Available online at http://www.journalijdr.com

IJDR

International Journal of
DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 06, Issue, 07, pp.8481-8484, July, 2016

Full Length Review Article

THE BASIC KNOWLEDGES ABOUT THE POSSIBILTY OF EMPLOY THE MICRONUCLEUS TEST FOR THE DIAGNOSIS OF THE DOUBTH CASES IN THE SCREENING FOR THE DETERMINATION OF BREAST CANCER

***[1]Menicagli, R. and [2]Menicagli, L.**

*[1]Senior Scientist in Medresearch Mediglia (Milan), Italy
[2]Department of Radiology Hospital San Donato University, Milan, Italy

ARTICLE INFO

Article History:
Received 20th April, 2016
Received in revised form
15th May, 2016
Accepted 18th June, 2016
Published online 31st July, 2016

Key Words:
Biodiversity,
Health, Southeast Asia, Ecology,
environmental law.

ABSTRACT

Introduction and Objectives: The aim of this study is to check the possibility to use the tests with micronuclei in saliva for cases doubts detected in screening for breast cancer
Material and Methods: The principal search on Pub Med (MEDLINE), Home Genetic Reference is for articles published from Jan 1, 2000 to Dec 31, 2001,for the keywords : "micronuclei in exfoliated buccal cells in breast cancer " The key words are used in the research in free text, and with cross –referencing method application. Another search it is been made for "MN in breast cancer ", from Jan1 2014 to today.
Results: Five studies show that in buccal cells, in breast cancer the MN are significantly higher than compared to benign cases as in six studies for the detection of micronuclei in needle aspirates ductal,. Contrasting results are for MN in peripheral blood lymphocytes
Discussion: The micronuclei scoring can be used as a biomarker on fine needle aspiration cytology smears of breast cancer while the tests in peripheral blood lymphocytes , have known reproducibility problems .The exfoliated buccal mucosa cells test, show an increase of MN. May be interesting to apply the MN scoring in cases of doubt, according to functional BI-RADS category 3 (probably benign), and which are sent to a control
Conclusions: it has verified that in buccal cells, in cases of breast cancer is significantly higher than the number of MN compared to benign cases, indicates the possibility of applying this technique in cases of doubt, in oral saliva.

INTRODUCTION

The micronuclei (MN) are small additional cores that are formed during anaphase from the condensation of acentric chromosome fragments or whole chromosomes that are not embedded in the main nuclei of the daughter cells. Responsible for the formation of MN are two different types of genetic damage: a) fragmentation of chromosomes by clastogenic agents, b) the damage of the mitotic spindle or the centromere of whole chromosomes by aneuploidogenic agents. The micronuclei contain fragments acentric (clastogenic mechanism) or whole chromosomes (aneuploidogenic mechanism) in migration delays during anaphase. The use of the technique as a measure of cytogenetic damage in peripheral blood lymphocytes in 1976, then the test was improved by Fenech and Morley through the use of cytochalasin B, inhibiting toxin cytokinesis. The formation of MN occurs because of genetic damage of the cell and this damage can be quantified by assigning a score (number of MN) as an indicator of genetic damage; in this way there is a possible correlation between genotoxic damage to the various diseases. There are two predominant mechanisms that lead to the formation of MN in a mitotic cell: the first concerns the chromosomal breakage due to clastogenic agents, the second a dysfunction of the mitotic process due to aneugenic agents which prevent spindle formation during mitosis. As a result of it there is a retard on the chromosomes formation in anaphase .and with the daughter cells that may have micronuclei containing whole chromosomes. There are also other training MN causes and what can be observed spontaneously in 'normal healthy individual subjected for example to environmental exposure to pollutants, radiation, bio-hazard materials, drugs, poisonous chemicals. Even the food /

