamine is a reasonable option for severe chronic pain. The mechanism of action in the reversal of opioid tolerance by ketamine is believed to

involve an interaction between NMDA receptors, the nitric oxide pathway and μ-opioid receptors. Ketamine also inhibits serotonin and dopamine reuptake and inhibits voltage-gated Na and K channels.[6] Takahashi etd reported complete pain relief using ketamine by the epidural route in patients refractory to other pain treatments.[7]

SCS is one of the most effective modalities for management of refractory neuropathic pain unresponsive to conservative therapies. The mechanism action is based on Gate control theory postulating a spinal modulation of noxious inflow. In combination with comprehensive medical management like physical and psychotherapy, SCS can provide long term pain relief with concomitant improvement in the quality of life, daily function and patient satisfaction. This technique is cost effective in the long term despite its high cost.[8] In a prospective study of 19 patients treated with SCS, Oakley et al shown that 80% of patients experienced at least 50% improvement in pain rating scale, Sickness impact profile and VAS after an average follow up period of 7.9 months.[9]

Pulsed RF lesioning of SG is useful in the management of neuropathic pain. It seems to alter the synaptic transmission, producing a neuromodulatory effect, thus providing analgesia without causing any tissue destruction or painful after effects. A retrospective study of 86 RF-SG block shown benefits for patients suffering from complex regional pain syndrome type 2, cervicobrachialgia or post thoracotomy pain.[10]

In patients with central deafferentation pain that persists and becomes intractable to other treatments, DREZ lesioning of cervical root gives long lasting satisfactory pain relief with acceptable morbidity rates.[3]

The concept of DREZ lesion involves surgical destruction of second order neurons of the ascending nociceptive pathway. Destruction of the DREZ and superficial dorsal horn is thought to abolish this abnormal electrical activity and thus help relieve pain.[1] Twenty-six patients with intractable deafferentation pain after brachial plexus avulsion lesion treated with DREZ lesioning showed that analgesic effect of DREZ micro coagulation surgery gradually decreased over the longer period of time. However, this technique is still effective treatment of brachial plexus avulsion pain, as most patients had >50% pain reduction even after 5 years of surgery without the need for additional analgesic therapy.[11]

In our case also the pain of brachial plexus injury was refractory to different medical and surgical pain modalities and was treated with DREZ lesioning of cervical root with excellent pain relief. After surgery patient developed pad like feeling and heaviness in right lower limb within 48 hours however he was able to walk for which he was treated with drugs pregabalin and vitamin B_{12}. At 6 months and 2 years follow up patient had complete pain relief and right lower limb problems gradually abated.

Conclusion

Patients with severe disabling pain of brachial plexus avulsion, refractory to various medical and surgical pain procedures benefit with DREZ lesioning of cervical root with an acceptable morbidity.

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Unusual Site Metastases of Gastrointestinal Carcinoma: Diagnostic Role of FNAC

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Summary

Cutaneous and subcutaneous metastases from internal malignancies are uncommon, with reported frequency of 0.2 - 10%. They can occur as a sign of disease recurrence or as a sign of terminal disseminated cancer or rarely , as first manifestation of an occult malignancy. Mostly, they present as subcutaneous or dermal nodules, close to the site of primary tumour. Usually, skin involvement is synonymous with extensive disease and frequently implies a poor prognosis. Therefore, such cases are treated with palliative therapy and so, fine needle aspiration cytology (FNAC), which is a minimally invasive procedure, can be used as a diagnostic modality for their detection. It is a simple and rapid procedure that can be used as first line of investigation in such cases. We hereby report two cases of gastrointestinal carcinoma who presented to us with cutaneous metastases, one in the popliteal fossa and another at upper back. Both of our cases were diagnosed by FNAC.

Keywords: Cutaneous metastases, FNAC, Gastrointestinal carcinoma

Introduction

The most common metastatic sites of gastrointestinal (GI) cancers are liver, regional lymph nodes and lung. Cutaneous metastasis of internal malignancies is quite uncommon, being discovered in only 0.2-10% of autopsies performed on patients with cancer.[1,3,5,9] Most of these cutaneous metastases present in the form of subcutaneous and dermal lesions. These nodules usually tend to be firm, rubbery and not painful, thus can mimic lipomas or neurofibromas.[2] Usually, for almost all cancers, skin involvement is synonymous with extensive disease and frequently implies a poor prognosis. Early detection and proper diagnosis of such lesions can significantly alter the treatment and prognosis. In rare cases, such metastasis may be the only sign of disease in postoperative patients or they may present as the first manifestation of unsuspected asymptomatic occult neoplasm in an otherwise healthy person. Herein, we present two different cases of gastrointestinal adenocarcinomas that subsequently developed cutaneous metastasis at unusual site.

Case reports

CASE 1: A 55-year-old male presented to us with a 1-month history of pain and subcutaneous swelling in the left popliteal fossa. No history of fever, any recent trauma or skin rash at local site was present. In his past medical history,he was operated for gastric adenocarcinoma (T3N0M0) 28 months back. Ten months later, he presented with recurrence at the local anastomotic site so was given chemotherapy as well as radiation. After two months, again he had metastasis at left supraclavicular region. So, he received palliative radiotherapy (30 Grays in 2 weeks in 10 #) and has demonstrated a good response to the treatment symptomatically. At present he has left popliteal fossa swelling. His post radiation CT showed no signs of any visceral metastases.

On physical examination, a 1x1cm^2 hard, non-tender, mobile swelling of the left popliteal fossa was detected. FNAC of the swelling was performed. Cytological smears were prepared and stained with modified Papaniculaou (Pap) stain.

CASE 2: A 42-year-old male came to us with complaint of multiple skin nodules at upper back. He was a known case of carcinoma rectum (mucinous adenocarcinoma) for which he underwent abdominoperineal resection in September 2013 and also received full course of adjuvant chemo and radiation therapy. During follow up examination in January 2014, CT scan revealed residual lesion in recto-sigmoid junction along with few enlarged lymph nodes in para-aortic and meso-rectal region but without any metastasis to other visceral organs. On physical examination, multiple firm, non-tender, immobile nodules varying in diameter from 0.3 – 1.0 cm were found. FNAC was performed from cutaneous nodules, smears were prepared and stained with modified Pap stain.

Cytomorphological findings

Cytology smears for the two cases showed malignant cells arranged in clusters, glandular pattern and scattered singly in the background of extracellular mucin (Figure 1). Tumour cells were medium sized with moderate amount of pale eosinophilic to finely vacuolated cytoplasm, vesicular chromatin and prominent nucleoli (Figure 2). Both the cases were diagnosed as metastatic adenocarcinoma.

Discussion

Cutaneous metastases from internal malignancies are rare, occurring in less than 5% of patients and are reported most frequently after the fourth decade of life.[2,3,7] It can occur anytime in the

course of malignancy. The incidences of various tumors that metastasize to skin correlates with a frequency of occurrence of the primary malignant tumor in each gender. Breast carcinoma (69%) is the commonest cause of cutaneous metastases in women followed by carcinoma of the large intestine (9%), lungs and ovaries (4%).The primary sites of carcinoma with cutaneous metastases among men in decreasing order are lungs (24%), large intestine (19%), oral cavity (12%), kidney and stomach (6% each).[4,5] They usually occur close to the site of primary tumour, that is, chest in lung and breast carcinoma, abdominal wall in gastrointestinal malignancies and lower back in renal carcinomas.[6]

Usually skin involvement of cancer cells can occur through several mechanisms, which includes; spread to regional skin via lymphatics, spread to distant sites due to hematogenous spread, direct contiguous tissue invasion or by implantation during surgery. Chest and abdomen followed by head and neck are the most common reported sites of cutaneous metastasis.[6] But in our cases, metastasis was in popliteal fossa (case 1) and upper back (case 2),which are unusual sites of cutaneous metastasis from GI carcinoma.

