Appendix

PUBLICATIONS

A) RESEARCH ARTICLES

1) **Hansa J**, kannan R and Ghosh SK. Screening of 185DelAG, 1014DelGT and 3889DelAG mutations of BRCA1 in Breast Cancer patients from North-East India: a hospital based study. *Asian Pacific Journal of Cancer Prevention*. 2012; **13**:5871-5874.
2) Ghosh SK, Choudhury B, **Hansa J**, Mondal R, Singh M, Duttagupta S, Das A, Kumar R, Laskar RS, Kannan R, Ghosh PR. Human Papillomavirus Testing for Suspected Cervical Cancer Patients from Southern Assam by Fast-PCR. *Asian Pacific Journal of Cancer Prevention*. 2011; **12**:749-751.

B) PROCEEDING ABSTRACTS IN INTERNATIONAL/NATIONAL CONFERENCES

1) **Jagadish Hansa** and Sankar Kr Ghosh (2012), "Mutation detection of *BRCA1* gene in breast cancer patients from Southern Assam, India", 2nd International Conference on Perspective of cell signaling and Molecular Medicine, during 8-11 January 2012, Bose Institute.
2) Bishal Dhar, **Jagadish Hansa**, Rosy Mondal and Sankar Kr Ghosh (2012), "COLD-PCR: a molecular tool for mutation detection in *TPO*, *BRCA1* & D-loop of mitochondrial DNA", 2nd International Conference on Perspective of cell signaling and Molecular Medicine, during 8-11 January 2012, Bose Institute.
3) Rajeev Kumar, R Ravi Kannan, Sankar K. Ghosh, Sumit Goyal, **Jagadish Hansa**, Aditi Bhowmik (2011), "Relationship between various Biochemical, Haematological, Co-morbid, Life-style, Demographic parameters in Gallbladder Carcinoma: A single centre retrospective study of 342 patients", AACR, 2011, New Delhi.

4) Rosy Mondal, **Jagadish Hansa**, Ravi Kannan, Sankar Kumar Ghosh (2011), "Mitochondrial DNA genome as biomarker in oral squamous cell carcinoma from Northeast India", AACR, 2011, New Delhi.

5) **Hansa Jagadish**, Mondal Rosy, Kannan Ravi and Ghosh K. Sankar (2011), "Mitochondrial DNA Detection at the D310 Region by COLD-PCR in Cancerous Tissue", 98th ISC, 2011, Chennai.

6) Mondal Rosy, **Hansa Jagadish**, Kannan Ravi and Ghosh K. Sankar (2011), "Detection of Mitochondrial Genome Variation in Head and Neck Cancer", 98th ISC, 2011, Chennai.

7) Singh M., **Hansa J.**, Choudhury B., Duttagupta S., Das A., Kumar R., Laskar S R., Kannan R., Ghosh K S., (2011), "PCR Based Diagnosis of HPV Infection in Suspected Cervical Cancer Patients for Southern Assam: A Report". 98th ISC, 2011, Chennai.

8) Mondal Rosy, **Hansa Jagadish**, Kannan Ravi and Ghosh K. Sankar (2010), "Prevalence of Oral Cancer and its Association with Mitochondrial DNA Mutation from Northeast India", 97th ISC, 2010, Tiruvanantapuram.

9) **Hansa Jagadish**, Mondal Rosy, Biswas R. and Ghosh K. Sankar (2010), "PCR Based Disgnosis of HPV from Cancer Patients in the Hospital Based Study from Southern Assam", 97th ISC, 2010, Tiruvanantapuram.

C) BOOK CHAPTERS

1) Pradosh Mahadani, Ksh Miranda Devi, Mridul M Das, Mohua Chakraborty, Fazlur Rahman, **Jagadish Hansa** and Sankar Kumar Ghosh BIOINFORMATICS IN DNA BARCODING. Chapter-5, Pp. 105-136. In: A **TEXT BOOK ON DNA BARCODING** (ed. Ghosh, S.K.), Book Space, Kolkata. **2012; ISBN: 81-922989-4-8**.

RESEARCH ARTICLE

Screening of 185DelAG, 1014DelGT and 3889DelAG BRCA1 Mutations in Breast Cancer Patients from North-East India

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Abstract

Around 1.35 million people of worldwide suffer from breast cancer each year, whereas in India, 1 in every 17 women develops the disease. Mutations of the Breast Cancer 1 (BRCA1) gene account for the majority of breast/ovarian cancer families. The purpose of study was to provide a prevalence of BRCA1 germline mutations in the North-East Indian population. In relation to the personal and family history with the breast cancer, we found mutations in 6.25% and 12.5% respectively. Three mutations, 185DelAG, 1014DelGT and 3889DelAG, were observed in our North-East Indian patients in exons 2 and 11, resulting in truncation of the BRCA1 protein by forming stop codons individually at amino acid positions 39, 303 and 1265. Our results point to a necessity for an extensive mutation screening study of high risk breast cancer cases in our North-East Indian population, which will provide better decisive medical and surgical preventive options.

Keywords: Breast cancer cases - BRCA1 - mutations - North-East India

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Introduction

Globally, breast cancer is the most common cause of cancer-related death in women, with around 327,000 deaths each year. Around 1·35 million cases of breast cancer have been found each year and 4·4 million women are believed to be live with breast cancer worldwide. It has been speculated that in 2020, around 1·7 million women will be diagnosed with breast cancer, which is an increased of about 26% in the developing world from current levels (Wong et al., 2009). In India, almost 100,000 women are diagnosed every year with breast cancer, and a rise to 131 000 cases is predicted by 2020 (Agarwal et al., 2008; Mangtani et al., 2010). And in North-East India breast cancer has always been a hotspot in comparison to rest part of the India because of genotoxic stress from tobacco exposure (Sunita et al., 2010).

Several environmental risk factors that may contribute to or hasten the development of breast cancer have been identified, including mainly lifestyle and reproductive factors. The factor with the strongest breast cancer risk association is a family history of breast and/or ovarian cancer, the associated risk being even higher for family history of early-onset disease (≤ age 40) (Datta et al., 2009). Genetic susceptibility to breast cancer is triggered in several ways; the best understood causal mechanism being due to germline mutations in tumor suppressor genes. Together, mutations in BRCA1 and BRCA2 genes account for the great majority of families with hereditary susceptibility to breast and ovarian cancer (Ford et al., 1998).

Among breast cancer patients, up to 5%~10% are considered directly relating to the inheritance of mutation in BRCA1 (MIM 113705, Genbank accession no. U14680) and BRCA2 (MIM 600185, Gene bank accession no. U43746), which accounts for most of the hereditary breast cancers (Claus et al., 1994). Moreover, women carrying these mutations have 60%~80% prone to breast cancer and ovarian cancer (Wooster et al., 2003). The BRCA1 gene is located on long arms of chromosomes 17 and it encodes a protein of 1863 amino acids (Hall et al., 1990). The protein physically associates with p53 and involved in homologous recombination (HR) and double-strand break repair in response to DNA damage (Greenberg 2008; Zhang et al., 2010). Miki et al. describes that BRCA1 is a strong candidate for the breast and ovary cancer (Miki et al., 1994). The spectrum of BRCA1 mutations has been characterized in different populations worldwide, with significant variation of the relative contribution of these genes to hereditary cancer between populations (Brozek et al., 2011). However, the contribution of mutations in these two genes to breast cancer patients in the Indian population remains relatively unexplored apart from a few small studies (Saxena et al., 2006). Thus, the screening of prevalence of mutations in BRCA1 gene will serve as a molecular predictor for women with breast cancer along with ovarian cancer in North-East Indian population.

