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"Evaluation Of Micronucleus Assay As A Biomarker For Early Diagnosis Of Cancer Breast"

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ABSTRACT-

Breast cancer has been alarmingly increasing over the last decades or so not only worldwide but also locally in Madhya Pradesh, India. The current researchwork aims to generate a novel tumor marker for early diagnosis of breast cancer in the form of Micronucleus Assay, which is an indicator of genomic damage. In the current study, a total of 200 volunteers participated in the study, patients of age groups 18 - 60 years.Approval from IEC ((Reg No.: 510/JNCH/RES/ 15/10/2022),- **100** Normal and **100** CA Breasts(with and without co-morbidities like Gallbladder and Thyroid issues), DNA damage and other cellular abnormalities calculated on the basis of MN.The Micronucleus assay showed a significantly higher micronucleus score in breast cancer patients when compared to healthy control subjects indicating genomic damage in cancer patients. Micronucleus frequencyof 0.64 ±0.178 (Mean ± SE value) and **Normal control group**with 0.08 ±0.55.Micronucleus formation was increased in **CA Breast with Gallbladder stone**group with 0.3 ±0.104. Micronucleus frequency and **CA Breast with Thyroid**shows lesser DNA damage as compared with the above groups.0.2 ±0.104, Micronucleus frequency.In the present study, **micronucleus analysis** of breast cancer as well as healthy control females demonstrated a higher genomic damage in breast cancer patients in the form of higher MN frequency, which in turn can be utilized as a potential biomarker for early diagnosis of breast cancer.

Keywords: Thyroidism, Gallbladder, Gallstone, High Risk, Carcinoma Breast

INTRODUCTION

The micronucleus assay is a crucial test used to evaluate the presence of micronuclei, which are small, additional nuclei that can form in cells as a result of genetic damage. This assay serves as a regulatory tool for assessing genotoxicity, which refers to the harmful effects of certain substances on genetic material. It specifically detects damage caused by two types of agents: clastogens and aneugens.A number of breast cancer patients are found to have a history of thyroid problems and/or gallbladder stones indicating a possible role of these diseases in the development of breast cancer.**(Ali MohsinHasan***et al.,* 2022) conducted a study on the formation of gallbladder stones in female breast cancer patients undergoing tamoxifen therapy in Iraq. The findings indicate a major association between tamoxifen analysis and the formation of gallbladder stones. Postmenopausal women appear to have a higher risk of increasing these stones. The prospective case-control study enrolled 14 premenopausal and 36postmenopausal females. It was noted that there is a significant relationship between the development of gallbladder stones and increased body weight during the course of therapy.**Wysowski, DK, Goldbery, EL, *et al.*, (1986)** conducted a study investigating the potential association between breast cancer and gallbladder disease. The research aimed to determine whether women with breast cancer have a higher risk of having a history of gallbladder disease. Their findings indicated that gallstones and breast cancer share several common risk factors.

2. MATERIALS AND METHODS-

Ethical Committee also approved (**IEC No: 510/JNCH/RES/ 15/10/2022**)the detailed health history questionnaire and informed consent form both in English & Hindi, which was signed by each enrolled subject. Peripheral blood was taken in heparinized sample tubes for further parameters like Micronucleus assay.**Peripheral blood culture**-After informed consent was taken, 100 blood samples(agegroups 18 – 60)were collected from breast cancer patientsof chemotherapy ward of JNCH and RC, Idgah Hills including the ones with gallbladder stone history.100 samples were also collected from healthy control females having no breast cancer and no other diseases for comparative study.

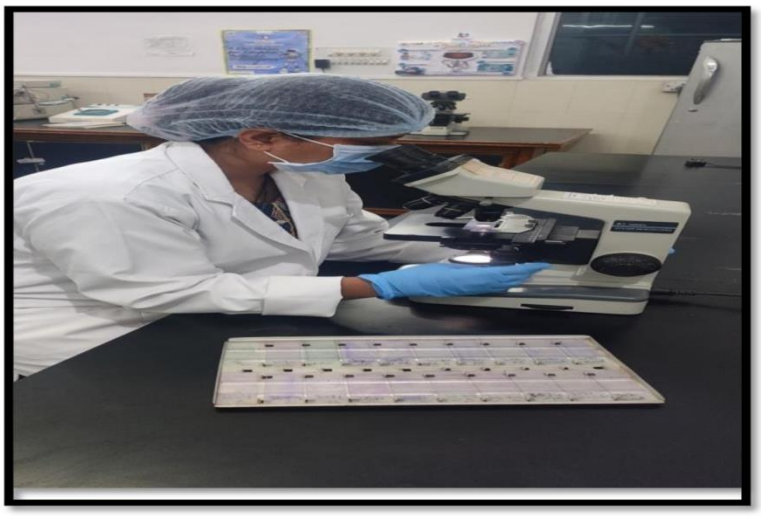
Micronucleus Assay (M.R. Scarfi, *et al.*, 1991):