There are several hypothesis described for metastases in different organs which can also hold true in case of cutaneous metastasis but further studies are required to establish the same. Stephen Paget first described the"seed and soil" hypothesis, which stated that metastasis is not due to chance events, but rather certain tumour cells (the seeds) grow preferentially in selective organs that have intrinsically favourable micro-environment (the soil).It is possible that the interaction between tumour cells and certain factors secreted from the dermis or epidermis play a crucial role in the skin homing mechanism of metastatic cells. Chemokines and their receptors have been shown to be involved in tumorigenesis and metastasis.[8]

It may be difficult sometimes to distinguish primary adnexal tumor from metastatic lesion. In the Present Study both of our patients were immunocompromised, so, secondary infective granuloma and primary adnexal tumours of skin were the mains differential diagnose:s .

These cutaneous metastases are easily accessible and palpable thus allowing us to use FNAC as first line of investigation. It also proves to be an efficient and quick method of microscopic confirmation and reduces the numbers of surgical biopsies. It is done on an outpatient basis with routinely available equipments. It is inexpensive, less traumatic and rapid procedure as compared to punch biopsies. The turnover time for the report is just 4–6 hours. Since the diagnosis is rapidly available on FNAC, appropriate therapy for the patient can be started earlier. According to the study of 83 cases of skin and subcutaneous metastasis from internal malignancy by Bansal et al, no false-positive and false-negative diagnoses were given. The study yielded a sensitivity and specificity of 100% for FNAC as a diagnostic modality in cutaneous and subcutaneous metastases of internal malignancies.[9] Moreover, if cell blocks are prepared, they can be used

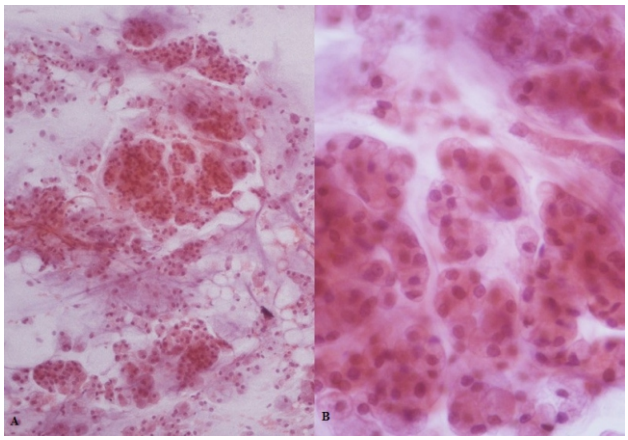

Figure 1: (A) Neoplastic cells arranged in glandular arrangement and in clusters in the background of extracellular mucin.(Pap, 10x). (B) Malignant cells with moderate to abundant cytoplasm and moderate nuclear atypia. (Pap, 40x).

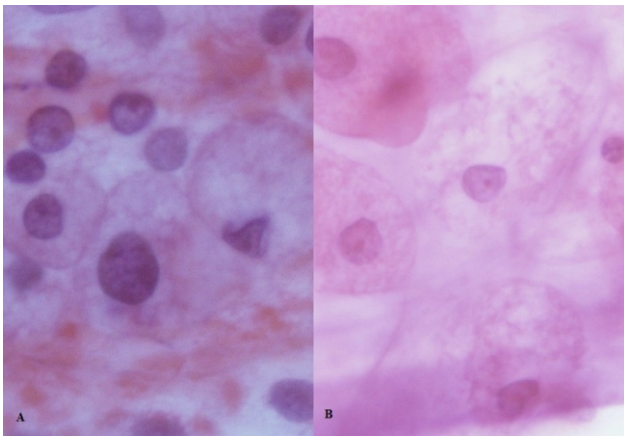

Figure 2: (A) and (B) Individual malignant cells are medium to large sized with moderate to abundant finely vacuolated cytoplasm, nuclear hyperchromasia and prominent nucleoli. (Pap, 100x).

for immunostaining and electron microscopy. This is particularly important in cases where skin metastases are the first sign of internal malignancy.

Conclusion

We conclude that skin metastases from internal malignancies are rare and may have an unpredictable presentation. Our study demonstrates the unequivocal role of FNA as a diagnostic modality for the detection and confirmation of cutaneous metastases from internal malignancies at unusual sites. It is safe and rapid procedure and recommended as a first line of investigation.

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"Meditation is the dissolution of thoughts in Eternal awareness or Pure consciousness without objectification, knowing without thinking, merging finitude in infinity."

Voltaire

Summaries of International Symposium on Recent Trends in Cancer Research: from OM to OMICS (awardees)

01. Expression of EGFR Isoforms - Efforts to Combat Gliomas in an Indian Scenario

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Summary

An all encompassing code- ATGC formulates the core of all life better known as the genome. For cancer research, especially brain tumors where conventional methods of diagnosis or treatment do not always translate to better prognosis, Omics has proven to be a beneficial tool. Here we have studied the Epidermal Growth Factor Receptor (EGFR) – the wild type (EGFR A) transcript and 3 isoforms (EGFR B; EGRFR C; EGFR D) missing the intra- and inter-cellular domain using real time PCR for copy number variation analysis. Seventy nine patients with gliomas were enrolled for this study. Out of this eight tumors were of pediatric origin. Hyper proliferation was observed in 8 adult tumors most of which were of male origin. EGFR B showed significant variation with increase in age and it also played a role with EGFR D in different genders. Cell types changed the expression levels where tumors of oligodendrocytic origin showed higher expression of all isofroms followed by astrocytic tumors. Ependymal and embryonal origin tumors showed comparatively lower levels of isoforms. glioblastoma multiforme, the most menacing form of brain tumors, showed a hyper expresssion of EGFR variants. Higher expression of all EGFRs was also observed in grade IV tumors and it was the lowest in grade I tumors. This is the first Indian study showing an impact of clinical factors and cell type on EGFR variants in patients suffering from glial tumors, a life threatening disease with very short life expentency.

02. Role of Poly (I: C) in Treatment of Oral SCC

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Summary

The role of Poly (I:C), a double stranded RNA which mimics viral infection in mice has long been implicated in experimental therapeutics which principally works by activating Iterferon-3 (IRF-3) in blood which in turn activates the natural killer cells (NK cells) that are cytotoxic to tumor cells. The role of transglutaminase 2 (TGM2) in exacerbating Oral Squamous Cell Carcinoma (SCC) is known and it acts by activating Tissue Growth factor-beta (TGF-B). We wish to establish a connection between Poly (I: C) and TGM2 and treat the oral SCC using gene therapy. This study is carried out to elucidate the connection between Poly (I:C) and transglutaminase 2 (TGM2) in oral squamous cell carcinoma and potential treatment in mice.

12 carcinogenic 4-nitroquinoline-1-oxide (4NQO) model of mice are to be used in study of which 6 are to be given Poly (I:C) and other 6 the vehicle. Post 15 days of the treatment, the level of TGM2 in blood and tumor tissue along with the area of tumor using ELISA, RT-PCR, Western blot and micrometer caliper, respectively are to be measured. Retinoic acid (RA), a TGM2 agonist and cystamine, a TGM2 specific antagonist are to be given to 3 mice of each group, respectively. Post 15 days, TGM2 expression in blood and tumor tissue is to be investigated using the same methods used above.

We expect to see the reduction in size of tumor and TGM2 expression in blood and tumor tissue in poly (I:C) treated mice. Therapeutically we also expect to see the increase in size of tumor in RA treated and decrease in size in cystamine treated mice. If the expected results fall in line with our hypothesis, it would be clear that TGM2 overexpression leads to oral SCC and that the poly (I:C) works by down regulating the expression of TGM2. Furthermore, knocking out TGM2 gene could be the potential therapy for oral SCC.