Accumulation of various environment and genetic factors during lifespan of an individual can combine to form pathogenesis in breast cancer. Direct analysis of the tumor genome can reveal the genomic events accumulated during tumor progression. Hence, we investigated genomic alterations in thirty two breast cancer participants of North-East India with consideration to the exon 2 and

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exon 11 of BRCA1 gene. The main focus behind the study is to estimates genetic influence of BRCA1 in North-eastern population, which would be able to make better decision about medical and surgical preventive options.

Materials and Methods

Case selection

Thirty-two breast cancer tissues of female patients' (aged 29-73 years) were randomly chosen and used in the study from the CCHRC in 2009-2011 along with their consent letter. To examine the population frequency of any sequence variants identified in the patients, a series of age matched control samples were also collected from women. The genetic background of the population studied was unknown prior to study. Patients' selection was mainly based on following observation: 1) Younger women effected from breast cancer (≤40). 2) Previous personal history of a patient related to breast cancer. 3) Family history of the breast cancer patients.

Methods

Fresh cancerous and matched normal (adjacent non-neoplastic) tissue's specimen as well as blood samples were collected into EDTA vials from thirty two randomly selected patients from Cachar Cancer Hospital and Research Centre (CCHRC). The study was carried out after taking written informed consent from the participant. The extraction of DNA from tissue and blood were done by phenol/chloroform method (Ghosh et al., 2011). The supernatant containing total genomic DNA was aliquot and stored at -20°C. The isolated DNA was checked by the spectrophotometer and gel electrophoresis; by the help of its purity and quantity, the DNA was processes for the PCR amplification. The quantity of DNA used for PCR reaction was about 50-100 ng. The coding regions of BRCA1 gene were screened for DNA sequence variants by PCR techniques using exon specific primers (Table 1). It was performed and monitored in Veriti PCR machine (Applied Biosystems Inc., CA, USA) for 30 cycles (Table 2). The reaction's mixture contained total volume of 30 μl. Then PCR products were eluted from the agarose gel by the help of QIA kit® (Cat. No. 28704, USA) and processed for bidirectional Sanger sequencing at Bose Institute, India

Bio-informatics Tools

Raw nucleotide sequences from the sequencing results were processed through Sequence Scanner v1.0 (Applied Biosystem). The exported sequences were analyzed by using BLASTN software at NCBI site. According to the highest similarities, we depict the peak through the Chromas 2 software, and show the variations among sequences in figure panel.

Results

This is the first report in BRCA1 mutations from breast cancer patients of North-East India. We have designed our experiment to perceive the spectrum of mutation in breast cancer patients as well as the age matched controls. In the present investigation, we had selected three patterns of observation, age, personal history and family history of the patient.

In consideration to the studied data, the most common age group consisting of women 46-57 years around 90.62%, with the mean age of onset for 32 women diagnosis with breast cancer surveyed during 2009-2011 was 48 years (Table 2). Approximately 9.37% of cases were diagnosed under the age of 40 years. In relation to the age and breast cancer, the percentage of breast cancer in the younger women is quite high in this region. 6.25% patients have the personal history of breast cancer, and they found out with the breast cancer disease (Table 2). Reminiscent of this, the personal history of a patient with breast cancer in one breast or related cancer in other parts of the body has a 3 to 4 fold increased risk of developing a new cancer in other breast or in another part of the same breast. From the present exploration of mutational research study, we have found that 12.5 \% of the patients, whose have breast cancer in first-degree relative (Table 2).

By the help of three sets of primer, we amplified the particular regions of the BRCA1 gene and screened for alterations of the particular position of 185DelAG, 1014DelGT and 3889DelAG of exon 2, 11a and 11d respectively (Figure 1). To facilitate the objective of this study, we used the PCR technique and DNA sequencing technology for screening of all patients for the particular region of mutation with this population. The mutation rate is high, more than 40% were found to be positive with

Table 1. Primer pairs for the amplification of desire nucleotide bases

Primers Name	Primers Sequence (5'-3')	Tm (°C)	Ta (°C)	Amplicon size (bp)
BRCA1-185DelAG-F	ATT GGA ACA GAA AGA AAT GG	51.2	50	180
BRCA1-185DelAG-R	CCT AGT ATG TAA GGT CAA TTC T	54.7		
BRCA1-1014DelGT-F	ACA GCA TGA GAA CAG CAG	53.7	54	195
BRCA1-1014DelGT-R	CAC AGG GGA TCA GCA TTC AGA	59.8		
BRCA1-3889DelAG-F	TCT ACT AGG CAT AGC ACC GTT	57.9	53	192
BRCA1-3889DelAG-R	CTT CCA ATT CAC TGC ACT GTG	57.9		

*Tm - Melting Temperature, Ta - Annealing Temperature

Table 2. Characteristics of Breast Carcinoma Patients

Age	Total No. of patients	Mutation Positive cases	Family History and Personal History of patients F.H	No F.H	P.H	No P.H
≤40	3 (9.37%)	1(33.33%)	1 (3.12%)	2 (6.25%)	0	3 (9.37%)
≥40	29(90.62%)	12(37.5%)	3 (9.37%)	26 (81.25%)	2(6.25%)	27(84.37%)
Total	32	13(40.62%)	4(12.5%)	28 (87.5%)	2(6.25%)	30(93.75%)

*F.H: family history, No F.H: without reported family history, P.H: Personal history, No P.H: without reported personal history

Table 3. *BRCA1* Deleterious Mutation in North-Eastern Breast Cancer Patients, India

Gene	Exon	NT	Base change	Codon	AA change	BIC Designation	Variation Type	Reported	Cases found	Mutation Rate
BRCA1	2	185	DelAG	23	Stop 39	185DelAG	Truncated protein	Ashkenazi, Jews, others	1	3.12%
BRCA1	11a	1014	DelGT	299	Stop 303	1014DelGT	Truncated protein	Various, Pakistani	3	9.37%
BRCA1	11d	3889	DelAG	1257	Stop 1265	3889DelAG	Truncated protein	Various, Chinese	9	28.12%

*NT: Nucleotide Position, AA Change: Amino Acid Change, BIC Designation: Breast Cancer information core Designation

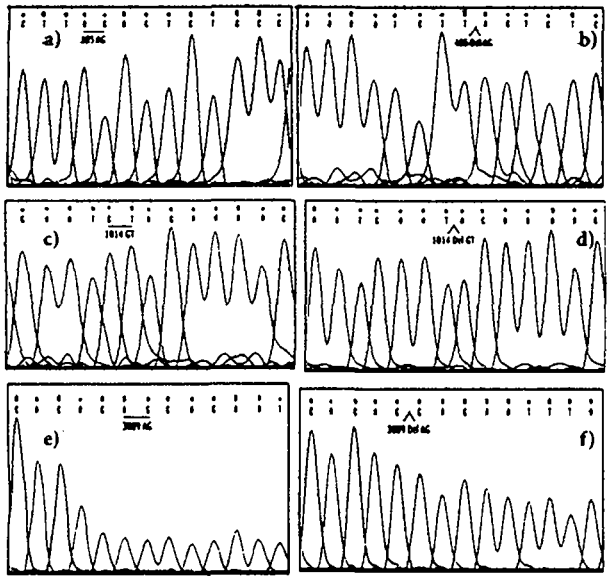

Figure 1. Variation in the Sequence Chromatograms of BRCA1 Gene Amplified and Screened for Alterations of the Specific Position at 185 Del AG, 1014 Del GT and 3889 Del AG of Exon 2, 11a and 11d Respectively from Breast Cancer Patients. a) Normal sequence, no deletion at 185 AG position from exon 2. b) Mutated sequences, deletion at 185 AG position, from exon 2. c) Normal sequence, no deletion at 1014 GT position from exon 11a. d) Mutated sequence, deletion at 1014 GT position from exon 11a. e) Normal sequence, no deletion at 3889 AG position from exon 11d. f) Mutated sequence, deletion at 3889 AG position from exon 11d.