The in vitro micronucleus assay is a mutagenicity testing system used to detect chemicals that induce the formation of small, membrane-bound DNA fragments known as micronuclei in the cytoplasm of interphase cells. These micronuclei can arise from acentric fragments—chromosome fragments that lack a centromere—or from whole chromosomes that fail to migrate with the rest of the chromosomes during anaphase of cell division.This assay is viable to identify the activity of both clastogenic and aneugenic chemicals. Procedure starts with the peripheral blood sample of healthy control. With Mitomycin-C as negative control was added in normal samples (3.0 µg/ml) and Cytochalasin B (0.033µg/ml) treatment after 48 hrs of incubation at 37°

to development of the using cytokinesis-block methodology, addition of the actin polymerisation inhibitor Cytochalasin B during the targeted mitosis,enables identification of nuclei that have undergone one division as binucleates.The integer of nuclei per cell specifies the amount of nuclear divisions that have arisen since the addition of Cytochalasin B. (**M.R. Scarfi, *et al.*, 1991)**

For the experimental part of the study, the blood samples were processed for Micronucleus Assay which was

- Showing incubation of Blood culture tubes at 37° for 72hrs,

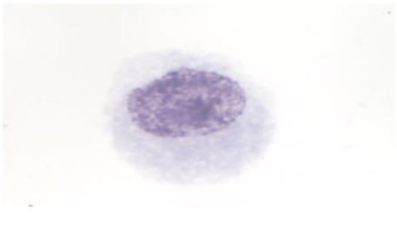


- Microscopy observation of slide

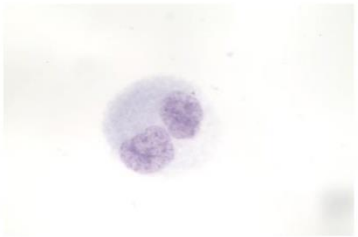
performe**d** by following the standardized protocol of **M.R. Scarfi, *et al.*, 1991.**in this method, blood samples are taken in a heparinized vacutainer and processed for Cytochalasin B Micronucleus Assay in which after culturing the blood in culture media like RPMI 1640 for 48 hours, Cytochalasin B was added to block cytokinesis and then further incubated and processed for MN Scoring. After centrifuging the sample and discarding supernatant, hypotonic solution KCl is added and incubated followed by further centrifuging and washing the solution in Carnoy's fixative (Methanol& Acetic Acid). The sample is then ready for staining by Giemsa stain and **Preparation of slides:**The slides prepared by the air-drop method observed under microscope for MN Scoring. Micronucleus observed are counted for each sample and recorded. **(Table no 1 or 2 .**

FIGURES

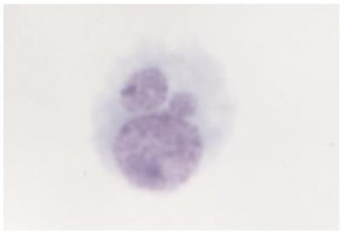
Figures showing normal and abnormal cell