03. Design, Synthesis and Anti-cancer Activity of Novel Pyrazole Derivatives as c-MET Receptor Tyrosine Kinase Inhibitors

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Summary

Cancer is very complicated disorder seen in human beings which is a leading cause of death worldwide. Lung, stomach, liver, colon and breast cancer cause the most deaths due to cancer each year. One of the important therapeutic targets with its aberrant signaling mediating invasive cellular program in cancers is c-MET Receptor Tyrosine Kinase. c-MET pathway is responsible for triggering various signaling pathway like RAS-MAPK pathway, PI3K pathway, STAT3/5 pathway and ALK pathway. c-MET triggers tumor growth, formation of new blood vessels that supply the tumor with nutrients and

thus, cancer spreads to other organs. In this present studies, computational followed by synthetic approaches were used to design and synthesized small molecules targeting c-MET kinase. The generation of pharmacophore was carried out using GALAHAD module of Sybyl in order to identify essential features required to be present in c-MET kinase inhibitors. All the generated models of pharmacophore were validated using required validation protocol. The best model was subjected to virtual screening in which about 18000 molecules were retrieved. 3D-QSAR studies were performed on a potent series of pyrollotriazine targeting c-MET kinase. Based on results of pharmacophore and 3D QSAR studies, 25 molecules were designed having pyrazole ring and docked onto respective c-MET kinase (PDB ID: 3DKF). Based upon docking score, 4 molecules were synthesized and characterized using physical and spectral methods. *In-silico* ADMET were also done to predict the pharmacokinetic and toxicity profile of synthesized molecules. The anti-cancer activity was executed on MCF-7, HCT-15, A-375 cell lines showing prominent results.

04. Prediction of potential phytochemical interaction towards Synovial Sarcoma Protein TLE-1

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Summary

Synovial sarcoma is a soft tissue malignancy. Transducer-like enhancer of split 1 (TLE1) is overexpressed in synovial sarcomas. TLE1 is an excellent discriminator of synovial sarcoma from other sarcomas, including histologically similar tumors, such as malignant peripheral nerve sheath tumor. TLE proteins (human homologues of Groucho) are transcriptional corepressors that inhibit Wnt signaling and other cell fate determination signals, and so have an established role in repressing differentiation.

In the present study, investigation of some selected phytochemicals viz, β-sitosterol, Septicine, Stigmasterol, α-amyrin, Hypophyllanthin, Tylophorinidine, Phyllanthin, Ursolic acid, Berberine, Xanthatin,and Tylophorinine towards Synovial Sarcoma Protein TLE-1 has been carried out via molecular docking studies.

The *In silico* effectiveness of selected phytochemicals were studied based upon the interaction with the protein's active site residues with less binding energy. Interaction energy estimates of the compounds within the active site of protein showed that these compounds are more selective towards TLE-1. Clusters of conformations with their free energy of binding were analyzed which clearly demonstrates a potential channel and by this means the translocation across the channel in TLE-1 is anticipated. We also performed the protein-protein interaction of TLE-1 and Wnt. This was further docked with the selected phytochemicals to identify the changes in the interaction so that Wnt signaling can be activated, so that further apoptotic mechanism may be initiated in the sarcoma cells.

05. Anti-Cancer Potential of Symplocos Racemosa Bark On Human Liver Cancer Cellline(Hep3-B)

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Institute of Pharmacy, Nirma University[1], Ahmedabad, Sun Pharmaceutical Advanced Research Centre[2], Vadodara. Pharmanza Herbal Pvt. Ltd[3]., At Post Kaniya, Dist. Anand. Pioneer Pharmacy Degree College[4], Vadodara.

Summary

Hepatocellular carcinoma (HCC) is the fifth most common ubiquitous deadliest cancer worldwide with poor diagnosis and accounts for approximately 5,49,000 deaths each year. It is imperative to search alternative drugs for the treatment of HCC to replace the currently used drugs of less efficacy and safety. Traditional plants are valuable source of novel cytotoxic agents and are still playing greater role in health care. The plant *Symplocosracemosa* (Family: Symplocaceae) is a low under shrub, mostly found in South India and Himalayas. The bark is used traditionally for various including cancer and reported to contain many triterpenes and steroidal compounds. The present study is intended to investigate the in vitro cytotoxic study of the different extracts and fractions of *Symplocosracemosa Roxb bark*. In pharmacological screening, the cytotoxic activity of different extracts using human liver cancer cell line (Hep3-B) was evaluated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay. The results of ethyl acetate soluble fraction of crude extract of bark showed potent cytotoxic effect on Hep3-B cell line with IC_{50} 32.55µg/ml compared to other fractions. The ethyl acetate soluble fraction was loaded on column and eluted with varying proportion of Hexane: Ethyl acetate and different fractions were collected and evaluated with MTT assay on Hep3-B and normal cellline (Vero). Total three fractions out of all, showed potent cytotoxic effect on Hep3-B cell line withIC_{50}32.34, 49.69, 42.98 µg/ml. The work for the isolation of bioactives for promising cytotoxic effect is going on active fractions for purification of

compounds and structure elucidation. This study provides scientific basis for the reported use of bark of S. *racemosa* in Indian traditional system of medicine.

06. OM to OMICS : Interaction of Ancient and Modern Sciences to fight Cancer

Bhimani Dhara, Bhatt Nirzary
M.Sc Cancer Biology Semester IV, Department of Cancer Biology, Gujarat Cancer and Research Institute, Ahmedabad

Summary

This is the modern age of information explosion where there is importance of sciences related to personalised medicine. Various omics likes, genomics, proteomics, pharmacogenomics, transcriptomics, metabolomics etc. are useful in achieving our goals. Genomics is study of genomes of living organisms. Proteomics is study of proteomes. Pharmacogenomics is study of drug metabolism and interaction inside body; which is a combine study of pharmacodynamics and pharmacokinetics. All these sciences and branches of them are interdependent. The main focus is to develop an effective drug against cancer.

My efforts:

"Genetic polymorphism in genes involved in detoxification pathway and risk of oral cancer"

Now a days tobacco chewing is considerate etiological factor in risk of oral cancer. As an xenobiotic compound when it enters into living system it goes through the path of activation and detoxification. The enzyme play a major role here is Cytochrome P450 family and Glutathione-s-transferase family respectively. If there is any alteration in the functioning of these enzymes it causes accumulation; and if it do not follows by elimination then it ultimately leads to occurance of cancer. Gene polymorphism occurs which is a germ line mutation in population at frequency of >1%.

"Role of thymidylate synthase in Colorectal cancer"

There is brief introduction on role of thymidylate synthase in colorectal cancer and its effect on chemotherapy.

07. Leukemia treatment from Om to Omics

Dave Meera, Brahmbhatt Dhruva
M.Sc Cancer Biology Semester I, Department of Cancer Biology, Gujarat Cancer and Research Institute, Ahmedabad

Summary

Leukemia is a type of cancer that affects the blood and bone marrow, the spongy center of bones where our blood cells are formed. The disease develops when blood cells produced in the bone marrow grow out of control. The four most common types of leukemia are: The four most common types of leukemia are: acute myeloid leukemia (AML) acute lymphoblastic leukemia (ALL) chronic myeloid leukemia (CML) chronic lymphocytic leukemia (CLL).

Most forms of leukemia are first treated with pharmaceutical medication in 1940s and 1950s, typically combined into a multi-drug chemotherapy regimen in 1960s. In the early 1970s some are also treated with radiation therapy. Unfortunately, many leukemias developed drug resistance to chemotherapy when a patient relapsed. One solution was more intensive chemotherapy and total body irradiation followed by bone marrow transplantation or stem cell transplantation.