these three variations of the BRCA1 after screening. And from that, most of the mutation occurrences were from the exon 11, out of the 32 patients 12 have the mutation which was around 37.5% and in exon 2, the incidences of mutations were quite low (around 3.12%) to the other part of world ethnicity. In commencing exon 11, we have screened two parts of the partial sequence; those were exon 11a and 11d of 192 and 194 bp product of amplicon respectively (Table 1). The mutation rate of 1014DelAG and 3889DelAG was 09.37% and 28.12% correspondingly (Table 3).

In summary, we found two mutations of two base pair deletion in exon 11 among 12 patients (1014 Del GT {3 Patients} and 3889DelAG {9 patients}) which results in protein truncation of BRCA1 protein by forming stop codons at 303 and 1265 position of amino acid respectively and one mutation of two base pair deletion at exon 2 (185DelAG) results in a stop codon at 39 position of amino acid in BRCA1 protein. Cachar Cancer Hospital and Research Centre support the observations, evaluations and findings of the research study undertaken among breast cancer patients from the North-East India.

Discussion

In the last few years many studies have focused in screening of mutations in breast/ovarian cancer. In this study majority of patients were at the fourth stage and large percentage of the patients who come to the CCHRC seeking care owing to their nominal incomes. Generally, breast cancer can occur at any age but younger women are less susceptible to ward's breast cancer (Mathew et al., 2004). Our study comprises a lower mean value of age, which is revealed that the disease occurs a decade earlier, as compared to western countries (Sandhu et al., 2010). The probable reason for the early onset of this dreaded disease in the younger women may be due to personal history with a breast cancer/ovary cancer (Liang et al., 2011), family history of breast cancer, particularly in a mother, sister and daughter (Metcalfe et al., 2010), history of radiation therapy to the chest before age 40. (Narod, 2011)

The aim of the mutational research analysis was to study the incidences and distribution of mutations in North-Eastern region of India concerning the other factors relating for breast cancer. For the diagnosis of breast carcinoma, FNAC technique was used as it is a useful diagnostic tool because of its cost efficient and rapidness (Sandhu et al., 2010). Three deleterious nonsense mutations resulting in a premature termination codon were identified in BRCA1: 185DelAG in exon 2; 1014DelGT and 3889DelAG in exon 11, rather absent in the observed control group. Nonsense mutations of these three specific mutations are very detrimental to the protein; it can render the resulting protein non-functional due to formation of stop codon at the early stage. Unexpectedly, we have gathered an 185DelAG in North-East Indian Hindu patient residing in Cachar district who claimed to have family history but not to Jewish ancestry (Figure 1b). In India, 185DelAG has been reported in all populations studied (Kumar et al., 2002; Hedau et al., 2004; Valarmathi et al., 2004; Saxena et al., 2005). Similarly, Lakhotia et al. found the same mutation by the help of conformation sensitive gel electrophoresis in four Indian breast cancer families (Lakhotia et al., 2010). Worldwide population studies have revealed that the 185DelAG mutation predates the severance of Sephardi and Ashkenazi Jewish populations and is probably 2000 years old (Bar-Sade et al., 1998). BRCA1 1014DelGT was detected in two Muslim index cases of without any family history from Karimganj District, but both have the personal history of ovary cancer (Figure 1d). Interestingly, the same mutation was reported in a heterogeneous Pakistani population of Muslim religious (BIC-NHGRI). The specific position of 1014DelGT comes under the part of a DNA binding region where other tumor suppressor proteins could not bind and unable to form complex protein for the downstream act of protein. These observations suggest that 1014DelGT might be a common mutation in the Muslim community and might have migrated to the Indian population through a pool of Muslim immigrants (Liede et al., 2009). In North-Eastern region, the mutation of 3889DelAG is higher than the rest of the mutation found (Figure 1f), out of nine, three have the family history and found scattered in studied population; it also found in various populations of the world (Thirthagiri et al., 2008; Farooq et al., 2011). The location of 3889AG is towards the C terminus of BRCA1, within the transcriptional activation domain, a region as well reported to interact with the BRCA2 protein, which plays an important role in double stranded break (DSB) repair (Roy et al., 2012). Nevertheless, the number of mutations identified in the studied North-East Indian population is higher; it may be due to the selected

candidate who comes under the three patterns of the study. This significant proportion of mutation from BRCA1 suggests one of the several possibilities for genetic predisposition in the North-East Indian population.

The results of this primary study put forward that the mutational spectrum in exons 2 and exon 11 of BRCA1 gene in this population may be at variance from what has been observed in other Indian populations. Further studies or mutational screening of the whole BRCA1 gene from different geographical regions of India will help in identifying the mutations may provide the knowledge of biological properties of the protein corresponding to polymorphism. Through this aspect of proper counselling, patients and pre-symptomatic mutations carriers' studies would be able to make better decision about medical and surgical preventive options.

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RESEARCH COMMUNICATION

Human Papillomavirus Testing for Suspected Cervical Cancer Patients from Southern Assam by Fast-PCR

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Abstract

World-wide epidemiological studies have shown that cancer of the uterine cervix is the second most common malignant disease in women. Virtually every cervical cancer (99.7%) is HPV-positive, indicating that the presence of HPV is an obligatory element in their development. The present study was conducted by Fast-PCR (within 15 min.) based diagnosis of HPV 16 and HPV 18 infection amongst patients of suspected cervical cancer, confirmed by cytological methods. Twelve women, out of a total of fifty studied cases who had positive cervical pap smears (24%) were found to be positive for HPV 16/HPV 18 infection when PCR based technique was applied. The results indicate, perhaps, a greater specificity of PCR based diagnosis, or presence of other HPV subtypes as etiological factors in the present study group confined to Southern Assam.

Keywords: Fast-PCR - cytological methods - cervical cancer - HPV - sensitivity - specificity - Southern Assam

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Introduction

World-wide epidemiological studies have shown that cancer of the uterine cervix is the second most common malignant disease in women (Ogunmodede et al. 2007). Virtually every cervical cancer (99.7%) is HPV-positive, indicating that the presence of HPV is an obligatory element in the development of cervical cancer (Walboomers et al., 1999). Specific anogenital types of human papillomavirus (HPV) cause the initiating infection that leads to cervical cancer. More than 100 HPV types are known, of which at least 70 infect the anogenital tract. Knowledge of HPV status is becoming increasingly important as a triage screen after detection of atypical cells of undetermined significance (Bollmann et al., 2003) and as a primary screen for cervical cancer detection (Cuzick et al., 2000).

