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GENOMIC INSTABILITY IN PERIPHERAL BLOOD LYMPHOCYTES OF CANCER PRONE SYNDROMES

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ASBSTRACT

It is well known that several chromosomal syndromes as well as chromosomal instability syndromes are cancer prone. A thorough cytogenetic study (in certain cancer prone syndrome) that may bring out any associated factors if any in the transformation of precancerous status to cancerous status making use of both chromosomal aberrations (CA) analysis and cytokinesis block micronucleus cytome assay (CBMN cyt) was undertaken. Blood samples were collected from the cancer prone chromosomal syndromes- Down syndrome (n=20); Gonadal Dysgenesis (n=5); Chromosomal instability syndromes - Aplastic Anemia (n=5). Age and sex matched ethnic controls (n=30). A significantly higher frequencies of CA, micronuclei (MNi) and various nuclear anomalies were observed in the patients in comparison to the controls ($p<0.001$). The Aplastic Anemia patients showed a higher frequency of genomic instability followed by Gonadal Dysgenesis and Down syndrome. Therefore these biomarkers could be used to predict the risk of malignant transformation of cancer prone chromosomal syndromes.

KEYWORDS: Genomic instability, cancer prone syndromes, chromosomal aberrations, CBMN cyt assay

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INTRODUCTION

The last two decades have seen accumulation of the data on the chromosomes of human tumors but still there are many gaps in our knowledge. While revealing great variations from tumor to tumor, the data have failed to disclose any simple change common to all cancers. It must be emphasized that the improved resolution that can be achieved with the use of chromosome banding technique alone as yet made little impact on the field of cancer cytology[4]. Hence, it was planned to make a thorough study on the cytogenetics of certain cancer prone syndromes by making use of advanced giemsa techniques and the recently developed and effectively used cytokinesis blocked micronucleus cytome assay (CBMN cyt). It is well known that several chromosomal syndromes such as Down, Turner, and Klinefilter as well as several chromosomal instability syndromes such as Ataxia Telengectasia (AT) Bloom Syndrome (BS) and Fanconi Anemia (FA) and certain chromosomal breakage syndromes such as 13q- leading to retinoblastoma, 11p13 syndrome leading to Wilm's tumour are cancer prone. Several hereditary disorders including immuno deficiency syndrome (ID) Nijmegen breakage syndrome (NBS) were also associated with an elevated risk of cancer. However it is disappointing to note, that no serious study was attempted to understand the factors that may be involved in the transformation of precancerous status to cancerous status. Hence it is proposed to undertake at least few cancer prone syndromes for the transformation study. As almost all the cancer prone syndromes are associated with chromosomal abnormalities, it was reasonable to take a thorough cytogenetic study (in certain cancer prone syndrome) that may bring out any associated factors if any in the transformation of precancerous status to cancerous status making use of both CA analysis and CBMN cyt assay.

MATERIALS & METHODS

The subjects were selected with the help of Medical practitioners. The patient samples were categorized as follows and analysed. The cancer prone chromosomal syndromes- Down syndrome (n=20); Gonadal Dysgenesis (n=5); Chromosomal instability syndromes - Aplastic Anemia (n=5). Age and sex matched ethnic controls (n=30) were included in this study.
Informed written consent was obtained from all the individuals and peripheral blood samples were collected by venipuncture in heparinised vacutainers. The ethical clearance was obtained from the University for conducting this study.

CYTOGENETIC STUDIES

Peripheral blood samples were processed immediately on sampling and cultured. Chromosome preparations were made according to the standard procedure[1].Cultures were set up by mixing 0.5 ml of whole blood with 6 ml of Ham's F10 media (PAN Biotech GmBH), supplemented with 1.2 ml of fetal bovine serum (Hi Media) and 0.3 ml of phytohemagglutinin (GIBCO).

