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Alteration In Baseline Somatic DNA Damage In Patients With Type 2 Diabetes After A Non-Experimental Intermittent Fasting.

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ABSTRACT

Diabetes mellitus can lead to the generation of ROS leading to increased oxidative stress which worsen diabetic complications and DNA damage. A change in circadian pattern of food intake and sleeping has a major role in progression of this disease. Ramadan fasting is considered as an intermittent fasting therefore it is important to assess the somatic DNA damage in type 2 diabetic patients who observe this fast. The present study assessed extends of somatic DNA damage in type 2 diabetic patients who observe Ramadan fasting by cytokinesis block micronuclei assay method. The micronuclei frequency was increased in test group and control groups. However comparison of the difference in test group with control group was not significant. In diabetic non fasting group there was a marked increase in the micronuclei frequency. But similar change was not noticed in diabetic fasting group. The possible reason could be that contribution of fasting to DNA damage is negligible in diabetic patients. It is also revealed that diabetic subjects have more DNA damage when compared to nondiabetic subjects. Ramadan fasting does not alter DNA damage in Type 2 diabetic patients.

Keywords: DNA damage, Ramadan fasting, CBMN assay, Diabetes Mellitus.

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INTRODUCTION

Diabetes Mellitus (DM) refers to a group of metabolic disorders with multiple etiologies. It is characterized by chronic hyperglycemia which results from defects in insulin secretion, insulin action, or both. The defect in the insulin action and secretion leads to various metabolic derangements in carbohydrate, protein and lipid metabolism as well as water and electrolyte regulation. There are mainly two types of DM based on etiology, type 1 and 2. In type 1 DM there is a complete absence or severe deficiency of insulin secretion or the insulin is defective. Type 2 diabetes is characterized by insulin resistance, a relative insulin deficiency and increased glucose production, which encompasses 90 -95% of the diabetic individuals [1].

Reactive oxygen species (ROS) which is generated in the body is normally defended by the antioxidant system of the body [2, 3]. DM can lead to the generation of ROS leading to increased oxidative stress which has a crucial role in diabetic complications and DNA damage [4-6]. Several factors such as lifestyle modifications can lead to the development of DM [7]. The change in the number, timing and portioning of the meals can affect various metabolism [8]. Ramadan fasting, observed by Muslims is considered as intermittent fasting, because the abstinence from food and drink is only between dawn and sunset [9]. Not only food and drinks, drug intake is also restricted during fasting hours of Ramadan [8]. It is important to assess the somatic DNA damage in type 2 diabetic patients while observing this type of non experimental intermediate fasting as there are alarmingly increasing number of diabetic observants of Ramadan fasting [9]

MATERIALS AND METHODS

MATERIALS

The study subjects were selected from the patients and their relatives who visited Dr. Suresh's Diabcare India, (A holistic centre for diabeto-cardiology and diabetic foot care) Calicut, Kerala, India. Informed consent was obtained from the volunteers of the study and detailed demographic data were collected. Relevant medical history and medications if any were also recorded. Height and weight measurement for calculation of body mass index (BMI) were also done in subjects. The blood pressure of the subjects was also recorded to verify hypertension. The study was approved by the Institutional Ethics Committee. The blood samples collected from the subjects were considered as the material for the present study. Clinically proven diabetic and non diabetic subjects for the present study were categorized as following.

Group A: Diabetes patients who observe Ramadan fasting (considered as test group for the present study)

Group B: Diabetes patients who do not observe Ramadan fasting (considered as diabetic control group for the present study)

Group C: Non diabetic people who observe Ramadan fasting (considered as fasting control group for the present study)

Group D: Non diabetic people who do not observe Ramadan fasting (considered as normal control group for the present study).

Non smoking, nonalcoholic male subjects above 18 yrs old, without any auto immune diseases, cancer or any chronic or acute infections, who follow a nonvegetarian diet were included in this study. Subjects above 60 yrs of age and females were excluded from the study.