****Corresponding author: Menicagli, R.,***
Department of Radiology Hospital San Donato University 'Milan, Italy.

beverage and lifestyles with free radicals formation, leading to the formation of MN. Among other causes, (Verma , 2014) in the formation of MN we can cite: chronic inflammation, heavy metal poisoning, chemotherapy, radiation injuries, various pre cancer conditions, a large number of genetic diseases, defects in nutrition, direct damage to DNA or breakage, chromosomal aberrations, malfunctioning mitotic apparatus, and interference with DNA synthesis .It 'clear, therefore, as well as cancer conditions induce the formation of MN. The great majority of cases of breast cancer are derived from the possible inheritance (familial high risk) attributable primarily to the mutation of BRCA genes * 1, BRCA 2, and TP53***, genes that are required to repair the double strand in the case genotoxic damage. The DNA breakage and loss of some functions can lead to genetic instability due to multiple chromosomal breaks. There is a possibility that in the cases of breast cancer may have in general an increase in the number of genetic damage and the number of cells with micronuclei can be increased, for example, not only in the sampling by needle aspiration, or in lymphocytes from peripheral blood, but also in the epithelial cells of buccal smear. But to better understand the possible applications with the MN test is useful to examine the correlation between genetic mutations and the formation of the breast cancer and particularly for BACR1, BACR2, TP53, referring to the clinical certainties highlighted for these genes in the Data Base:" Home Genetic Reference"

Home genetic references for BRCA1

The BRCA1 gene provides instructions for making a protein that acts as a suppressor tumorale.che helps cells in the prevention of uncontrolled division and growth. The BRCA1 protein is involved in repairing damaged DNA. In the core of many types of normal cells, the BRCA1 protein interacts with several other proteins for repairing breaks in DNA. These breaks can be caused by natural and medical radiation or other environmental exposures, and also occur when chromosomes exchange genetic material, in preparation for cell division. Helping to repair DNA, the BRCA1 protein plays a key role in maintaining the stability of the genetic information of a cell. Research suggests that the BRCA1 protein also governs the activity of other genes and plays an essential role in embryonic development. To perform these functions, the BRCA1 protein interacts with many other proteins, including tumour suppressors, and other proteins that regulate cellular activity.

Below other health conditions related to genetic change of brca1 Ovarian cancer; prostate cancer; other cancer

Home genetic reference BRCA2

The BRCA2 gene provides instructions for making a protein that acts as a tumour suppressor. Helping as the BRCA1 gene to prevent the uncontrolled cell growth. The BRCA2 protein is involved in repairing damaged DNA. In the core of many types of normal cells, the BRCA2 protein interacts with several other proteins for repairing breaks in DNA. These breaks can be caused by natural and medical radiation or other environmental exposures, and also occur when chromosomes exchange genetic material, in preparation for cell division. Helping to repair DNA, the BRCA2 protein plays a key role in maintaining the stability of the genetic information of a cell.

Researchers suspect that the BRCA2 protein has additional functions within cells. For example, the protein can help to regulate the process of cytokinesis, which is the key step in cell division, usually at the end of telophase, which means when the cytoplasm divides to form two separate cells.

Genetic change of BRCA2 in breast cancer

Researchers have identified more than 1,800 mutations in the BRCA2 gene. Many of these mutations are associated with an increased risk of breast cancer in men and women, as well as several other types of cancer. Most BRCA2 gene mutations lead to the production of an abnormally small, non functional version of the BRCA2 protein from one copy of the gene in each cell. As a result, less of this protein is available to help repair damaged DNA or fix mutations that occur in other genes.

Below other health condition associated to genetic change of BRCA2 Fanconi anemia; ovarian cancer; Prostate cancer other cancer

Home genetic reference TP53

The TP53 gene provides instructions for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing too fast or in an uncontrolled way. The p53 protein is located in the nucleus of cells throughout the body, where it attaches (binds) directly to DNA. When the DNA in a cell becomes damaged by agents such as toxic chemicals, radiation, or ultraviolet (UV) rays from sunlight, this protein plays a critical role in determining whether the DNA will be repaired or the damaged cell will self-destruct (undergo apoptosis). If the DNA can be repaired, p53 activates other genes to fix the damage. If the DNA cannot be repaired, this protein prevents the cell from dividing and signals it to undergo apoptosis. By stopping cells with mutated or damaged DNA from dividing, p53 helps prevent the development of tumors. Because p53 is essential for regulating cell division and preventing tumor formation, it has been nicknamed the "guardian of the genome."

