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Original Research Article

IDENTIFICATION OF MICRONUCLEUS IN BUCCAL MUCOSA OF TYPE -2 DIABETIC PATIENTS: AN EARLY DIAGNOSTIC MARKER

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ABSTRACT

Background and Aims: The elevated levels of DNA damage, decreased efficacy of DNA repair and high susceptibility to mutagens have been associated with type 2 diabetes mellitus. The Micronucleus (MN) is a condensed form of chromatin with the appearance of small nuclei present in the cytoplasm and it is formed from chromosomal fragments or intact whole chromosomes that lag behind at the anaphase stage of cell division. So the presence of MN in cells reflect structural and numerical chromosomal aberrations arising during mitosis.

Materials and Methods: In the present study 50 cases of diabetic patients and 50 non diabetic patients were taken for screening for Micronuclei (MN).

Results: In diabetic patients, the frequency of micronuclei were more than the control group. So it is significantly elevated in type 2 diabetes patients. This is due to the elevated level of DNA damage in these patients.

Conclusion: The presence of micronuclei indicates the genomic instability and chromosomal damage. This can be a predisposing factor for development of cancer. So this can be used as a biomarker for diagnosis of type 2 diabetes mellitus.

KEY WORDS: Micronucleus, Buccal Mucosa, Type -2 Diabetic Patients.

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INTRODUCTION

The high prevalence of type 2 diabetes mellitus (DM2) is a public health problem in India. Diabetes mellitus is a syndrome characterized by chronic hyperglycemia due to defects in insulin secretion, insulin action, or both [1]. The elevated levels of DNA damage, decreased efficacy of DNA repair and high susceptibility to mutagens have been associated with type 2 diabetes mellitus [2]. This genomic instability leads to development of cancer.

The most important cause for development of type 2 diabetes mellitus is oxidative stress [3]. Microvascular complications like neuropathy, renal disease and macrovascular disease like coronary artery disease, cerebrovascular disease

and peripheral vascular disease are major cause of morbidity and mortality in patients with type 2 diabetes mellitus [4]. There is an evidence of an increased cancer risk in diabetic patients, being more evident for primary liver cancer and pancreatic cancer [5].

Micronucleus (MN) is a condensed form of chromatin with the appearance of small nuclei present in the cytoplasm and it is formed from chromosomal fragments or intact whole chromosomes that lag behind at the anaphase stage of cell division [6]. So the presence of MN in cells reflect structural and numerical chromosomal aberrations arising during mitosis, thus it can be used as a diagnostic marker for cancer screening [7] Elisabeth mullner et al., stated that the levels of buccal MN in diabetic individuals are approximately 2-fold higher than in nondiabetic participants [8]. Shen Z. MN in exfoliated buccal cells are a novel and non-invasive biomarker of genomic stability, formed during mitosis and represent the loss of chromosome fragments or a whole chromosome that failed to be incorporated in the main nuclei. Genomic instability is a hallmark of tumourigenesis [9,10]. Maria Grazia Andreassi et al., stated that a significant correlation between MN frequency and telomere shortening that lead to genetic instability and it is a biological marker for cardiometabolic diseases and diabetes mellitus [11]. Martinez-Pérez L et al., revealed that type 2 diabetes mellitus patients have significantly more genetic damage (in terms of MN frequency) than nondiabetics. This indicates that MN may be a useful constituent in a panel of biomarkers for the risk of diabetes [7].

AIMS AND OBJECTIVES

1. To identify micronucleus in the buccal cells of diabetic patients and non-diabetics

2. To explore the possibility of application of obtained data as screening test in community

MATERIALS AND METHODS

This is a case control study and it has been conducted on 50 Type 2 diabetic patients and age matched 50 controls. Buccal smears were taken by wooden spatula and it was smeared over the glass slide then fixed with methanol and glacial acetic acid in the ratio of 3:1 and stained with Giemsa and May-Grunwald's stain**.** About 1000 cells have been screened under the microscope from each person. Obtained data were analyzed and compared.

OBSERVATIONS AND RESULTS

The prepared slides were screened by microscope and according to the The Fenech criteria: (a) a diameter between 1/16 and 1/3 of the mean diameter of the main nucleus; (b) non-refractivity; (c) no linkage or connection to or overlap with the main nucleus; (d) the same staining intensity as the nucleus; and (d) location within the cytoplasm, the MN were identified.

Table 1: Total number of Micronuclei (MN) identified in both cases and controls.

Study group	Total No. of cells screened	Total No. of Micronuclei seen	Percentage
Cases	52000	151	0.29
Controls	50000	13	0.026

Table. 1 shows the total number of MN identified in case group was 151 and in control it was 13 only. The percentage of identified micronuclei was more in cases than control though the number of cells screened in the control group was lesser than the case group.

Table 2: Presence of MN in case and control group.

Study group	Micronuclei		
	Present	Absent	Percent
Cases	47	3	94%
Controls	6	44	12%

Table 2. shows the maximum percentage of MN were observed in the cases(94%) than control group (12%).

Table 3: Distribution of Micronuclei (MN) according to duration of Diabetes.