A stem cell transplant (sometimes called a bone marrow transplant) is a medical procedure in which diseased bone marrow (leukemic condition) is replaced by highly specialized stem cells that develop into healthy bone marrow. There are two main types of stem cell transplants: Autologous and Autologous. During a stem cell transplant diseased bone marrow (the spongy, fatty tissue found inside larger bones) is destroyed with chemotherapy and/or radiation therapy and then replaced with highly specialized stem cells that develop into healthy bone marrow. The severity of side effects and the success of the transplant vary from person to person and sometimes can be difficult to predict before the transplant.

It can be discouraging if significant challenges arise during the transplant process. However, it is sometimes helpful to remember that there are many survivors who also experienced some very difficult days during the transplant process but ultimately had successful transplants and have returned to normal activities with a good quality of life.

In proteomics, Plasma membrane proteome analysis of leukemia cells can be used to define biomarkers for diagnosis, classification, prognosis and progression monitoring, as well as to predict therapeutic response or resistance. Ayurveda or natural medicine has always helped so many cancer patients to fight with this dreadful disorder. Uptake of some nourishing ayurvedic supplements which are helpful in protecting the healthy cells and regulating the metabolism of the body. Stop progression or acceleration of cancer. This ayurvedic supplements along with chemotherapy and radiotherapy improve health condition of leukemia patient.

08. Development of Biodegradable Nanoparticle for Delivery of Anticancer Drug

Gulati Pallavi, Gupta Dikshi, Tyagi Priyanka, Kapoor Sumeet, Singh Harpal
Centre for Biomedical Engineering, Indian Institute of Technology, Delhi

Summary

Paclitaxel is considered as a promising anticancer agent. It is one of the most effective broad spectrum anticancer agents used for the treatment of lung, pancreatic, ovarian, breast, head, neck cancer and Karposi Sarcoma. It acts as a mitotic inhibitor and prevents the depolymerisation of microtubules. Due to the substantial challenges associated with Paclitaxel which includes limited aqueous solubility in blood, lack of selectivity towards cancerous and non- cancerous cells, multidrug resistance and hypersensitivity reactions, there is an urgent need of a cancer treatment which is less toxic and specific towards the cancer cells. Therefore, nanoparticle delivery system is gaining attention worldwide due to its favourable properties of improved pharmacokinetic profile, sustained circulation and better accumulation of drug in the cancerous tissue due to EPR effect.Our aim is to encapsulate Paclitaxel in block-copolymeric(PLA-PEG-PPG-PEG)nanoparticle which is FDA approved and has a biodegradable shell of size ranging from 70-100 nm. The nanoparticle was synthesised by solvent evaporation method and was characterized by Fourier Transform Infrared Spectroscopy(FTIR),Nuclear Magnetic Resonance(NMR) and Scanning Electron Microscopy (SEM). In vitro analysis of these nanoparticles in cancerous cell line showed increased cytotoxic effect and effective cellular uptake as compared to free drug. These drug loaded proposed nanoparticle system used in the present study will be explored in in-vivo model for their therapeutic efficacy.

09. Targeting Cancer Stem Cells in Solid Tumors: Novel Approach

Mirza Sheefa[1], Rakesh Rawal[2], Nayan Jain[1]

Dept. of Life Sciences, Gujarat University[1], Ahmedabad, The Gujarat Cancer and Research Institute[2], Ahmedabad

Summary

Solid tumours, including sarcoma, carcinoma and lymphoma contributes to a larger proportion of cancer burden and poses a major therapeutic challenge. There is increasing evidence that diverse solid tumours are hierarchically organized and sustained by a distinct subpopulation of CSCs. These cancer stem cells (CSC) account for the therapeutic refractoriness and subsequent relapse locally or metastasize to distant organs. The clinical relevance of CSCs is undisputedly the key rationale which can be achieved by developing novel approaches that facilitate the identification, targeting and eventually elimination of these CSCs specifically. Most research into the resistance of cancers to chemotherapy has concentrated on molecular mechanisms of resistance, whereas the role of limited drug distribution within tumours has been neglected. In order to highlight later phenomenon, we have derived a unique workflow combining various isolation, identification and therapeutic strategies. The freshly resected tumour tissue is made to single cell suspension. Later CSCs are isolated by immunomagnetic cell separation and further expansion ex-vivo till desired cell count is achieved for downstream applications. Partly the cells were used for 3D culture on collagen scaffold for in-vitro screening using classical chemotherapeutic drugs routinely opted for the patient and/or phytochemicals for various time intervals and allowed to grow further. Later these cells are grown in cellulose containing media for generating tumorosphere (CFUs). Our preliminary findings are suggestive of selective elimination of CSCs using classical drugs and phytochemicals in combination than any single regimen. These CFUs containing CSCs can be utilized for further genomic characterization and cell based therapy e.g. autologous dendritic cell based targeting of residual tumour cells.

"It is of great importance, when we begin to practise prayer, not to let ourselves be frightened by our own thoughts."
Teresa of Ávila

Summaries of Published Articles

01. Breast Disease 00 (2014) 1–15 1
DOI 10.3233/BD-140395

Role of PRL-3, Snail, Cytokeratin and Vimentin Expression in Epithelial Mesenchymal Transition in Breast Carcinoma

Patel Nupur A [1], Patel Prabhudas S [2], Vora Hemangini H [1]
[1]Division of Immunohistochemistry and Flow Cytometry
[2]Cancer Biology Department, Gujarat Cancer and Research Institute

Summary

This study evaluated epithelial mesenchymal transition (EMT) in breast cancer, molecules such as PRL-3, Snail, Cytokeratin and Vimentin involved in EMT. In this study, m-RNA expression of PRL3 and Snail by RT PCR, protein expression of PRL-3, Snail, Cytokeratin and Vimentin by immunohistochemistry were evaluated on paraffin-embedded tissue sections of 100 patients with breast cancer. PRL3 m-RNA expression (above cut off level > 2487301.00) and PRL-3 protein expression was noted in 52% and 70% of breast carcinoma patients, respectively. The higher incidence of PRL3 protein than m-RNA expression could be due to post translation modification. Further, Snail m-RNA expression (above cut off level > 1285142.00) and Snail protein expression was noted in 53% and 54% of breast cancer patients respectively and Snail protein expression was found significantly higher in patients with pre-menopausal status. The loss of cytokeratin expression in 32% and gain of vimentin expression in 17% was noted in these patients. Vimentin expression was found significantly higher in patients with stage IV disease, BR score 4 and PR negativity. In multivariate survival analysis, Vimentin expression found as strong indicator of biologically aggressive breast cancer predicting reduced disease free survival (DFS) and overall survival (OS). In our study reveals that Vimentin expression emerged as significant biomarker for predicting reduced DFS and OS in breast cancer. The study proposes routine evaluation of Vimentin with other predictive parameters can allow use of EMT inhibitors with conventional therapy to revert EMT in breast cancer.

02. Journal of Cancer Research and Treatment, 2014, Vol. 2, No. 2, 28-40 Available online at http://pubs.sciepub.com/jcrt/2/2/2 © Science and Education Publishing DOI:10.12691/jcrt-2-2-2

Study of Immune Effector Cells in Leukoplakia and Oral Cancer

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Received September 05, 2014; Revised September 16, 2014; Accepted September 18, 2014

Summary

Immunosuppresion in oral squamous cell carcinoma (OSCC) is related to high degree of recurrence and believed to develop from premalignant lesion. Leukocytes especially T cell subsets are important in immune surveillance during malignant transformation. This study has been planned to observe changes in systemic immune response in premalignant and malignant oral lesions. The proportions of Neutrophils, Monocytes, Lymphocytes, total T cells, T cell subsets including αβ/γδ T cells, Cytotoxic T cells, Helper T cells, Naive/ Effector/Memory T cells, Regulatory T cells and NK-T subpopulations were analysed in peripheral circulation of healthy donors (N= 49), Leukoplakia (N=20) and OSCC patients (N=100) by flowcytometry. In comparison with healthy donors, decreased Lymphocytes, Naive Helper cells, NK T subpopulations and increased Effector cells were observed in Leukoplakia patients. Similarly, decreased Lymphocytes, NK T subpopulations and increased Neutrophils, Monocytes, Helper and Regulatory T cells were observed in OSCC patients as compared to healthy controls. Moreover, Lymphocytes were decreased and Regulatory T cells were increased during the progression of Leukoplakia to OSCC. Further, in relation with clinicopathological parameters, Cytotoxic cells were found to be reduced with increasing histological grade. Also, Helper cells were found to be decreased in patients with tobacco and alcohol habit and also with increasing tumor size. Further, in univariate survival analysis, increased incidence of relapse was observed in patients with low γδ and NK T cells. In multivariate survival analysis, low γδ T cells emerged as poor prognosticator for disease free survival and high Regulatory T cells emerged as poor prognosticator for predicting overall survival. Altered systemic immune response was seen during malignant transformation and also found to be associated with patient's survival. Thus, investigation of circulating Leukocyte and T cell subsets seems to be useful for predicting patient's survival and to identify immunosuppressed patients who may be benefited with immunotherapy.