ANALYSIS OF CHROMOSOMAL ABERRATIONS

Lymphocyte cultures were harvested after 72 hours from the time of initiation. They were arrested at metaphase with 0.001% colchicine (Sigma). The cells were treated hypotonically for 11 minutes with 0.075 M KCl (Merck), followed by fixation with methanol-acetic acid (3:1). Fixed cells were dropped onto clean microscopic slides, air dried and stained with Giemsa solution (4%). For each sample, 100 well spread metaphases were scored for all types of chromosomal aberrations.

CBMN CYT ASSAY

For this assay, lymphocyte cultures were set up as described above. Cells were blocked

in cytokinesis by the addition of Cytochalasin B (6μg/ml final concentration, Sigma) at the 44th hour. Harvesting was carried out at the end of 72 hours. The cells were treated hypotonically with 0.075 M KCl for 8 minutes, followed by fixation with methanol-acetic acid (3:1). Subsequently, slides were prepared and stained with Giemsa solution (4%). For each sample, 1000 binucleated cells were scored for abnormalities following the criteria specified by Fenech M.[2]

STATISTICAL ANALYSIS

A comparison between the data obtained from the patient groups and the control group was analyzed using the t-test. A p value of < 0.001 with confidence limit of 95% was defined as statistically significant. Numerical data are presented as mean±S.D. Microsoft Excel and WinStat were used for statistical analysis.

RESULTS AND DISCUSSIONS

The frequency of genomic instability was analyzed by using both the cytogenetic techniques such as, CA analysis and CBMN Cyt assay in patients and controls.

CYTOGENETIC STUDY IN CHROMOSOMAL SYNDROMES

The frequency of CA (ploidy, chromosome break, and dicentrics) per cell in Down syndrome (0.10±0.02) was significantly higher than that of the controls (0.001±0.003). In Gonadal Dysgenesis patients also the frequency of CA per cell was significantly higher (0.11±0.02) than that of the controls (0.001±0.003) (Table 1, figure 1)

Table 1
The frequency of variations in genomic instability in selected study subjects compared to controls

Study groups	Total Frequency of Chromosomal abnormalities	Total frequency of nuclear anomalies(CBMN Cyt Assay)
Down Syndrome	0.10±0.02 (N=20)	0.01±0.003 (N =10)
Gonadal Dysgenesis	0.11±0.02 (N=5)	0.02±0.007 (N=5)
Aplastic Anemia	0.19±0.04 (N=5)	0.02±0.006(N=5)
Control Subjects	0.001±0.003(N=30)	0.001±0.001 (N=30)

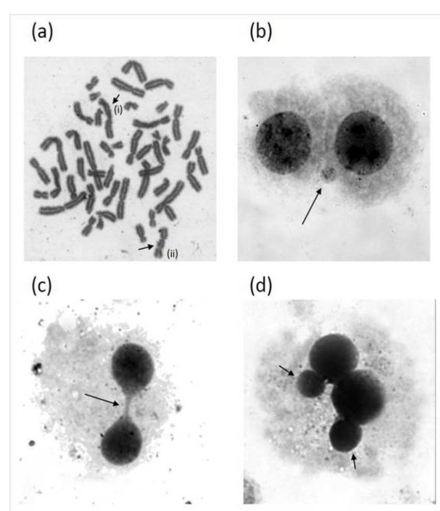

Figure 1
(a) Metaphase showing Dicentric Chromosome (i) and Chromatid Break (ii)., (b) A Binucleate cell with one micronuclei (Mni)., (c) A Binucleate Cell with Nucleoplasmic Bridge (NPB)., (d) A Binucleate Cell with Nuclear Buds(NBUDs)

CBMN Cyt assay was used to analyze the frequency of various nuclear anomalies(Figure1) in patients and controls. The frequency of MNi per cell was significantly higher in the patient groups than that of the controls (p<0.001). In Down syndrome, the mean frequency of MNi per cell was 0.006±0.002, nucleoplasmic bridges (NPBs) were 0.003±0.001 and nuclear buds (NBUDS) were 0.001±0.001 and in the controls, they were 0.0003±0.0006, 0.0007±0.0008 and 0.0006±0.0008 respectively. The mean frequency of various nuclear anomalies was significantly higher (0.011±0.003) than that of the controls (0.001±0.0001) (Table 2 and Figure 2).