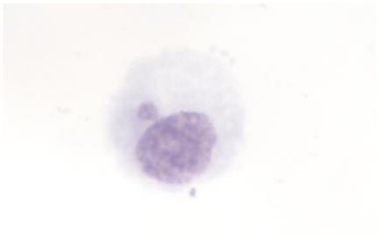
Normal mononucleated cell



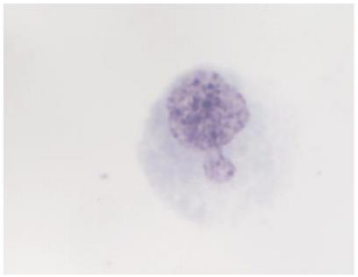
normal binucleated



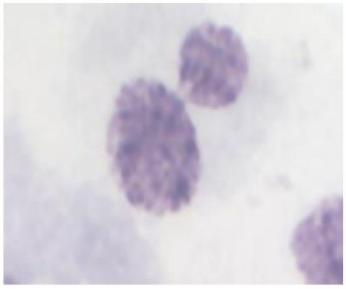
cell Micronucleus cell



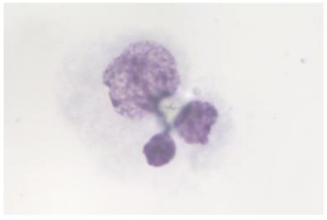
Mononucleated Micronucleus



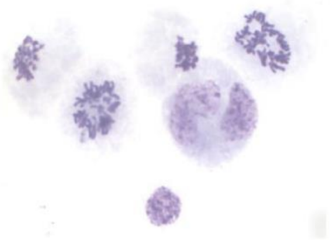
cell Binucleated buds



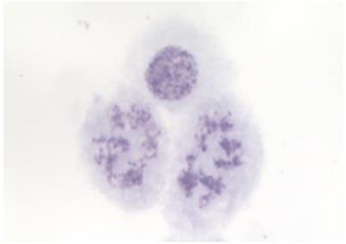
Bridge



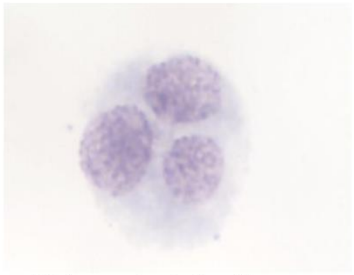
Neoplasmic Bridge



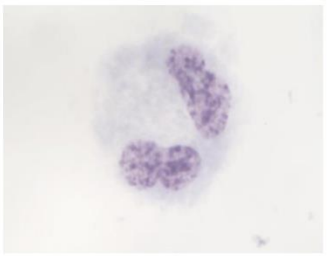
Polarised cell



polarised nucleus



Tri_ nucleated cell



Quadera_nucleated cell

Result -Micronucleus Assay:

Table 1: Mean ± SE (Standard Error) value of different group with normal and abnormal cell

Cell Type	Positive Control	Negative Control (MMc)	Ca Breast with Thyroid	Ca Breast with Gallbladder stone
MNC	34.8 ±0.721	11.8 ±1.266	35.8 ±1.266	24.8 ±1.266
BNC	55 ±1.04	12.5 ±0.92	40.6 ±0.75	22 ±1.04
MN.Mn	1 ±0.388	21 ±1.04	4.2 ±0.858	11.4 ±0.75
BN.Mn	1.4 ±0.75	31.6 ±1.515	2.2 ±0.858	14 ±1.04
BNB	1 ±0.465	3.8 ±1.266	6 ±1.04	7.8 ±1.266
MNB	2.8 ±1.266	4.6 ±1.364	3.8 ±0.551	8.6 ±1.515
QNC	5 ±1.04	8 ±1.04	4.6 ±1.364	6.6 ±0.75
BN.Bd	2 ±1.04	8.4 ±1.364	2.4 ±0.75	4 ±1.04

Cell Types:MNC- Mononucleated cells, **BNC**- Binucleated cells, **MN.Mn**- Mononucleated cell with Micronucleus,**BN.Mn**- Binucleated cells with Micronucleus,**BNB**- Binucleated cells with Nuclear Bud ,**MNB**- Mononucleated cell with Nuclear Bud,**QNC**- Quadra nucleated Cells ,**BN.Bd**- Binucleated cells with Nuclear Bridge