03. Asian Pac J Cancer Prev, 15 (20), 8549-8556

Cancer Stem Cells and Stemness Markers in Oral

Squamous Cell Carcinomas

Patel Shanaya S, Shah,Kanisha, ShahManoj J, Kothari Kiran C, Rawal Rakesh M

Summary

Head and neck squamous cell carcinoma (HNSCC) is one of the world top ten most common cancers with its highest occurrence in the Indian subcontinent and different aggressive and etiological behavioral patterns. The scenario is only getting worst with the 5 year survival rates dropping to 50%, persistent treatment failures and frequent cases of relapse/recurrence. One of the major reasons for these failures is the presence of cancer stem cells (CSCs), a small population of cancer cells that are highly tumourigenic, capable of self-renewal and have the ability to differentiate into cells that constitute the bulk of tumours. Notably, recent evidence suggests that cancer stem cells are especially resistant to conventional therapy and are the "drivers" of local recurrence and metastatic spread. Specific markers for this population have been investigated in HNSCC in the hope of developing a deeper understanding of their role in oral cancer pathogenesis, elucidating novel biomarkers for early diagnosis and newer therapeutic strategies. This review covers the fundamental relevance of almost all the CSC biomarkers established to date with a special emphasis on their impact in the process of oral tumourigenesis and their potential role in improving the diagnosis, prognosis and treatment of OSCC patients.

04. DOI 10.1007/s13205-014-0267-0

Tobacco Habituated and Non-habituated Subjects Exhibit Different Mutational Spectrums in Head and Neck Squamous Cell Carcinoma

Rawal Rakesh M, Joshi Madhvi N, Bhargava Poonam, Shaikh Inayat, Pandit Aanal S, Patel Riddhi P, Patel Shanaya, Kothari Kiran, Shah Manoj, Saxena Akshay, Bagatharia Snehal B

Summary

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common non-skin cancer in the world. Tobacco chewing is implicated with most of the cases of HNSCC but this type of cancer is increasing in non-tobacco chewers as well. This study was instigated to provide comprehensive variant and gene-level data in HNSCC subjects of the Indian population and fill the gap in the literature on comparative assessment of gene mutations in cancer subjects with a habit of tobacco and those without any habit using targeted amplicon sequencing. We performed targeted Amplicon sequencing of 409 tumor suppressor genes and oncogenes, frequently mutated across many cancer types, including head and neck. DNA from primary tumor tissues and matched blood was analyzed for HNSCC patients with a habit of tobacco and those without any habit. PDE4DIP, SYNE1, and NOTCH1 emerged as the highly mutated genes in HNSCC. A total of 39 candidate causal variants in 22 unique cancer driver genes were identified in non-habitual (WoH) and habitual (WH) subjects. Comparison of genes from both the subjects, showed seven unique cancer driver genes (KIT, ATM, RNF213, GATA2, DST, RET, CYP2C19) in WoH, while WH showed five (IL7R, PKHD1, MLL3, PTPRD, MAPK8) and 10 genes (SETD2, ATR, CDKN2A, NCOA4, TP53, SYNE1, KAT6B, THBS1, PTPRT, and FGFR3) were common to both subjects. In addition to this NOTCH1, NOTCH2, and NOTCH4 gene were found to be mutated only in habitual subjects. These findings strongly support a causal role for tobacco, acting via PI3K and MAPK pathway inhibition and stimulation of various genes leading to oncogenic transformations in case of tobacco chewers. In case of non-tobacco chewers it appears that mutations in the pathway affecting the squamous epithelial lineage and DNA repair genes lead to HNSCC. Somatic mutation in CYP2C19 gene in the non-habitual subjects suggests that this gene may have a tobacco independent role in development and progression of HNSCC. In addition to sharing high mutation rate, NOTCH gene family was found to be mutated only in habitual sample. Further, presence of mutated genes not earlier reported to be involved in HNSCC, suggest that the Indian sub-continent may have different sets of genes, as compared to other parts of the world, involved in the development and progression of HNSCC.

05. Curr Stem Cell Res Ther. 2014 Oct 20. [Epub ahead of print]

Epigenetic Regulators Governing Cancer Stem Cells and Epithelial-Mesenchymal Transition in Oral Squamous Cell Carcinoma.

Patel S, Shah K, Mirza S, Daga A, Rawal R

Summary

Oral squamous cell carcinoma (OSCC) is amongst the most prevalent form of cancer worldwide with its predominance in the Indian subcontinent due to its etiological behavioural pattern of tobacco consumption. Late diagnosis, low therapeutic response and aggressive metastasis are the foremost confounders accountable for the poor 5 year survival rate of OSCC. These failures are attributed to the existence of "Cancer Stem cell (CSC)" subpopulation within the tumour environment. Quiescence, apoptotic evasion, resistance to DNA damage, abnormal expression of drug transporter pumps and in-vivo tumorigenesis are the defining hallmarks of CSC phenotype. These CSCs have been distinguished from the tumor mass by determining the expression

patterns of cell surface proteins, specific stemness markers and quantifying the cellular activities such as drug efflux & aldehyde dehydrogenase activity. Hence, it is necessary to understand the underlying mechanisms that regulate the CSC features in tumour development, metastasis and response to chemotherapy. Increasing evidence suggests that majority of malignant cells eventually undergoing Epithelial-Mesenchymal transition (EMT) share many biological characteristics with CSCs. Thus, this review encompasses the functional relevance of CSC and EMT markers in OSCC population with a hope to elucidate the fundamental mechanisms underlying cancer progression and to highlight the most relevant epigenetic mechanisms that contribute to the regulation of CSC features. We further aimed to explore the causal effects of nicotine, a major tobacco carcinogen, on epigenetic mechanisms regulating the OSCC CSCs and EMT markers which unravels the undisputable contribution of tobacco in oral carcinogenesis.

"We are all too often told by someone that we are too old, too young, too different, too much the same, and those comments can be devastating."
Sharon Salzberg

Summaries of Presentations at Clinical Meetings

01 Haematopoeitic stem cell transplantation – A GCRI Experience study of 224 patients.

Patel Kinnari A
Department of Medical Oncology

Summary

The aims was the study of Haematopoeitic Stem Cell Transplantation. We studied 224 cases of haematopoetic stem cell transplantation done in our institute from 1999 to 2014. Allogenic transplantation was done in 38.8% and Autologus transplant in 61.2% of patients. Out of 224 patients, Leukemia patients were 32, lymphoma patients 66, Multiple myeloma 62, Thalassemia major 29, Aplastic anemia patients 18 and others were 8 of Allogenic & 8 Autologus BMT. Autologus transplantation was done in 137 patients. Most common indication was multiple myeloma (45.2%) while HL (29.9%), NHL (18.2%) and others were (6.5%). Allogenic transplantation was done in 87 patients, common diseases were Thalassemia major (33.3%), AML (31%), Aplastic anemia (20.6%), CML (5.7%) & others (9.1%).