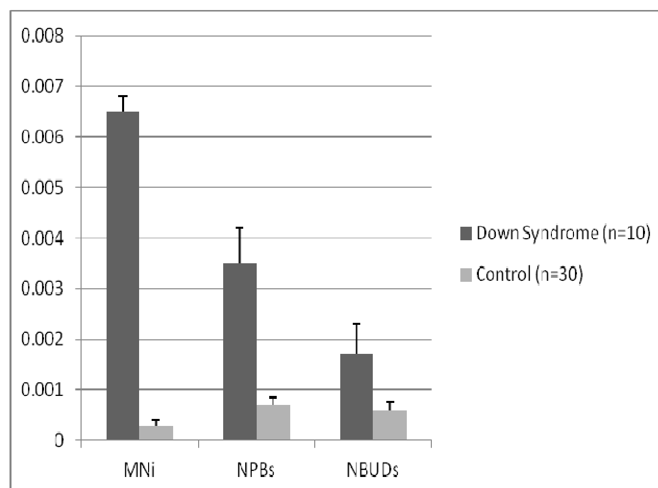

Figure 2
The frequency distribution of various nuclear anomalies observed by applying CBMN Cyt technology in Down Syndrome and controls

In Gonadal Dysgenesis patients, the mean frequency of MNi per cell was 0.011±0.003, NPBs was 0.004±0.001 and NBUDS was 0.005±0.003 and in the controls they were 0.0003±0.0006, 0.0007±0.0008 and 0.0006±0.0008 respectively. The mean frequency of total nuclear anomalies was significantly higher (0.021±0.007) than that of the controls (0.001±0.0001) (Table 2 and Figure3)

Table 2
The Frequency of various nuclear anomalies observed by applying CBMN Cyt technology in Down Syndrome , Gonadal Dysgenesis, Aplastic Anemia and controls.

	Affected Study Subjects			Healthy Study Subjects
	Down Syndrome(n=10)	Gonadal Dysgenesis (n=5)	Aplastic Anemia (n=5)	Control (n=30)
Total number of Binucleates (BN) scored	10000	5000	5000	30000
Total number of Micronuclei (MNi)	65	58	74	11
Mean frequency of MNi per Individual	6.5±2.06	11.6±3.64	14.8±4.00	0.37±0.61
Mean frequency of MNi per cell	0.006±0.002	0.011±0.003	0.014±0.004	0.0003±0.0006
Total number of Nucleoplasmic bridges (NPBs)	35	22	27	23
Mean frequency of NPBs per	3.5±1.58	4.4±1.67	5.4±1.14	0.77±0.81

Individual				
Mean frequency of NPBs per cel	0.003±0.001	0.004±0.001	0.005±0.001	0.0007±0.0008
Total number of Nuclear buds (NBUDs)	17	28	39	18
Mean frequency of NBUDs per Individual	1.7±1.15	5.6±3.43	7.8±2.77	0.6±0.8
Mean frequency of NBUDs per cell	0.001±0.001	0.005±0.003	0.007±0.002	0.0006±0.0008
Total number of MNi, NPBs and NBUDs	117	108	140	52
Mean frequency of MNi, NPBs and NBUDs per individual	11.7±3.86	21.6±7.43	28±6.63	1.7±1.311
Mean frequency of MNi, NPBs and NBUDs per cell	0.011±0.003	0.021±0.007	0.028±0.006	0.001±0.001