HPV typing has an important prognostic or therapeutic value, as it can distinguish between HPV types of high and low oncogenic risks. Identification of high-risk HPV genotypes may permit selection of those patients who are at increased risk for disease and may therefore provide additional clinical value. An important requirement for this approach is that HPV testing and identification of high-risk HPV types should be highly sensitive and specific (Speich et al., 2004). The types associated with diseases of the anogenital tract can be classified on the basis of phylogenetic relationship (Zur Hausen, 1996) and of associated frequencies with benign or malignant cervical lesions as high-risk types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -54, -56, -58, -59, and -66) and low risk types (HPV-6, -11, -34, -40, -42, -43, -44). The most common HR types are HPV-16, -18, -31, -33, and -45 (Bosch et al., 1995; Nobbenhuis, 1999). HPV genome can be divided into three different regions including early, late and long control regions (Moosavi et al., 2008). Early region proteins are classified into two groups: E1, E2, E3 and E4 proteins, and E5, E6 and E7 oncoproteins (Dyson, et al., 1989). E5 oncoproteins stimulate the growth of epithelial cells and in many cancers, lead to increased cellular mitosis and consequently cause papilloma lesions (Prayitino, 2006). E6 oncoprotein has been implicated in causing chromosomal abnormalities and progression of cells to neoplasia. E6 is also responsible for causing damage to P53 (Pei, 1996). E7 oncoprotein binds to Rb gene products and other similar proteins thereby inactivating them (Kim, 2001).

The detection and type-specific classification of HPV infection by in vitro viral culture is not possible and serological tests are still ineffective. Direct hybridization based assays, such as Southern blotting and in situ hybridization have been described, but lack sensitivity and specificity (Szuhai, 2001). It has been reported in many studies that target DNA amplification may offer more specificity and sensitivity in detecting HPV infection (Malloy, 2000). In this study we have compared the diagnostic efficacy of PCR based techniques over cytological examination in the direct detection of HPV infection involving HPV 16 and HPV 18 as well as evaluated the relevance as a screening technique.

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Materials and Methods

Samples

Samples were collected from fifty patients attending Cachar Cancer Hospital, Silchar, Silchar Medical College and Hospital, Silchar and different infertility clinics of Southern Assam, who had positive pap smear during May-November, 2010. Considering the short duration of the study, no follow-up was done as part of this study in patients who were found positive for HPV infection by cytological/PCR methods except being referred for further appropriate clinical consultation. Samples were collected with informed consent of subjects.

Cytological Methods

Smears were collected with Ayer's spatula and immediately fixed in absolute alcohol for minimum 6 hours. The smears were then washed with distilled water and dipped in hematoxylene for 7 to 10 minutes. These were subsequently washed in running tap water and dipped in 1% acid alcohol for decolourization, and repeated twice. After third washing, the smears were dipped in 50% alcohol and transferred to O.G 6 solution and kept for 2 to 3 minutes. These were then dipped in absolute alcohol and transferred to EA 50 solution and kept for 20 to 30 minutes. After final dipping in absolute alcohol, the smears were cleared with xylene and mounted for viewing under optical microscope.

DNA extraction

Cervical samples were taken with sterilized swab and put in sterilized collection tubes. The samples were stored at 04°C until DNA isolation. To isolate DNA, sample was taken and mixed with 500μL of TES buffer. Then 30/50μL of 10% SDS and 2/5μL of proteinase K was added to it and kept in a water bath for 1Hr at 57°C. Equal amount of phenol chloroform isoamylalcohol (25:24:1) was added and centrifuged at 12000rpm for 8mins. The supernatant was taken and equal amount of chloroform isoamylalcohol (24:1) was added and further centrifuged. The supernatant was again taken and twice the amount of absolute alcohol was added and kept in refrigerator for some time. The refrigerated supernatant was next centrifuged, alcohol discarded and washed with 200 – 500 μl of 70% alcohol and kept for drying. After that 20 – 50μL nuclease free water was added and left overnight. Isolated DNA was checked under agarose gel electrophoresis in a 1% gel and observed in UV transilluminator for purity.

PCR Method: HPV DNA was demonstrated by performing PCR with consensus primers. To identify the specific HPV types (16 and 18) in the HPV positive specimens further PCR was performed using HPV 16 (5' GCC TGT GTA GGT GTT GAG G 3' -Forward and 5' TGG ATT TAC TGC AAC ATT GG 3' -Reverse) and HPV 18 (5' GTG GAC CAG CAA ATA CAG GA 3' -Forward and 5' TCC AAC ACG TGG TCG TTG CA 3' -Reverse) specific primers. The HPV 16 primer pairs were designed from the L1 region and HPV 18 primer pairs were from the E1 region of the HPV genome. Each amplification reaction mixture of GeneAmp® Fast PCR Master Mix (2X) [Applied Biosystem] 20μL contained buffer, Mg2+, dNTPs, Gold enzyme, sets of Primers and isolated DNA from different cervical samples in 96 wells Veriti Thermal cycler (Applied Biosystem).

FAST PCR amplification using AmpliTaq GOLD (2X):The following PCR steps were employed for FAST PCR (AmpliGOLD-Applied Biosystem) of the total volume of 20 ul.

Step	Reactivation of Enzyme	PCR (30 cycle)			Final step	
	Hold	Denaturation	Annealing	Extension	RAM	Hold
Temp	95°C	95°C	58	68°C	72°C	4°C
Time	10 min	3 sec	3 sec	3 sec	15 sec	15sec

Total Time taken- Reactivation of Enzyme (10 min) + PCR for 30 cycles [3+3+3=9 second X30=270 sec (4½ min)] + 30 sec (½ min)= 15 min

Results and Discussion

In our study, samples were collected from fifty patients as described previously. To avoid false positive results, and more confirmation of HPV genotype, we use this new technique to detect HPV. The mean age of patients was forty five years (ranging from twenty to seventy) and the highest number of HPV positive cases belonged to patients aged forty to fifty years. The presence of amplifiable DNA, using primers for HPV 16 and 18 was confirmed in twenty four (24%) out of the fifty samples. Out of the twelve positive cases HPV type 18 was present in ten cases and HPV type 16 was present in two cases. The PCR was carried out using Fast-PCR method by Applied biosystem reagents (details in Materials and Methods) within in 15 min. with the primers specific to the subtype 16 and 18 and the product sizes were 246 bp (product from L1 region base 379 to 624 with forward – reverse primers) and 162 bp (product from E1 region base 1448 to base 1609 with forward – reverse primers) respectively, depicted in Figure 1.

Our result showed that the occurrence of affected percentage of HPV type 18 is high in comparison to the HPV type 16 in our geographical region. In nineteen cases (76%), it was not possible to identify the virus genotype which we attributed to possibly false positive results of cytological tests or may be due to the presence of other

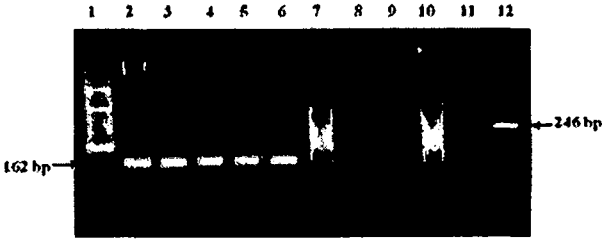

Figure 1. Agarose Gel Electrophoresis (2%) of PCR Products of HPV Type 16 (246 bp) and 18 (162 bp). Lane-1-DNA marker, Lane- 2,3,4,5 and 6-amplified DNA (162 bp) of HPV18 and Lane-7, 8, 9, 10 and 11- no amplified (absence of HPV), 12- amplified DNA (246 bp) HPV type 16

genotypes.