In Multiple Myeloma 61.9% are in CR, 23.8% Relapsed & alive & 11.13 relapsed & exp.and 3.17% TRM. In Hodgkin's Lymphoma 63.1% are in CR, 18.4% relapsed & alive, 8% relapsed & expired and 10.5% TRM. In NHL 39.2% are in CR, 35.8% relapsed and 25% TRM. In Thalassemia 51,7% are in CR, 34.6% relapsed and 13.7% TRM. In AML 32% are in CR, 24% relapsed and 16% TRM,20% GvHD,8%exp due to infection (patients were in CR).In other leukemia (CML+ALL) 40% in CR, 40% expired due to GvHD and 20% exp due to persistent disease. In Aplastic Anemia 72.2% are in CR and 27.8% relapsed or not responded but no TRM.

HSCT is used in various benign and malignant haematological conditions and as well as solid tumors. Due to improvement in conditioning regimen, supportive care and treatment of GVHD, the overall results are improved and TRM rate has reduced. In future more patients will be eligible for Allogenic transplant because of the availability of donor registry program and umbilical cord blood banks.

02. Study of Prognostic Factors in Endometrial Cancer with Special Reference to Accuracy of Imaging and Intra Operative Gross Visual Inspection

Desai Arun J
Department of Gynaecologic Oncology

Summary

This study was carried out with aims to decide accuracy of gross visual inspection and imaging in predicting prognostic factors in endometrial cancer. 25 cases of endometrial cancer were studied. Preoperative imaging (MRI/CT scan) and intra-operative gross visual inspection (GVI) were individually compared to final histology to determine their accuracy for size, myometrial invasion and cervical extension. Sensitivity and specificity of MRI and GVI for detecting myometrial invasion were 80.95% / 50% and 86.36% / 66.67% while positive and negative predictive value 89.47% / 33.33% and 95% / 40% respectively. Both were statistically significant. Sensitivity and specificity of MRI and GVI for detecting size of tumour were 92.86 % / 45.45% and 95.45% / 66.67% while positive and negative predictive value were 68.42 % / 83.33% and 95.45% / 66.67 % respectively. Both were statically significant.

03. Relevance of Interleukin-1α, Interleukin-1β, Interleukin-8 and Interferon-α in Thyroid Diseases

Darshita Patel
Division of Molecular Endocrinology

Summary

Cytokines act as important modulators of tumorigenesis. They are thought to modulate development and growth of both normal and neoplastic thyroid cells. Therefore, the aim was to explore the occurrence of Interleukin-1α, Interleukin-1β, Interleukin-8 and Interferon-α levels in serum of 19 healthy individuals, 21 patients with goitre, 16 with autoimmune diseases and 32 thyroid carcinoma patients. IL-1β, IL-8 and IFN-α were significantly higher in patients with thyroid disease as compared to healthy individuals while, IL-1α was significantly higher in patients having goitre. IL-1α was significantly inversely correlated with tumor size, lymphatic permeation, and differentiation status, while IL-1β significantly correlated with the nodal and tumor differentiation status. IL-8 levels showed significant positive correlation only with disease stage while IFN-α levels demonstrated no significant correlation with the studied clinicopathological parameters. Finally it may be concluded that, the studied cytokines have a significant role in thyroid cancer pathogenesis and may represent useful serum biomarkers in patients with thyroid diseases.

04. Supramajor Surgeries in High Risk Situations-Is it Worth?

Karthikeyan R

Department of Surgical Oncology

Summary

In cancer patients with high risk conditions, there is always a dilemma whether to operate the patient or not, because of the fear that patient may die of high risk condition than cancer. Here, we present two cases of periampullary carcinoma- one with liver cirrhosis and other with cardiac co morbidity / heart failure being taken up for supramajor surgery (Whipples procedure). Both the patients are alive and free of tumor. So, in an institutional setting with due precautions, competent multidisciplinary team efforts and patient's understanding of involved risk, we can operate upon these patients.

05. A Comparative Study of Intraperitoneal Nebulization Versus Intraperitoneal Instillation of Ropivacaine Hydrochloride 0.75% and Morphine Sulphate 1% for Postoperative Analgesia in Laparoscopic Surgeries

Solanki Rekha
Department of Anaesthesiology

Summary

The aim of this study was to evaluate the effects of intraperitoneal local anaesthetic administration for postoperative pain relief after laparoscopic surgeries. Thirty patients of ASA I and II, aged 18-60 years were divided in two groups. At the end of surgery Ropivacaine (0.75%) 2 mg/kg and morphine 2 mg was nebulized in group N and instilled in group I. Visual analogue score (VAS) for static and dynamic pain, duration of analgesia, total opiate consumption during first twenty four hours, and complications were noted. VAS (at rest) at 0, 12 and 18 hours was 5.29±1.03 vs. 16.30±3.61, 15.23±2.25 vs. 32.21±1.53 and 29.46±2.05 vs. 38.60±2.71 respectively (P<0.05). VAS (dynamic) at 0, 12 and 18 hours was 12.21±5.05 vs. 23.21±8.65, 20.40 ±4.87 vs. 41.02±3.45 and 30.81±5.03 vs. 40.30±9.21 respectively (P < 0.05). Incidence of shoulder pain was 33% in I group and 0% in N group. (P< 0.05). Duration of analgesia was 17.57±0.02 hours in group N and 13.48±0.05 hours in group I (P<0.05). Morphine consumption in N group was 2 times lower (P< 0.05). Intraperitoneal nebulization with ropivacaine and morphine reduces postoperative pain and referred shoulder pain as well as morphine consumption after laparoscopic surgeries as compared to instillation. It can be used as an alternative to epidural technique.

06. Retrospective Study of Operated Cases of Cerebellar Metastasis

Mody Paresh
Department of Neuro oncology

Summary

Cerebellar (brain) metastasis are the commonest solid tumor in neurosurgical practice. Metastasis in cerebellum need special emphasis as 15-18% are involving cerebellum. Surgery and Radiotherapy are main stay of treatment. Surgery confers several advantages to other therapies as:
1 Immediate relief of symptoms,2 Diagnosis,3 Relief from steroid dependence,4 Avoid csf diversion, shunt for hydrocephalus

We retrospectively analysed so cases of cerebellar metastasis who underwent microsurgical exicision with or without csf diversion (External ventricular dranaige, omaya or ventriculoperitoneal shunts) Evaluated the presentation, diagnosis (Histopathology), postoperative complication, over all outcome We concluded that surgical resection of cerebellar matastasis is effective and safe for selected cases with higher performance status (kps >70) and are associated with long term survival.

07. Cancer Scenario in Population Based Cancer Registry: Ahmedabad Urban Agglomeration Area 2007-2011

Shah Janmesh
Department of Community Oncology and Medical Records

Summary

The main objective of this study is to assess the magnitude and type of various cancers in Ahmedabad urban areas and to provide a framework for controlling the impact of cancer on the community. An attempt has been made to study the trends in the Age Adjusted Incidence Rates for the leading cancer sites for the period 2007 to 2011. Five years data from the period 2007 to 2011 were reviewed and their frequency, proportion and Age Adjusted Incidence rates by sites and gender were calculated and represented. Cancer of mouth and tongue continue to be the first two leading sites among males whereas cancer of breast and cervix were the leading sites in females. The study will be helpful to understand the cancer burden and patterns among the population of Ahmedabad city. Preventive measures must be taken to reduce the incidence of cancers.