Graph 1: (a) Showing bar graph with percentage of normal & abnormal cells

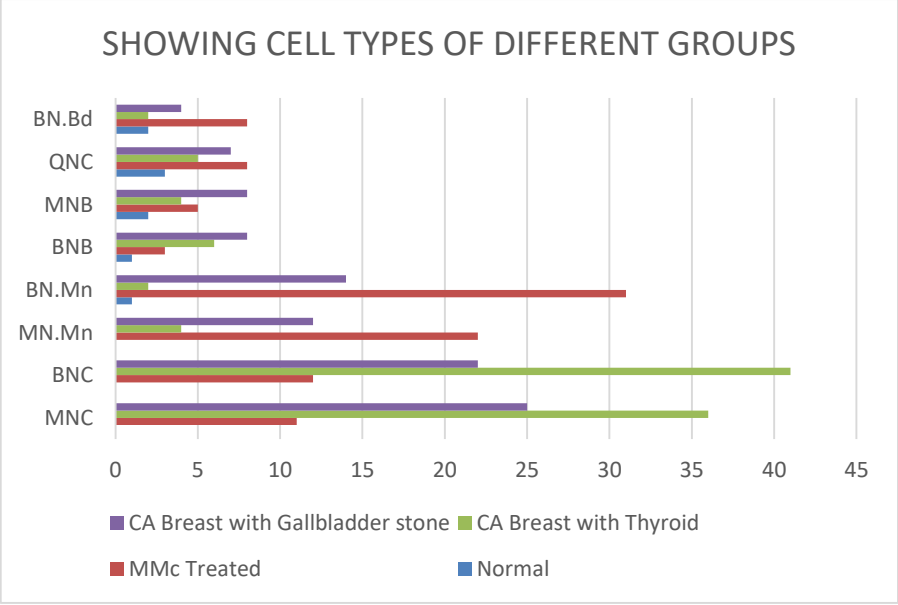
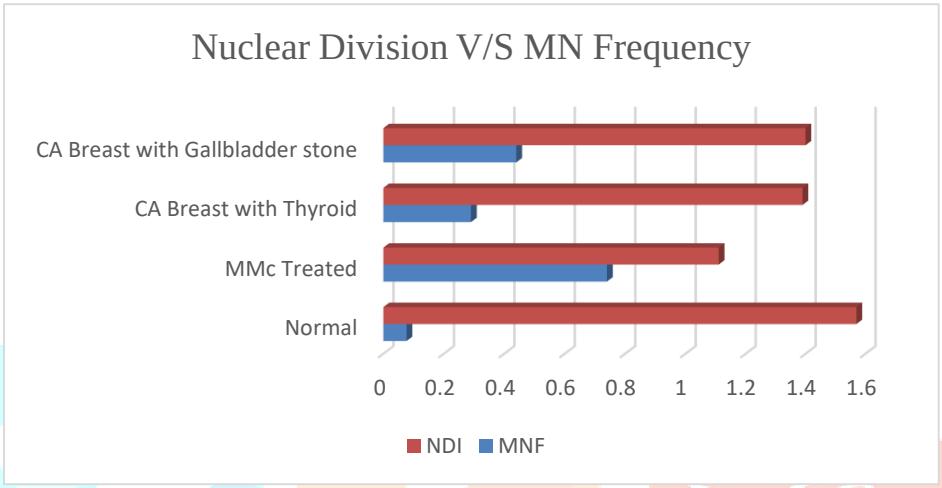


Table 2: Mean ±SE value of different group showing MN frequency V/S Nuclear division index

INDEX	Normal	MMc Treated	CA Breast with Thyroid	CA Breast with Gallbladder stone
MNF	0.08 ±0.551	0.64 ±0.178	0.2 ±0.104	0.3 ±0.104
NDI	1.4 ±0.104	1.14 ±0.075	1.22 ±0.0858	1.4 ±0.0806

Graph 2 :(B)Showing bar graph withMN frequency V/S Nuclear division index



OBSERVATION ANDRESULT: A total of **200** individuals were enrolled in the study**, 100** Normal and **100** CA Breast patients(with and without Gallbladder and Thyroid issues).The purpose of evaluating breast cancer patients with some common co-morbidities like gallbladder stones and thyroid problems that are supposed to increase the risk of breast cancer was to evaluate the role of these diseases in further increasing genomic damage (Wysowski et al., 1986). As per data observed about DNA damage based on CBMN assay compared with **Negative control** (Mitomycin-C treated group), the findings showed highest Micronucleus frequency of 0.64 ±0.178 (Mean ± SE value) and **Normal control group**with 0.08 ±0.55,Micronucleus formation was increased in **CA Breast with Gallbladder stone**group with 0.3 ±0.104 Micronucleus frequency and **CA Breast with Thyroid**showed lesser DNA damage as compared with the above groups.0.2 ±0.104, Micronucleus frequency.

DNA damage based on CBMN assay shows that the **CA Breast with Gallbladder stones** individuals have higher level of DNA damage, which can be due to either long exposure of treatment drugs or chronic inflammation.

CONCLUSION:

In conclusion, while breast cancer remains a major health challenge, increased awareness, screening strategies, and advancement in biomarker analyses together can combat this deadly disease more effectively. In this regard, the current study has generated a very useful biomarker in the form of MN assay, which is a useful assay in studying toxicity and genomic damage, can be utilized in cancer detection as well.

Micronucleus Assay is thus an indicator of genomic damage that can enhance cancer risk eventually if accumulated over a long period of time and therefore a good screening tool for cancer diagnosis. The current study evaluated micronucleus in both cancer patients and healthy control and successfully demonstrated a higher score in cancer patients proving its usefulness as a biomarker.

FUTURE PROSPECTS

The *in vitro***Micronucleus Assay**should be utilized in long term studies and in larger samples to validate the utility of this highly useful diagnostic tool.

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