Molecular (PCR) tests may accurately identify different types of HPV (of low and high cancer risks) in cells from cytological screening of cervical lesions and, due to their high sensitivities, have been the focus of attention of many studies (Bauer et al., 1992). The purpose of our study was to detect the HPV 16 and 18 strains by PCR technique with the help of two pairs of primers. The advantage of this PCR based assay, unlike the other currently available assays, is that it is able to report the actual genotype of the HPV detected, rather than issue a broad based ambiguous diagnosis of HPV infection. In the present study, cervical smears were simultaneously subjected to cytological examination as well as PCR technique of detection. The traditional way of classifying tumors is by histopathology. The ability to analyze change in the levels of the transcripts and/or protein products for literally thousands of genes promises interesting possibilities as a research tool for understanding the underlying molecular mechanisms, but also for automated tissue diagnosis (Drain et al., 2002; Oh et al., 2009). HPVs frequently infect humans. They are classified into categories of low risk types responsible for the most common sexually transmitted viral infections and high risk types which are crucial etiological factors in cervical cancer development (McFadden and Schumann, 2001). The direct detection of HPV in cervical specimens may offer an alternative (?) or complement to population-based cytological screening. Recent studies have demonstrated that HPV test results are more sensitive (although they are less specific) than Pap smears in detecting high-grade dysplasia in older women (Schiffman et al., 2000; Wright et al., 2000). In most scenarios women with positive HPV tests still have Pap tests or a diagnostic procedure to provide cytological or histological confirmation of their disease.

We conclude that PCR based diagnosis may be a more specific test for detection of HPV subtypes. Contrary to many studies, it seems that PCR based diagnosis of HPV may not be ideal as a screening test in comparison to conventional pap smear studies, although it may be complementary. However, the use of PCR technique, covering all strains, may be a more sensitive diagnostic procedure that can help to differentiate between suspicious lesions and confirmed HPV infection at risk of subsequent malignancy. We suggest that further studies, covering larger study groups in other geographical locations, are required to draw a firm conclusion as to the diagnostic significance/advantage of PCR techniques over conventional methods.

Acknowledgement

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P- 11

Mutation detection of *BRCA1* gene in breast cancer patients from southern Assam, India

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Each year 10.9 million people suffer from breast cancer worldwide that result in 6.7 million deaths from the disease. In India, one in 17 women is suffering from breast cancer during their lifetime. The breast cancer susceptibility genes, *BRCA1* and *BRCA2* are thought to account for the majority of breast/ovarian cancer families. Here, we examined the partial coding sequence of the *BRCA1* gene taking exon 2, 11 and 20 as these exons are mostly affected, globally reported from breast cancer information core databases. Genomic DNA was isolated from 32 patients' tissue with matched blood diagnosed by Fine Needle Aspiration Cytology (FNAC) and biopsy. With the help of 3 sets of *BRCA1* primers, mutation analysis was carried out using polymerase chain reaction (PCR) followed by DNA sequencing. We found two mutations of two base pair deletion in exon 11 among 12 patients (1014 delGT {3 Patients} and 3889 delAG {9 patients}) which results in protein truncation of *BRCA1* protein by forming stop codons at 303 and 1265 position of amino acid respectively and one mutation of two base pair deletion at exon 2 (185delAG) results a stop codon at 39 amino acid of *BRCA1* protein which has been described in Ashkenazi Jewish families. Northeast Indian populations have not been extensively studied, for prevalence and spectrum of mutations in cancerous patient. This sequenced based analysis will give ideas to understand the early onset of familial breast cancer.

P- 9

COLD–PCR: a molecular tool for mutation detection in *TPO*, *BRCA1* & D-loop of mitochondrial DNA

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DNA mutations occur in a wide variety of diseases, sudden infant death and cancer, such mutation may occur in nuclear DNA (nDNA) or in mitochondrial DNA (mtDNA). Of the diseases, cancer and thyroid dysfunction (hypothyroidism) is one of the major health issues which may occur due to genetic polymorphism apart from environmental stress. Detection of mutations in *TPO* , *BRCA1* gene and D-loop region of mtDNA in case of thyroid dysfunction, breast & oral cancer respectively and developing an effective technique called COLD-PCR (Nature Medicine;14;2009), for the enrichment and detection of mutation without sequencing. PCR amplification was done using respective primers for the desired gene followed by direct sequencing of the amplified products for detecting mutations in different samples. For large scale of samples mutation detection was done using COLD-PCR technique, for which primers were designed in such a way that will amplify only the mutation point in the gene which gave product size 100bp in all three cases. The COLD-PCR was performed with our clinical samples (mutated DNA) with the normal samples (wild type DNA) for further validation of the Indels. In TPO gene insertion mutation was found in exon 8 (7bp ins at 1183_1190 Ins GTGCATG and 4bp ins 1203_1207 Ins TGGT) and deletion mutation in exon 9 (1bp del at 1562 i.e. 1562delA and 6bp del at 1583 i.e. 1583_1589 del CATGCC) respectively. In case of *BRCA1* gene three mutations were found, out of which two of them were mutations of two base pair deletion in exon 11 (1014 delGT and 3889 delAG) and one was two base pair deletion at exon 2 (185delAG). In D-loop region mutations at nucleotide position 146 (TàC) and 152(CàT) occurred simultaneously in all the cases which were hotspot mutation in oral cancer and were detected using COLD-PCR in large number of samples. Thus, COLD-PCR is a reliable technique for detection of mutation in different genes without doing direct sequencing and therefore, reducing time and expenses for molecular diagnosis of diseases like thyroid dysfunction, detection of somatic mutations in cancer in diverse clinical samples.

B43 A novel bioactive principle from *Piper longum*-induced G1 arrest and apoptosis in human bladder cancer T24 cells. Venkatakrishnan V[1], Ramadevi S[2], Senthilkumar S[3], Jeevarathinam K[1], Lakshmi B.S[2]. [1]Pondicherry University, Pondicherry, India, [2]Anna University,, Chennai, India, [3]SASTRA University, Thanjavur, TN, India.

The antineoplastic potential of bioactive molecules from herbal medicine has been greatly concerned. In this study, 1-C 3' 4'-Methylenedioxy phenyl dodec-1-en-10-one (MDPD), isolated from *Piper longum*, was evaluated for its antiproliferative potential in vitro. MDPD induced cell death in T24 human bladder cancer cells in a time and dose-dependent manner. Intranucleosomal cleavages and PI staining confirmed that the cell death initiated by MDPD was due to apoptosis. Further, regulatory on G0/G1 arrest was proved by flow cytometric studies. RT-PCR and Immunoblotting studies showed the level of p53 was increased upon treatment with MDPD. Simultaneously the level of cyclin D and cdk2 were decreased. MDPD induced bax up-regulation, an apoptotic regulatory marker accompanied by an apparent down-regulation of Bcl-2 at genetic level. A good correlation between loss of mitochondrial membrane potential and cytochrome c release was observed indicating the participation of mitochondria-related mechanism. Treatment of T24 cells with MDPD also induced the activation of initiator caspase-9 protein levels which led to induction of apoptosis. In addition, MDPD also promoted caspase-3 activity after exposure for 6, 12 and 24 h, validating the apoptotic cell death. Furthermore, the MDPD-induced apoptosis on T24 cells was blocked by the broad-spectrum caspase inhibitor (z-VAD-fmk). Taken together, the results clearly demonstrate that MDPD effected the arrest of T24 cells in the G0/G1 phase and induced apoptosis through intrinsic pathway.