08. Prosthetic Rehabilitation

Soni Dipan N
Cosmetic Prosthetic Laboratory

Summary

Prosthetic department's main goal is to provide patients cosmetic contentment. We have different categories of patients like handicap from birth, burns patients and those who have accidentally or surgically lost limbs/organs. Majority of the patients are of breast prosthesis followed by ear eye, nose, chin, cheek, fingers and toes. Prosthesis helps to give proper shape and refashion with consideration to the age of the patient. Before making prosthesis

patients are explained about the procedure. After examining the patient whichever part's prosthesis is to be made, measurement to be taken includes diagram, depth, height, width and thickness. These prosthesis are prepared by impression taking, mould making, modeling, mould cast either silicone or acrylic, color mixing and last finishing patient's prosthesis. Patient has to take an appointment, so that enough time can be spared and privacy can be maintained. Patients' psychological feelings should be considered and he/she must be satisfied with the shape and color of prosthesis. The silicone prosthesis is very soft, smooth and mild like skin and look natural. The acrylic prosthesis is very hard and strong. For breast prosthesis, it can be prepared either from sponge, U foam or silicone material. The prosthetic department provides the satisfaction to the patient after getting the prosthesis. They can face the people without hesitation.

09. Re-Irradiation in Head and Neck Malignancy- A Prospective Study

Poddar Jyoti
Department of Radiation Oncology

Summary

Analysis of efficacy of external beam re-irradiation in patients with recurrent and second primary head and neck carcinoma in previously irradiated area, with respect to acute and late radiation reactions, loco-regional control and disease free survival. A single arm prospective study, with 47 patients of recurrent or second primary malignancy of head and neck region, in previously irradiated area(>45Gray) was conducted between August 2012 – August 2013. They were treated with re-radiation, either post-operatively or as definite treatment with or without chemotherapy. Median disease free interval between primary treatment and re- radiation was 60 months All patients received 60-66 Gray in 30-33 # with daily dose of 2 Gray for 6.5weeks on Linear accelerator with 6 MV photons with shrinking field technique. Disease free survival at 6 months, 9 months and 12 months was 72.3%, 53.1% and 40% respectively. Acute reactions comprised of mucositis, dysphagia and skinreactions. Long term toxicity included subcutaneous fibrosis and trismus. Second course of radiotherapy is feasible in recurrent and second primary tumours of head and neck with acceptable toxicity and disease free survival.

"With the practice of meditation we can develop this ability to more fully love ourselves and to more consistently love others."
Sharon Salzberg

Presentations at the Clinical Meetings

(July 2014 to December 2014)

Sr. No.	Date	Speaker/Department	Title
1.	12.07.14	Patel Kinnari A Medical Oncology	Haematopoetic Stem Cell Transplantation - A GCRI Experience Study of 224 Patients
2.	26.07.14	Desai Arun J Gynecologic Oncology	Study of Prognostic Factors in Endometrial Cancer with Special Reference to Accuracy of Imaging and Intra Operative Gross Visual Inspection
3.	09.08.14	Patel Darshita Division of Molecular Endocrinology	Relevance of Interleukin-1α, Interleukin-1β, Interleukin-8 and Interferon-α in Thyroid Diseases
4.	23.08.14	Karthikeyan R. Surgical Oncology	Supra Major Surgeries in High Risk Situations - Is it Worth?
5.	13.09.14	Solanki Rekha Anaesthesiology	A Comparative Study of Intraperitoneal Nebulization Versus Intraperitoneal Instillation of Ropivacaine Hydrochloride 0.75% and Morphine Sulphate 1% for Postoperative Analgesia in Laparoscopic Surgeries
6.	11.10.14	Mody Paresh Neuro Oncology	Retrospective Study of Operated Cases of Cerebellar Metastasis
7.	08.11.14	Shah Janmesh Community Oncology and Medical Records	Cancer Scenario in Population Based Cancer Registry: Ahmedabad Urban Agglomeration Area 2007- 2011
8.	29.11.14	Soni Dipan N Cosmetic Prosthetic Laboratory	Prosthetic Rehabilitation
9.	27.12. 14	Poddar Jyoti Radiation Oncology	Re - Irradiation in Head and Neck Malignancy – A Prospective Study

Journal Club/Guest Lecture/ Review Lecture Presentations

(July 2014 to December 2014)

Sr. No.	Date	Presenter/ Department	Topic	Authors	Citation
1.	26.07.14	Girdhar Gopal Surgical Oncology	Cytoreductive Surgery Plus Hyperthermic Intraperitonial Chemotherapy with Oxaliplatin for Peritonial Carcinomatosis Arising From Colorectal Cancer	Mai-Kim Gervais, Pierre Dube, Yerrow Mcconnell, Pierre Drolet, Andrew Mitchell, Lucas Sideris	Jounral Of Surgical Oncology 2013; 108 : 438-443
2.	23.08.14	Shah Manali Physiotherapy	Spinal Accessory Nerve Neuropathy Following Neck Dissection	Luciana Pereira de Lima, Ali Amar, Carlos Neutzling Lehn	Brazillian J of Otorhino-laryngology 2011 Mar/ Apr; 77(2): Sao Paulo
3.	27.09.14	Bhardava Vishal Radio Diagnosis	Multiparametric MRI of Prostate Cancer	Sandeep S Hedgire, Tamara N Oei, Shaunagh Mcdermott, Kai Cao, Zena Patel M, Mukesh G Harisinghani	Indian Journal of Radiology and Imaging 2012 August: 22 (3);
4.	13.12.14	Patel Kinjal D Biochemistry Research Division	Prognostic Value of Human Apurinic/Apyrimidinic Endonuclease- 1 (APE1) Expression in Breast Cancer	Joohyun Woo, Heejung Park, Sun Hee Sung, Byung-In Moon, Hyunsuk Suh, Woosung Lim	PLOS ONE 2014: 9(6): e99528
5.	27.12.14	Patel Pratik Pathology	The New IASLC/ATS/ERS Lung Adenocarcinoma Classification From a Clinical Perspective: Current Concepts and Future Prospects	Jon Zugazagoitia, Ana Belen Enguita, Juan Antonio Nuñez, Lara Iglesias, Santiago Ponce	Journal of thoracic disease 2014 October: 6(5); S526-S536

Case Presentations for Morbidity, Mortality at Clinical Meetings

(July 2014 to December 2014)

Sr No	Date	Presenter/Department	Case discussion
1	26.07.2014	Kartik Surgical Oncology	Case Presentation: Mortality of Operated Case of Ca Oesophagus Lower One Third
2	26.07.2014	Modi Hardul Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
3	23.08.2014	Pol Dhiraj Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
4	23.08.2014	Shah Sanket Medical Oncology	Complications In A Case of Acute Lymphoblastic Leukemia
5	27.09.2014	Pol Dhiraj Anesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
6	27.09.2014	Singh Shweta Surgical Oncology	Case Presentation: Morbidity of Operated Case of Radial Forearm Flap
7	29.11.2014	Shah Nishita Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
8	29.11.2014	Pol Dhiraj Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Department
9	29.11.2014	Madabhavi Irappa Medical Oncology	Burkitt's Lymphoma of Oesophagus: Common Tumour at an Uncommon Site
10	27.12.2014	Shah Nishita Anaesthesiology	Mortality and Morbidity Data Presentation of Surgical and Medical Departments
11	27.12.2014	Pol Dhiraj Anaesthesiology Gandhe Ravindra Gastroenterologist	Case Presentation on Post ERCP Pancreatitis

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 Index Coperinicus.**

The journal's full text is available online at http://www.cancerindia.org

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

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- 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)
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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.

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

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The title page should include

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3. The name by which each contributor is known (Last name, First name and initials of middle name), with institutional affiliation;
4. The name of the department(s) and institution(s) to which the work should be attributed;
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Language and grammar

- Uniformly American English
- Abbreviations spelt out in full for the first time

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

Summary 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.

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Standard Journal

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

Online journal article

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

Chapter in a book

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

Online book or website

Garrow A, Weinhouse GL. Anoxic brain injury: assessment and prognosis. In: 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.