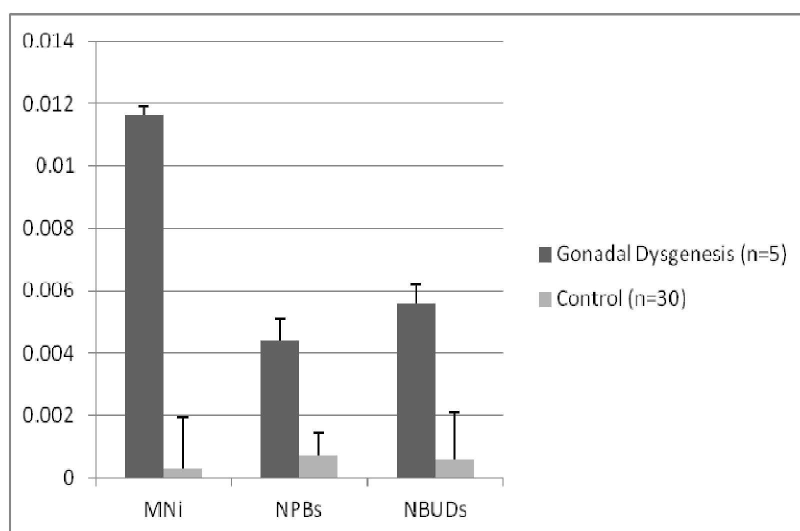

Figure 3
The Frequency distribution in the various nuclear anomalies observed by applying CBMN Cyt technology in Gonadal Dysgenesis patients and controls

CYTOGENETIC STUDY IN CHROMOSOMAL INSTABILITY SYNDROMES

In Aplastic Anemia the frequency of CA (0.19 ± 0.04) per cell was significantly higher than that of the controls (0.001±0.003) (Table 1). In Aplastic Anemia patients the mean frequency of MNi per cell was 0.014±0.004, NPBs was 0.005±0.001 and NBUDS was 0.007±0.002 and in the controls they were 0.0003±0.0006, 0.0007±0.0008 and 0.0006±0.0008 respectively. The mean frequency of total nuclear anomalies was significantly higher (0.028±0.006) than that of the controls (0.0017±0.0001) (Table2 and Figure 4).

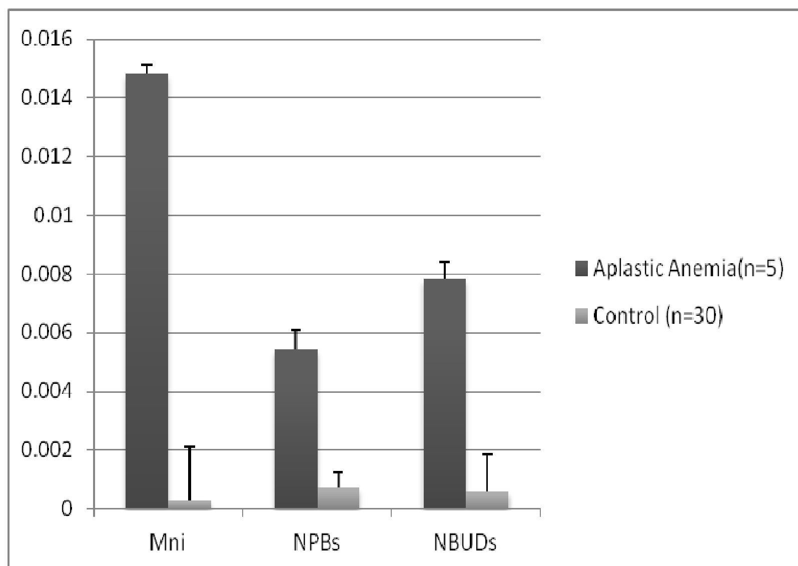

Figure 4
The Frequency distribution in the various nuclear anomalies observed by applying CBMN Cyt technology in Aplastic Anemia Patients and Controls

The Aplastic Anemia patients showed a higher frequency of genomic instability followed by Gonadal Dysgenesis and Down (Figures 5 and 6)