B44 Relationship between various biochemical, hematological, comorbid, lifestyle, and demographic parameters in gallbladder carcinoma: A single-center retrospective study of 342 patients. Raleev Kumar[1], R Ravi Kannan[1], Sankar K. Ghosh[2], Sumit Goyal[1], Jagadish Hansa[1], Aditi Bhowmik[2]. [1]Cachar Cancer Hospital & Research Centre, Silchar, Assam, India, [2]Assam University, Silchar, Assam, India.

Incidence of gallbladder cancer in northeastern India ranges from AAR 2.3 to 6.9 in males to AAR 4.7 to 14.7 in females. In the single center retrospective study of 342 gallbladder cancer patients that were reported between 2008 and 2010, the relevance of various biochemical, hematological, demographic parameters and dietary and personal habits were analyzed. 90% of gallbladder cancer patients were diagnosed at stage IV. 80% of patients were reported to have elevated level of alkaline phosphatise and total bilirubin. Hemoglobin level was found elevated in 40%. 60% were reported II on ECOG performance status. 85% of patients with gallbladder cancer were diagnosed at the age of more than 40 years. The peak incidence was in the 5th decade. Male to female ratio was 1:2. 80% of cancers were seen in association with cholelithiasis. 98% of gallbladder cancers observed in this study were in Bengali ethnic group. There did not appear to be any religious predilection. 10% of them have family history of cancer. 70% were tobacco users, whereas 53% were areca nut users. It was observed that 25% of the gallbladder cancer patients were seropositive for hepatitis B infection. A model for development of gallbladder cancer was discussed.

B45 Hydroxamic acids analogues as inhibitor of neoplastic human HeLa cells: A self-organizing molecular field analysis (SOMFA) approach. Ram Prakash Balwade, Jr. National Institute of Technology, Raipur, Chhattisgarh, India.

Hydroxamic acids have been recognized as compounds of pharmacological and toxicological and pathological importance. Taking the pharmacological potential of this class of compound in account, a series of N-arylsubstituted hydroxamic acids with general formula, R1NOH•R2C (=O), where R1 and R2 are phenyl substituted phenyl groups were synthesized.

The antiproliferative activity of 18 hydroxamic acids has been tested in vitro towards human cervix carcinoma, Hela cells by MTT assay. The IC_{50} values were found to be in the range from 32.38 to 276.77μM.

Molecular modeling was performed using Hyperchem Release 7.5 software. The structure of these compounds were built and optimized by molecular mechanics MM+ force field and assigned charge by the AM1 and PM3 Hamiltonian semiemperical method using MOPAC 2007. The final geometries are then performed RMS overlapping to fit with N-p-chlorophenyl-4-bromobenzohydroxamic acid, which has the best antitumor activity. Using Vega program the final overlayed geometries are converted into CSSR file format.

The three dimensional quantitative structure-activity relationship (3D-QSAR) studies were performed on hydroxamic acids analogues using self organizing molecular field analysis (SOMFA) method with their antitumor activities against HeLa cells.

The best 3D-QSAR model obtained with cross-validation q^2 (0.779) and cross-validation r^2 (0.838) show a good predictive ability of potency of hydroxamic acids.

The analysis of SOMFA contour maps provided insight into the possible modification of the molecules for better activity.

B46 Gene expression profiling of cervical cancers to delineate prognostic molecular markers. Asha Thomas[1], Umesh Mahantshetty[2], Shyam Kishore Shrivastava[2], Kedar Deodhar[2], Rita Mulherkar[1]. [1]ACTREC, Navi Mumbai, India, [2]Tata Memorial Hospital, Mumbai, India.

Cervical cancer is a major cause of cancer deaths among women in developing countries, accounting for 34% of all women's cancers and is the most common cancer in Indian women. Clinically localized cervical cancer can be effectively ablated using surgical or radiation treatments. Metastatic disease, however, affecting 15-20% of patients, is essentially incurable. Recent studies have shown the use of gene expression profiling by DNA microarray in the classification of various cancers and also in the prediction of disease outcome and treatment response. Such a study in cervical cancer may provide some insight into the mechanism of resistance to treatment and to predict the treatment outcome.

To identify a set of genes related to disease prognosis of cervical squamous cell carcinomas and to establish a predictive method, pretreatment samples of FIGO stage IIIB of the disease were analyzed using DNA microarray with Agilent 4*44K chip. The samples were grouped with respect to prognosis of the disease, as having no evidence of disease (NED) after a median follow up of 65 months or had disease recurrence (DIS) during the follow up period. Total samples were randomly grouped into training set (N=33) and test set (N=8) and all the analysis were done on training set of samples. Raw data obtained were normalized, filtered and analyzed using Genespring GX11 software.

Gene sets that are differentially expressed between the two groups at different p values and fold change were selected for clustering and Treeview analysis. Gene sets giving good clusters were selected and prediction models were established using support vector machine algorithm. These models were then used to predict the prognosis in test set (n=8) of samples. The gene set obtained after analysis could clearly differentiate good and bad prognosis group of samples and could predict the prognosis of test samples with 87.5% accuracy. The gene set was further subjected to receiver operator characteristic (ROC) curve analysis to check the individual predictive capacity of the genes. Further analysis of the data to

insufficient.

Objective: The objective of this study was to identify genes differentially expressed in non-small cell lung carcinoma patients in high-risk Northeast Indian population.

Material and Method: Gene expression profiles of five NSCLC samples compared to their matched normal adjacent tissues were generated using cDNA microarray. The results of cDNA microarray analysis were validated by real-time PCR in 30 samples of NSCLC. Differentially expressed genes (≥1.5 fold, $p<0.05$) were subjected to gene ontology and pathway analyses using DAVID software and ingenuity pathway analysis (IPA) tool.
Result: Transcription profile of NSCLC patients showed the expression of 734 genes to be differentially expressed (≥1.5 fold, $p<0.05$). Of these, 311 genes were overexpressed and 423 were underexpressed. DAVID analysis revealed significant enrichment in genes related to epidermal growth factor, homeobox related transcription activity and transcription cofactor activity. Moreover, MAPK signaling pathway was identified as significant biological cluster by the KEGG pathway annotation component in DAVID analysis. IPA analysis showed that the differentially regulated genes belong to distinct functional types such as cell death, cancer, cell cycle and cellular assembly and organization. Up-regulated genes associated with these terms included FGFR2, IFNG, RAG2, MAP3K2, TMSB10, CANT1, COL3A1, CXCL9, BRIP1, TOP1, and AGPAT6. Down-regulated genes associated with above biological functions were BCL2, CD44, NGFR, PDGFRA, CASP10, MALAT1, MPL, PDPN, CNN1, ID3 and ADAMTS9. Genes associated with cell death, cell cycle, cellular growth and proliferation, cell signaling, inflammatory response appear to occupy prominent position in the network analysis.

Conclusion: Gene expression profile of NSCLC patient may provide insight into the molecular mechanisms underlying NSCLC pathogenesis and provide basis to the development of diagnostic biomarkers in NSCLC in the high-risk study population.

C32 The effect of betel quid and tobacco chewing, GSTM1, methylation of p16 gene, and Epstein-Barr virus infection on esophageal carcinoma: A case control study in high-incidence region of NE India. Ruhina Shirin[1], Fazlur Rahman[1], Rosy Mondal[1], Ravi Kannan[2], Sankar Kumar Ghosh[1]. [1]Assam University, Silchar, Assam, India, [2]Cachar Cancer Hospital and Research Centre, Silchar, Assam, India.