Total No. of cases	Duration of Type 2 Diabetes		
	< 5yrs	5-10yrs	>10ys
	11	21	18
No. of Micronuclei	21	54	76
Average no. of MN/case	1.9	2.57	4.22

Table 3. shows the distribution of MN in various cases according to the duration of diabetes mellitus. In which , the maximum number MN was present in patients having the type 2 DM more than 10yrs. In case of 5-10yrs duration,

the average no. of MN was 2.57. but it was very less in patients having type 2 DM for less than 5yrs.

Table 4: Comparision of mean MN frequency between case and control group.

Group	Mean	SD	t' value	df	p value
Case	3.02	1.463	11.6941	98	<0.0001
Control	0.26	0.803			

Table.4 shows the mean MN frequency is more in diabetic patients than control group and the 'p' value is < 0.0001 . This shows that it is statistically significant

Fig. 1: Showing the Distribution of MN according to duration of Type 2 DM.

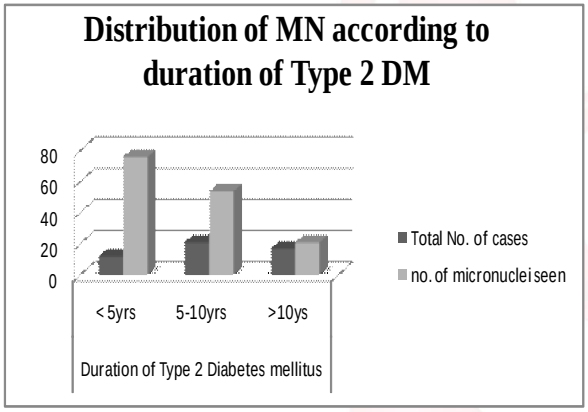


Fig. 2: Showing the Micronucleus.

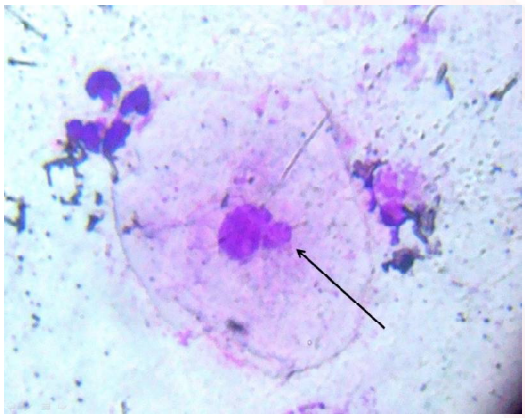
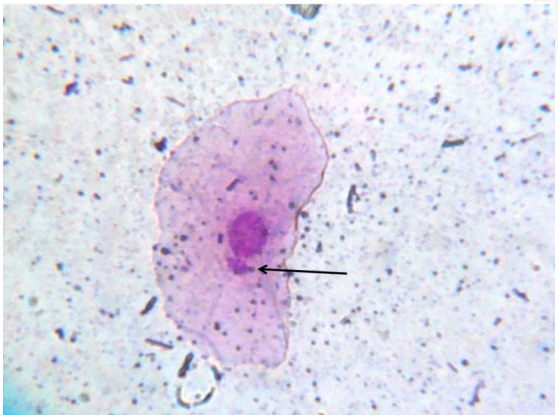


Fig. 3: Showing the Micronucleus.



DISCUSSION

The diabetes mellitus is a major cause for increase in morbidity and mortality around the world. Identification of MN in exfoliated cells of diabetic patients was done in this study. In case group MN were found in 47 patients and in control only from 6 persons we could find. In this study, the mean MN frequency was more (3.02) in case group whereas in control group it was less (0.26). This is consistent with a study done by Martínez-Pérez L. M et al [7].

The average number of MN was more in patients with more than ten years of duration of diabetes. But in case of duration of less than 5yrs the MN frequency merely decreases. This is due to the drug effect. According to Shaik NA et al, the frequency of MN is more in patients treated with glimepiride and pioglitazone [12]. Zuniga-Gonzalez *et al* found that the increased frequency of MN levels in exfoliated buccal cells in type1 and type 2 diabetic patients compared with control group [13]. Elisabeth mullner et al., stated that the levels of buccal MN in diabetic individuals are approximately 2-fold higher than in nondiabetic participants [8]. This is consistent with this study. Shen Z. MN in exfoliated buccal cells are a novel and non-invasive biomarker of genomic stability, formed during mitosis and represent the loss of chromosome fragments or a whole chromosome that failed to be incorporated in the main nuclei. Genomic instability is a hallmark of tumourigenesis [9,10]. Maria Grazia Andreassi et al., stated that a significant correlation between MN frequency and telomere shortening that lead to genetic instability and it is a biological marker for cardiometabolic diseases and diabetes mellitus [11].

Martinez-Pérez L et al., revealed that type 2 diabetes mellitus patients have significantly more genetic damage (in terms of MN frequency) than nondiabetics. This indicates that MN may be a useful constituent in a panel of biomarkers for the risk of diabetes [7].

Cairns stated that assessment of micronuclei (MN) in exfoliated buccal cells, might be of high relevance in future as up to 90% of all cancers are of epithelial origin [14].

CONCLUSION

The micronuclei were observed in type 2 diabetes mellitus patients. The frequency of micronuclei in diabetic patients was more than that of the non diabetic individuals. The presence of micronuclei indicates the genomic instability and chromosomal damage. This can be a predisposing factor for development of cancer. So this can be used as a biomarker for diagnosis of type 2 diabetes mellitus.

Conflicts of Interests: None

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