Genetic change of TP53 in breast cancer

Inherited changes in the TP53 gene greatly increase the risk of developing breast cancer, as well as several other forms of cancer, as part of a rare cancer syndrome called Li-Fraumeni syndrome These mutations somatic in the TP53 gene are much more common than inherited mutations, occurring in 20 to 40 percent of all breast cancers. These somatic mutations are acquired during a person's lifetime and are present only in cells that become cancerous. Compared with other breast cancers, those without TP53 gene mutations, tumors with these genetic changes, as BRCA2, tend to have a poorer prognosis. They are more likely to be aggressive, to be resistant to treatment with certain anti-cancer drugs and radiation, and to come back (recur) after treatment. Below other heath conditions associated to genetic change of TP 53 Bladder cancer; ovarian cancer; Head and neck squamous cell carcinoma; Other cancers Consideration on correlation of genetic mutations and MN formation

As we can see the genes identified as the main initiators of the breast carcinogenesis process, are all members of the family of tumor suppressor. These mutations if present in all the genes are also present in every cell in the body and can be passed from one generation to the next. As a result, they are associated with cancers as that familiarly cluster. More dangerous are the hereditary mutations , especially compared to the p53 gene, such as the one leading to the Li-Fraumeni syndrome, responsible for several types of cancer, but also those concern the BRCA1 and BRCA2.gene These inherited mutations in fact involve an increased risk, because the cells, having inherited the first mutation (loss of the homozygosity , heterozygosity for the healthy allele), only require the other, loss of heterozygosity (LOH), to eliminate the expression of tumor suppressor genes with the cell control loss .In any case in most of the mutations of the genes involved, the next is crucial presence of exogenous toxic factors and / or endogenous, which determines for the mutation of the other allele the negative homozygosis with the beginning of the process of carcinogenesis.

These data are very important because in the assessment of a possible alteration in the frequency of MN, we should take into account that it may occur, as previously said, even just for those factors that can be a contributory cause of a possible tumor formation, but also to damage not extremely relevant. Although few recent studies have shown that the frequency of micronuclei in exfoliated buccal cells, greatly increases in cases of breast cancer. The aim of this study is to propose, according to some of our initial studies (data not published), that the MN test can also be performed on the whole saliva...The principal aim, however, it is to propose such a study for doubtful cases of breast cancer detected in the screening

MATERIALS AND METHODS

Search strategy

We searched on Pub Med (MEDLINE) for articles published in English from Jan 1, 2000 to Dec 31, 2014.The following keywords was used: "micronuclei in exfoliated cells in breast cancer “,” We also searched on the Cochrane library, Google, Gene, Home Genetic Reference. The key words are used in the research in free text, and with cross –referencing method application. We searched also on Pub Med, the articles from Jan 1 2014 to March 2016, using as key words micronuclei in breast cancer finding seven articles

RESULTS

As we can see in the references, we show ten studies, also obtained by cross referencing, and in particular only four of them concern only the monitoring and examination of the frequency of the Micronuclei and other nuclear anomalies in exfoliated buccal mucosa cells in the women with breast cancer. Five study has verified that in buccal cells, in cases of breast cancer is significantly higher than the number of MN compared to benign cases, indicates the possibility of applying this technique in cases of doubt, for whom a certain percentage. The research results on Pub Med for the key words "micronuclei in breast cancer from Jan 1 2014 to March 2016", have identified six studies for the detection of micronuclei sampling by needle aspiration, and three for MN test in peripheral blood lymphocytes