Tobacco smoking, betel quid chewing and alcohol consumption are the most established risk factors for esophageal cancer. Apart from the environmental factors, genetic variants, infection with Epstein Barr virus (EBV) and promoter hypermethylation of several tumor suppressor genes are also considered to play an important role in the development of this cancer. Recent evidences show that the presence of promoter hypermethylation of p16 gene is influenced by tobacco smoking, germline deficits of glutathione S-transferase (GST) gene families and EBV infection. However, the exact mechanism is still not well understood. In this study, data and samples were collected from 53 histologically confirmed esophageal cancer cases and 58 age- and sex-matched controls from a high-incidence region of India, where widespread use of smoked and smokeless form of tobacco along with betel quid and fermented areca nuts are prevalent. The habit related data were collected in a face-to-face interview. Logistic regression model was used to determine the odds ratio (OR) for the various parameters under study. We investigated the relation of smoking, betel quid, and tobacco chewing with GSTM1 genotypes; aberrant promoter hypermethylation of p16 gene and EBV infection. Among all the factors under the study, betel quid and tobacco chewing was the main risk factor with an age adjusted odds ratio (OR) of 3.25 ($p<0.001$) when adjusted for smoking and alcohol consumption. Another striking finding was an elevated association of EBV infection (60% vs 35%) in the esophageal cancer cases as compared to the controls (OR=3.11, $p=0.01$). A significant elevated risk for p16 methylation was associated with EBV infection (OR=5.25, $p=0.05$), however, no association between GSTM1 null genotype and p16 methylation was found. The risk for developing esophageal cancer significantly increased by 5.14 folds with promoter hypermethylation of p16 gene in tobacco and betel quid chewers with a null GSTM1 genotype and positive EBV infection ($p<0.01$). Our study suggests a strong combined effect of betel quid and tobacco chewing, GSTM1 germline deficits, EBV infection and promoter hypermethylation of p16 gene in the pathogenesis of esophageal cancer.

C33 Mitochondrial DNA genome as biomarker in oral squamous cell carcinoma from Northeast India. Rosy Mondal[1], Jagdish Hansal[1], Ravi Kannan[2], Sankar Kumar Ghosh[1]. [1]Assam University, Silchar, Assam, India, [2]Cachar Cancer Hospital and Research Centre, Silchar, Assam, India.

Mitochondrial dysfunction is a hallmark of cancer cells and mitochondrial DNA (mtDNA) mutations are strewn throughout the coding and noncoding regions. Tobacco consumption in various forms is the major risk factor for the development of OSCC which not only causes single strand breakage in the DNA but also makes the mitochondrial DNA susceptible to damage by reactive oxygen species (ROS). Mitochondrial DNA function is also impaired at certain complex I (ND2) and complex III (succinate-CoQ reductase) which contains the majority of the cytochromes. However the biological relevance of these mutations is not well understood. Here we tried to detect hot spot mutations in the d-loop region of the mtDNA of the 25 matched tissue samples of OSCC patients using PCR and direct sequencing. We found somatic D-loop mutations in all the patients, particularly in the mononucleotide repeat which were either insertions or deletion. Several mutations were found outside the D310 region among which mutations at nt146 and nt152 found to be hot spot in the oral cancer patients of this region. Also we observed that with the advancement of tumor stage, the number of mutations increases significantly in C-tract ($P<0.0001$) region. Our results demonstrate that these mutations can be used as a possible biomarker for the early diagnosis of OSCC. Furthermore, a large scale study of the mitochondrial genome is needed to establish the association of these mutations between the genotype/phenotype and the mitochondrial mutations.

C34 Induction of apoptosis by *Sesbania grandiflora* in human leukemia U937 cells. Rajnesta Roy, Deepak Kumar, Chinmay Chowdhury, Padma Das. Indian Institute of Chemical Biology, Kolkata, West Bengal, India.

Background: Leukemia is the most common hemato-oncological disorder that affects different age groups. Chemotherapy for leukemia is still far from producing a satisfactory result especially in underdeveloped countries due to its adverse effects, high cost, prolonged treatment protocol and alarming increase in the incidence of drug resistance. Phytochemicals are increasingly becoming an important source for cancer chemopreventive agents. *Sesbania grandiflora* commonly known as "sesbania" and "agathi" is widely used in Indian traditional medicine for the treatment of a broad spectrum of diseases including inflammation, leprosy, gout, and rheumatism. The present study provides evidence for the anti proliferative effect of methanolic extract of *S. grandiflora* flowers (SG) in leukemic cell lines and delineates the underlying involvement of various apoptotic pathways.

Methods: Cytotoxicity of SG was evaluated in U937 cells by MTT assay along with flow cytometric measurement of reactive

mutations in many cancer types. In this study, we analyzed tissue samples of head and neck cancer patients from Barak Valley using PCR and direct sequencing and found three common mutations in nucleotide position 73, 263 and 489 with insertions and deletion within the polycytidine stretch in all the individuals so far analyzed. We observed that with the advancement of tumor stage, the number of mutations increases significantly in C-tract. The nucleotides may prove to be the biomarkers for early diagnosis of cancer.

72. Mitochondrial DNA detection at the D-310 region by COLD-PCR in Cancerous tissue

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Keywords: *mtDNA, D-loop, D-310, COLD-PCR, Somatic Mutation, Head & Neck Cancer.*

Mitochondrial DNA mutations occur in a wide variety of degenerative diseases, sudden infant death, ageing and cancer. Many of the somatic mtDNA mutations in human cancers are located in the displacement loop (D-loop) and particularly at polycytidine stretch (C-tract) referred as D310. Most of the somatic alterations found in tumors showed deletion / insertions of 1- or 2-bp generating D310 variants identical to constitutive polymorphisms. To ensure highest sensitivity in mutation detection and overcome from the polymorphism, we adapted COLD-PCR method followed by DNA sequencing. After analysis of all the sequences for C-Tract alteration, we got some severe changes in the region by means of deletions and insertions. Though the region has polymorphism about 6-9C in first stretches of sequence in D-310 but the sequence of C-Tract has found 30% changes in all cases of head and neck cancerous patient.

MATERIALS AND METHODS:

Blood samples are collected from 60 healthy controls and 60 histopathologically confirmed female breast cancer patients (premenopausal and postmenopausal women) aged 25 to 80 years. Serum LDH levels and lipid profile are investigated preoperatively and serum LDH levels are estimated 21 days after the surgery.

RESULT:

There is a significant increase in preoperative and decrease in postoperative serum LDH levels in breast cancer patients as compared to controls. The values of preoperative serum LDH are higher in postmenopausal cases. Breast cancer patients have high BMI with increased total cholesterol, TGL, LDL-C as compared to the controls which is statistically significant.

CONCLUSION:

The findings of this study suggest an immense potential for LDH as a prognostic marker for breast cancer and confirms the association between dyslipidemia, BMI and increased breast cancer risk.