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Physiotherapy Department

The physiotherapy department was started in 1980. Since then rehabilitation of patients were made in department on indoor and outdoor bases. Number of patients increasing years after years. At present there are three physiotherapists-One senior physiotherapist and two junior physiotherapists are looking after services offered by department.

Department receives references from surgical, medical, orthopaedic, gynecology, neurology, pediatric, BMT wards, etc. Specialized work is carried out for lymph edema and trismus patients.

Introduction

Patients are surviving longer with cancer due to early detection and treatment. Cancer patients may survive but not thrive. With longer survival, quality of life becomes very important. Living longer does not necessarily mean living better. While improvement in treatment add years to the life of patients living with cancer. In this situation rehabilitation add life to these years. Cancer rehabilitation is about moving patients out of sick role and into effective day- to- day self management of their illness. Cancer rehabilitation focuses on maximizing physical, psychological, social, vocational functioning-during and after treatment. In addition rehabilitation supports individual and their families through periods to change to ensure that optimal quality of life and sense of well being is achieved. Physiotherapists are an important part of the multi disciplinary team of experts in provision of cancer rehabilitation including occupational therapist, language and speech therapist, dietician and lymph edema therapist. The fact that more and more individuals with cancer are surviving many with residual impairments and disabilities which lends weight to the importance of receiving rehabilitation from the point of diagnosis to end life.

Role of physiotherapist

As the survival from the cancer continues to increase, people are seeking to restore quality to their lives. There are increasing numbers of cancer survivors, creating the imperative to look beyond just survival potentially, remediable problems may include pain, deconditioning and functional impairments with cancer of breast, head and neck cancer, musculoskeletal, central and peripheral nervous system cancer, prostate cancer, metastasis, spine, and gynecologic cancer may particularly benefit from rehabilitation. People with cancer may present with wide range of needs including respiratory, neurological, orthopaedic, lymphatic, pain, musculoskeletal etc. The fact that these adolescents are facing several months of chemotherapy/radiotherapy and usually major surgery as well as direct effect of immobility due to pain, means that muscle wasting, joint stiffness as well as deconditioning. The goal of physiotherapist is to maximize a person's functional ability; for these, referrals to physiotherapy are an appropriate way to address these concerns. Active engagement of oncologist, general physician, palliative medicine and rehabilitation specialist can be useful to assist in rehabilitation needs. Physiotherapists can make a meaningful contribution to the health care team caring for the patient with cancer. The growing global population with cancer faces unique challenges from their disease and treatments they receive. Physiotherapist can make a unique contribution in helping them to achieve health and good quality of life. The prescribed exercises and life style advised by physiotherapist help the people to assist them in regaining and maximizing their independence and thus reduce burden to the society. It is designed to help patients and family members to learn and adapt and improve quality of life while living with cancer. A physiotherapist has the major share in educating as well as motivating the individual to take part in exercise program, to remind him that he did not make it through the rough road of cancer or chemotherapy just to end up on the couch and to assure him that myriad of side effects can effectively managed with exercise interventions.

The absence of physiotherapy intervention would be detrimental to patient care and the ability of the patient/family to cope with effects of the disease on its treatment on their functional capacity and quality of life.

Cancer rehabilitation in country is not well established and there is no current existing protocol being observed in these respective hospitals. This is compounded by the limited referrals due to lack of awareness among oncologist as well as knowledge of general population about what physiotherapist can do for patients with cancer.

Awareness campaign must be done to promote this services that physiotherapists can provide specifically for patients with cancer among oncologist and general population.

Treatment plan in our department is as follows for indoor and outdoor patients with cancer:

- Positioning, movement, mechanical therapies, and electrotherapy agents to relieve and control pain.

- Respiratory care management of dyspnoea, removal of secretions, nebulized drugs and oxygen management.
- Neurological rehabilitation techniques.
- Complex physical therapy (a combination of physical therapies e.g. manual lymphatic drainage, compression bandages and garments, exercises and skin care) to control lymph edema
- Education of client e.g. in care of limb post-surgery, in energy conservation or adaptive strategies, in appropriate handling strategies.
- Education of carers in appropriate handling skills.
- Exercise therapy to improve flexibility, strength and function.
- Relaxation techniques to reduce levels of anxiety

Training Programme

Since 2006, students from Ahmedabad institute of medical science, Ognaj are getting exposure and get clinical training in this field.

- Nursing students and biomedical engineering students are also taking orientation of these services.
- Students doing masters of physiotherapy are also attending department as part of their dissertation topic for study purpose.

Other activities at department

- Presented paper on cancer rehabilitation in different national conferences.
- Published article in national journal.
- Conducted workshop on lymph edema.

Figure1: References from different department.

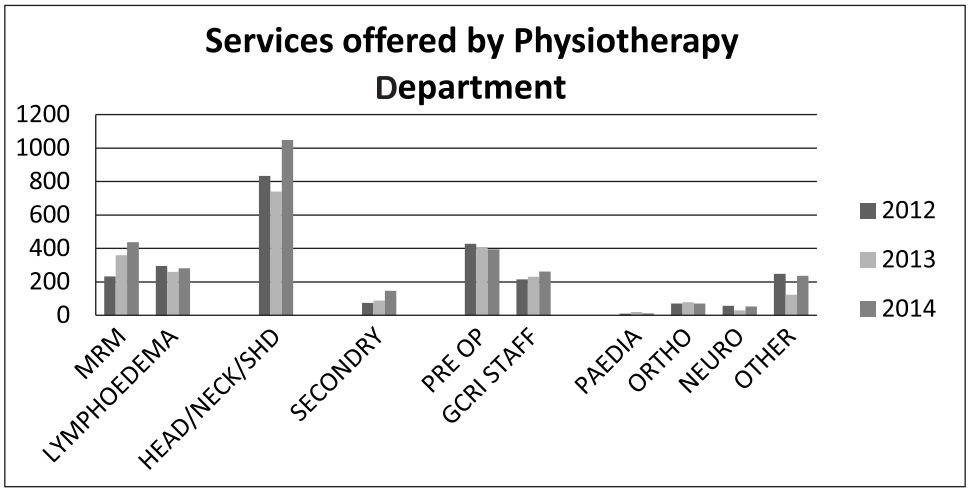
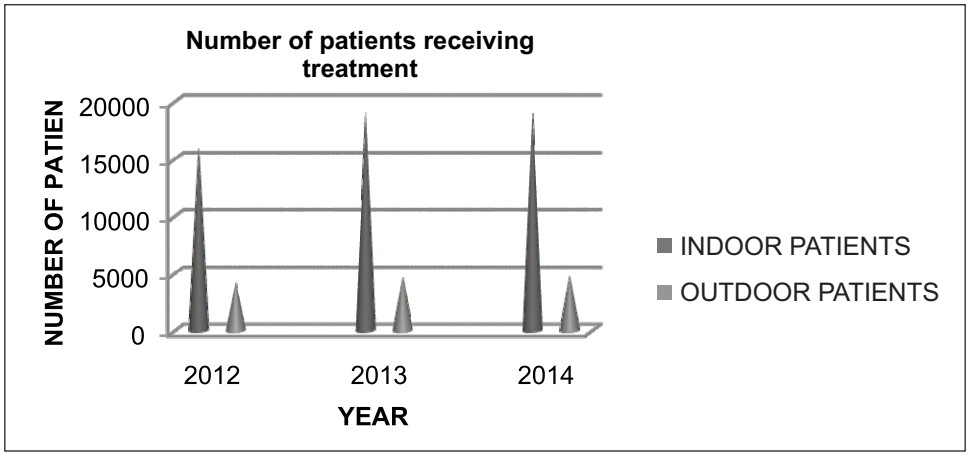


Figure 2: Average work load per year



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Exercises forTrismus

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