DISCUSSION

Before discussing the results obtained with the micronucleus test in buccal exfoliated cells in breast cancer, which presents the starting point for this proposal of investigation, we examine briefly the main results for the monitoring of the MN, for sampling by needle aspiration, or in lymphocytes from peripheral blood...In particular it can be noted that in searches was examined in which the frequency of the MN, in aspirates in ductal carcinoma in needle (Verma, 2014 ; Samanta and Dey, 2012 ; Goel *et al*., 2013; Samanta *et al*., 2011; Hemalatha *et al*., 2014; Tin *et al*., 2015), the study data indicate That micronuclei scoring can be used as a biomarker on fine needle aspiration cytology smears of breast carcinoma..Different is the comparison of the different experiences related to the test in peripheral blood lymphocytes, (Bolognesi *et al*., 2014 ; Varga, 2006; Koşar *et al*., 2016), .In a case-control study on the prediction of breast cancer risk / susceptibility., the results of this study do not support a significant role of micronucleus frequency as a biomarker of breast cancer risk / susceptibility.una recent research, () instead indicated That MN, may serve as sensitive biomarkers for routine detection of the genetic abnormalities in breast cancer,

Among the studies, (Dey *et al*., 2012; Flores-Garcia, 2014; Stefano Bonassib, 2015; Torres-Bugarín *et al*., 2014; Nersesyan and Adamyan, 2004), that have used the technique to detect the MN in exfoliated buccal mucosa cells we can consider the important the conclusions (1) relative to comparison from to a total 32 patients of carcinoma of breast and 49 patients of benign breast lesions diagnosed in fine needle aspiration cytology . The MN scoring in buccal smear was compared in malignant and benign breast cases, and shows a significantly high MN score in buccal smear of the cancer patients. The cells with MN are significantly increased in buccal cells of the breast carcinoma cases. The increased number of MN in buccal smears raises the possibility that the genetic damage in breast cancer patients is generalized. In another study (Samanta and Dey, 2012) it is been analyzed the frequencies of nuclear anomalies including MN, in exfoliated buccal mucosa cells of women with primary BC and healthy women. Buccal cells were collected from 21 BC patients (9 with stage I and 12 with stage II) and from 20 healthy females used as control group. The results of the evaluation of cells showed that the frequencies of MN were significantly increased in the pooled group of BC patients compared with the control In this research that is another result to evaluate for the aim of our study: patients with stage I BC was significant compared with controls and BC patients with stage II. a, but the number of cases examined is really small, and then the test protocol of the MN frequency is not rigorous. All of this research and also those related to other pathologies in which the test is looking for MN in exfoliated buccal cells in various diseases has been the subject of a "systematic review and meta-analysis" (Goel *et al*., 2013). In the review, we are told that the role of MN assay as a biomarker of chromosomal instability in buccal mucosa cells, alternatives such as surrogate tissue, reflecting the risk-susceptibility to cancer in

various organs other than the head and neck, and therefore also for breast cancer, it is not was well-designed studies available which also include those for breast cancer are extremely heterogeneous and includes several types of tumors. However, the meta-analysis of these studies reveals a meta-MR of 2.00 (95% CI 1.66 to 2.41), indicative of an effect that is worth further investigation. Without reporting the findings, practically the same of the other two studies, (Samanta *et al*., 2011; Hemalatha and Suresh, 2014) that indicate as in the future the MN scoring could be used as bio monitoring of DNA damage and in the early detection of high risk cases of carcinoma of breast. The MN test, as the first results of your research show (data not published), can also be performed on the whole saliva. We can say that this technique could be even higher importance in cases of doubt, those who are according to the international classification BI-RADS 3 (probably benign), and which are sent to a next control in six months. They concern in particular, according to the International Classification BI-RADS:

Images in MX exam of clustered micro calcifications not particularly indicative of cancer.

- considered benign nodules
- Distortion of parenchyma in MX, without to the same result with the ultrasound examination.
- hyperdense breasts that can present multi nodules whose single differentiation becomes difficult to interpret

Some tests detect calcifications BI-RADS 4 class; after failure in stereotactic biopsy, you should make a surgical biopsy, partly because the NMR has an index of about 20% false negative, but in clinical practice, the examination at six months is returned.