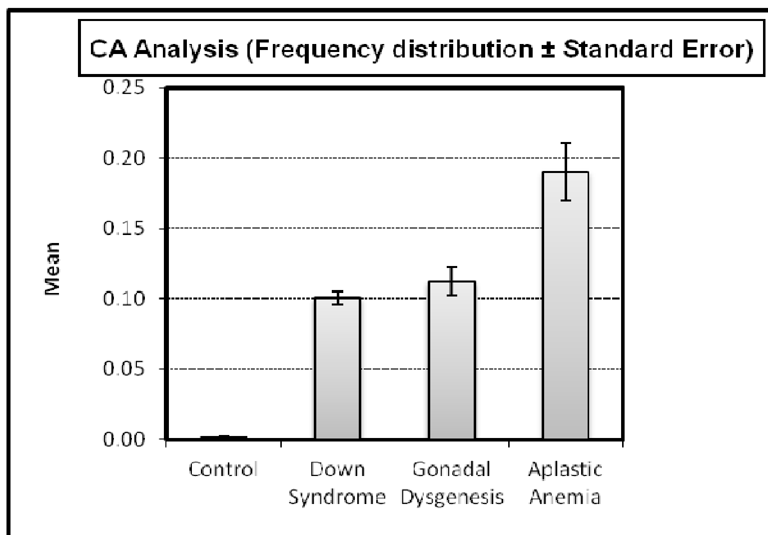

Figure 5
The frequency distribution of CA for patient groups and controls

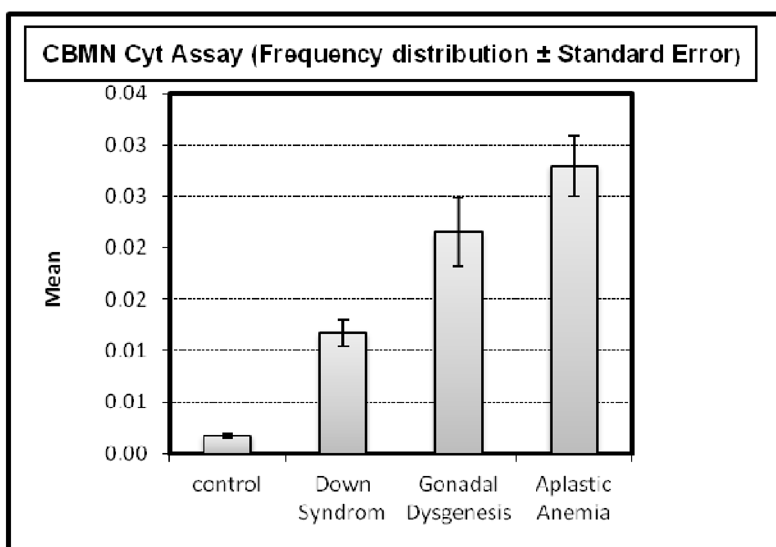

Figure 6
The frequency distribution of various nuclear anomalies for patient groups and controls

By making use of both the cytogenetic techniques- CA analysis and CBMN Cyt assay, a significant increase in CAs and MNi frequency has been confirmed in this study. Further, interestingly we observed in the patients of cancer prone chromosomal syndromes and chromosome instability syndrome there was significantly high frequency of CAs and MNi than in the controls, therefore these biomarkers could be used to predict the risk of malignant transformation of cancer prone chromosomal syndromes. Also an increase in the frequency of NBUDs a biomarker for gene amplification and NPBs a biomarker for dicentric chromosomes[3] observed in the patient groups strengthens hypothesis that this also may be a possible mechanism leading to cancer development. Micronuclei and other nuclear anomalies such as nucleoplasmic bridges and nuclear buds are biomarkers of genotoxic events and chromosomal instability. These genome damage events can be measured simultaneously in the cytokinesis-block micronucleus cytome (CBMN cyt) assay. MNi can originate during anaphase from lagging acentric chromosome or chromatid fragments caused by misrepair of DNA breaks or unrepaired DNA breaks. Malsegregation of whole chromosomes at anaphase may also lead to MNi formation. NPBs originate from dicentric chromosomes, which may occur due to misrepair of DNA breaks, telomere end fusions, and could also be observed when defective separation of sister chromatids at anaphase occurs due to failure of decatenation. NBUDs represent the process of elimination of amplified DNA, DNA repair complexes and possibly excess chromosomes from aneuploid cells[3]

A study with larger sample size and the application of sophisticated cytogenetic techniques such as fluorescent in situ hybridization technique will enable us to better understand the events that would lead to the initiation of cancer and may aid in developing better preventive and therapeutic strategies.

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