71. Detection of Mitochondrial Genome Variation in Head and Neck Cancer

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Keywords: *Mitochondrial DNA, C-tract, d-loop, Biomarker, Head and Neck cancer*

Mitochondrial dysfunction is a hallmark of cancer cells. Mitochondrial DNA (mtDNA) mutations strewn throughout coding and noncoding regions have been reported in cancer. The C-tract region within d-loop is composed of 12-18 cytidine bases that are interrupted by a thymidine base at position 310 and is found to be a "hot spot" region for somatic

Schirmer's test, were performed in 40 smokers (80 eyes) and 35 non-smokers (70eyes). Tear film BUT and basal tear secretion was 7.89±2.84sec, 6.38±2.94 mm, in smokers and 9.71±3.23 sec, 10.13±3.96 mm, in nonsmokers respectively ($p<0.05$).Our results lead us to conclude that quantity and quality of tear film significantly decrease with smoking and this decrease is further related to the amount of smoking.

19. PCR Based Diagnosis of Human Papillomavirus (HPV) Infection of Suspected Cervical Cancer Patient from Southern Assam: A Report

Memton Singh, Jagadish Hansa, *Biswadeep Choudhury*[1], Sumita Dutta Gupta[1], A Das[1], Rajeev Kumar[2], Ruhina S Laskar[2], Ravi Kannan[2], and Sankar Kumar Ghosh

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Keywords: *PCR, Cytological Methods, Cervical Cancer, HPV, Sensitivity, Specificity, Cancer Prevention Programmes, Screening.*

World-wide epidemiological studies have shown that cancer of the uterine cervix is the second most common malignant disease in women. Virtually every cervical cancer (99.7%) is HPV-positive, indicating that the presence of HPV is an obligatory element in the development of cervical cancer. The present study was done by PCR based diagnosis of HPV 16 and HPV 18 infection amongst patients of suspected cervical cancer, confirmed by cytological methods. Twelve women out of a total of fifty studied cases who had positive cervical pap smears (24%) were found to be positive for HPV 16/HPV 18 infection when PCR based technique was applied. The results indicate, perhaps, a greater specificity of PCR based diagnosis or presence of other HPV subtypes as etiological factors in the Southern Assam in which the study group was confined to.

be detected and accurately quantified in single tumour sections by use of nanoparticles conjugated to antibodies Nanoparticle-based DNA and RNA delivery systems offer several potential advantages for gene delivery to various human tumours, including breast cancer. Supermagnetic nanoparticles have exciting possibilities as contrast agents for cancer detection in vivo, and for monitoring the response to treatment. Several chemotherapy agents are available as nanoparticle formulations, and have at least equivalent efficacy and fewer toxic effects compared with conventional formulations. In the near future, the use of nanotechnology could revolutionise not only oncology, but also the entire discipline of medicine.

85. Pcr Based Diagnosis of HPV from Cancer Patients in the Hospital Based Study from Southern Assam

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Keywords: **HPV, Cervical cancer, PCR, Northeast, Red alert**

Cancer poses a global health problem and breast cancer is one of the leading causes of death among the women. It also poses serious challenge to India basically the south zone of northeast India. The national incidence of cancer is approximately 100 to 130 individuals per 1,00,000 but in the Northeast, according to the population-based cancer registry of Indian Council of Medical Research, the incidence is highest with Assam alone adding roughly 26,000 new cancer patients every year. The 16 and 18 subtype of the HPV are more effectively involve in making disorder of cell. A recent study from our laboratories identified HPV-16 and HPV-18 are also incorporated with

Mangostin has better binding energy than the Synthetic inhibitor SC-558 and can be used as a potent anti-breast cancer drug.

87. Prevalence of Oral Cancer and its Association with Mitochondrial DNA Mutation from Northeast India

Rosy Mondal, Jagadish Hansa, Ravi Kannan[1] and Sankar K. Ghosh

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Key words: **Mitochondrial DNA, D-loop, Mutation, Oral cancer, Squamous cell carcinoma (SCC)**

India has one of the world's highest incidences of oral squamous-cell carcinoma which constitutes about 20-30% of all cancers. It is believed that the widespread habit of betel quid chewing is an important risk factor as it exposes the oral mucosa to known carcinogens. It also induces physical abrasions, which may create mutagenic environments during wound healing as gateways for infections. Cancer begins with multiple cumulative epigenetic and genetic alterations that sequentially transform a cell or group of cells in a particular organ. The early genetic events might lead to clonal expansion of pre-neoplastic daughter cells in a particular tumour field. Subsequent genomic changes in some of these cells drive them towards the malignant phenotype. The association of Mitochondrial DNA instability in degenerative diseases, neurodegenerative diseases, sudden infant death syndrome, aging and longevity, and cancer has been reported. Mitochondrial DNA is more prone to mutation as it lacks efficient DNA repair mechanism. In addition mitochondrial D-loop region and ND2 gene is the mutation hotspot for oral SCC. Three mutation hotspots were observed in the D-Loop at nt 146, 152 and 186, two of which (nt 146 and 152) have also been implicated in oesophageal squamous cell

CHAPTER 5

BIOINFORMATICS IN DNA BARCODING

Pradosh Mahadani, Ksh Miranda Devi, Mridul M Das, Mohua Chakraborty, Fazlur Rahman, ***Jagadish Hansa*** *and Sankar Kumar Ghosh*

5.1 INTRODUCTION:

Bioinformatics is a biological science, the science of using information to understand biology. In a literal sense Bioinformatics is the integration of life sciences and information science. A common definition of Bioinformatics is the 'Science of organizing and analyzing increasingly complex biological data resulting from modern molecular and biochemical techniques'. But a classical definition describes it is the mathematical, statistical and computing methods that aim to solve biological problems using DNA and amino acid sequences and related information. Bioinformatics is conceptualizing biology in terms of molecules and applying 'informatics techniques' to understand and to organize the information associated with these molecules, on a large scale. The National Center for Biotechnology Information (NCBI 2001) defines "Bioinformatics is the field of science in which biology, computer science, and information technology merge into a single discipline". In Short, Information science is applied to manage and analyze the information generated a molecular biology to introduce the field called Bioinformatics. The functional roles of Bioinformatics are many. This branch of science has developed because of the need to handle staggering amount of biological data generated due to the technical advances in genome sequencing (genomics) and protein identification (proteomics). Bioinformatics employs computational power to catalogue, organize and structure these data into biologically meaningful information. This includes design of intelligent data.....

Homo sapiens partial BRCA1 gene for Breast cancer associated protein 1, isolate SGJH-AUBC1

GenBank: HE600032.1

LOCUS HE600032 137 bp DNA linear PRI 10-OCT-2011

DEFINITION Homo sapiens partial BRCA1 gene for Breast cancer associated protein 1, isolate SGJH-AUBC1.

ACCESSION HE600032

VERSION HE600032.1 GI:350540198

KEYWORDS .

SOURCE Homo sapiens (human)

ORGANISM Homo sapiens

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.

REFERENCE 1

AUTHORS HANSA, J., MONDAL, R. and GHOSH, S.K.

TITLE Detection of BRCA1 gene mutation in breast cancer patients from Northeast India

REFERENCE 2 (bases 1 to 137)

AUTHORS Ghosh, S.

TITLE Direct Submission

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FEATURES Location/Qualifiers

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HHL"
ORIGIN

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61 ttatcattga agaatagctt aaatgactgc agtaaccagg taatattggc aaaggcatct

121 caggaacatc accttag

//

GENE NAME	ACCESSION NUMBER	NO. OF SEQUENCES
BRCA1	HE600032-HE600038	7 Sequences
D-LOOP (HNC)	JN793569-JN793585	18 Sequences
D-LOOP (mtDNA)	JN603607-JN603629	23 Sequences

To access these nucleotide, please click to provided link :-

Website: www.ncbi.nlm.nih.gov/nuccore?term=Hansa%20J