Conclusion

The MN score can be a measure both for the breaking of chromosomes and chromosome loss and is a sensitive indicator of chromosomal damage. The MN test can be easily executed rapidly, without the need for cell culture and the cytogenetics is the least invasive method and is a very simple technique to study in comparison of the samples of blood for assay lymphocytes or tissue biopsy. Factors such as age, season, diet, oral hygiene, dental health and smoking can affect the MN frequencies in a person. These variables must be identified and controlled to achieve a more objective and reliable decision. However it has verified in many studies that in buccal cells, in cases of breast cancer, the MN number is significantly higher than compared to benign cases, and indicates the possibility of applying this technique in cases of doubt, for whom a certain percentage may present a chromosomal damage generalized. Test of MN in buccal cells of patients needs a large prospective study and to further conclusive opinion.

REFERENCES

Bolognesi, C., Bruzzi, P., Gismondi, V., Volpi, S., Viassolo, V., Pedemonte, S. and Varesco, L. 2014. Clinical application of micronucleus test: a case-control study on the prediction of breast cancer risk/susceptibility. PLoS One. 2014 Nov 21;9(11):e112354

Dey, P., Samanta, S. and Susheilia, S. 2012. Micronucleus assay in buccal smears of breast carcinoma patients; Diagn. Cytopathol. 2012 Aug;40(8):664-6.

Dis Markers. 2014;2014:956835

Flores-Garcia, A., *et al*. 2014. Micronuclei and other nuclear anomalies in exfoliated buccal mucosa cells of Mexican women with breast cancer. J BUON. 2014 Oct-Dec; 19(4):895-9.

Goel, S., Bhatia, A. Dey, P. 2013. Spontaneously occurring micronuclei in infiltrating ductal carcinoma of breast: a potential biomarker for aggressive phenotype detection?. Diagn Cytopathol. 2013 Apr;41(4):296-302.

Hemalatha, A., Suresh, T.N. and HarendraKumar, M.L. 2014. Micronuclei in breast aspirates. Is scoring them helpful ?, J Cancer Res Ther. 2014 Apr-Jun;10(2):309-11

Koşar, P.A., Koçer, M., Çelik, D.A. and Özçelik, N. Cytogenetic findings in patients diagnosed with breast cancer having undergone adjuvant chemotherapy regimens.Genet Mol Res. 2016 Mar 18;15(1).

Nersesyan, A.K. and Adamyan, R.T. 2004. Micronuclei level in exfoliated buccal mucosa cells of patients with benign and malignant tumors of female reproductive organs and breast; Tsitol Genet. 2004 May-Jun;38(3):72-5.

Samanta, S., Dey, P., Nijhawan, R. 2011. The role of micronucleus scoring in fine needle aspirates of ductal carcinoma of the breast; *Cytopathology,* 2011 Apr; 22(2):111-4.

Samanta, S.Dey P.. Micronucleus and its applications.Review; Diagn Cytopathol. 2012 Jan; 40(1):84-90.

Stefano Bonassib, *et al*. 2015. Review Clinical application of micronucleus test in exfoliated buccal cells: A systematic review and metanalysis .Mutation Research/Reviews in Mutation Research. Mutation Research 766 (2015) 20–31

Tin, S.S. and Wiwanitkit, V. 2015. Micronuclei in breast aspirates. J Cancer Res Ther. 2015 Oct-Dec;11(4):1042

Torres-Bugarín, O., Zavala-Cerna, M.G., Nava, A., Flores-García, A. and Ramos-Ibarra, M.L. Potential uses, limitations, and basic procedures of micronuclei and nuclear abnormalities in buccal cells.

Varga, D. 2006. et. On the difference of micronucleus frequencies in peripheral blood lymphocytes between breast cancer patients and controls; Mutagenesis. 2006 Sep; 21(5):313-20.

Verma, S.D. Correlation of morphological markers of chromosomal instability in fine needle aspiration cytology with grade of breast cancer; Cytopathology. 2014 Aug; 25(4):259-63

****