SAMPLE SIZE

One hundred and fifty three subjects participated in the present study. There were 41 subjects in Group A, 40 subjects in Group B, 34 subjects in Group C and 38 subjects in Group D. The blood samples were collected three days before and three days after Ramadan fast (sample I and II respectively). Five ml of venous blood was collected aseptically from all the subjects by venepuncture in heparinized vaccutainers

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METHODS

CYTOKINESIS BLOCK MICRONUCLEUS ASSAY (CBMN ASSAY) [10, 11]

The micronucleus (MN) assay detects DNA damage and considered as a best established biomarkers of chromosome damage. After isolating the lymphocytes, the cell pellet was suspended in RPMI 1640 medium and centrifuged for 10 minutes. Removed the supernatant and cultured the lymphocytes in sterile bottles using RPMI 1640 medium containing 15% foetal calf serum and incubated for 72 hours at 37ºC. Lymphocytes were stimulated to divide with phytohaemagglutinin (PHA). Fourty four hours after PHA stimulation, cytochalasin-B was added to the cultures to give a final concentration of 4.5µg/ml and this block the cytokinesis. After 70 hrs, the cells were harvested and treated with hypotonic solution of 0.075 M KCL. Then it is fixed with a mixture of methanol-glacial acetic acid (3:1) and dropped onto clean coded microscopic slides followed by Giemsa staining technique.

SCORING OF MICRONUCLEI

A total of 1000 binucleated cells were scored for each subject on coded slides to determine the frequency of binuleated lymphocytes with micronuclei. The following criteria were satisfied while scoring the micronuclei.

- The diameter of the MN was less than one-third of the main nucleus
- MN was either separated from or marginally overlapped with main the nucleus with a clear nuclear boundary.
- The staining of MN was similar to that of main nuclei.

STATISTICAL ANALYSIS

The data analysis was performed using SPSS version 22.0. Quantitative Variables were expressed as Mean ± SD. Comparison of quantitative variables of sample I and II were analysed by paired t test. Comparison of quantitative variable among more than two groups were analysed by ANOVA. A p-value of <0.05 was considered as the level of significance.

RESULTS

There were 153 male subjects in the study population in which the mean age as well as the BMI of the subjects in different groups was comparable. None of the subjects selected were hypertensive [Table 1].

Table 1: AGE, BMI, SBP AND DBP of different groups (MEAN ± SD)

Variables	A (N=41)	B (N=40)	C (N=34)	D (N=38)	p
Age	52.2 ±8.2	49.4±7.0	46.0±10.4	41.8±12.0	NS
BMI	25.1±3.0	24.5±2.9	25.2±3.8	25.1±4.0	NS
SBP	132.9±19.5	123.0±11.3	127.5±15.2	123.3±8.6	NS
DBP	86.6±9.0	82.3±10.1	82.4±8.3	81.0±6.2	NS
BMI- Body mass index, SBP- systolic blood pressure, DBP- diastolic blood pressure; A=diabetic fasting group, B=diabetic non fasting group, C = nondiabetic fasting group, D= nondiabetic nonfasting group, NS=non significant					

The mean CBMN frequency in group A in sample I and II was 14.2±1.1 and 14.3±0.9 respectively with mean increase of 0.1 in sample II as compared to sample I, which is statistically not significant. In group B mean CBMN frequency value was 13.6±0.9and 14.9±0.8 in sample I and II respectively, with a significant increase of 1.8(p=0.01). Mean CBMN frequency in group C subjects was 9.3±2.8 and 9.5±0.8 in sample I and II respectively, with non significant increase in CBMN frequency of 0.2 in sample II as compared to sample I. No significant difference in the micronuclei frequency was observed between sample I and II of group D [Table 2]. The difference in mean CBMN frequency level in sample II as compared to sample I in group A and group B was compared; the difference was statistically significant(p=0.002). Similarly the difference in MN of I and II of

group A and group C were compared and found statistically non significant.The difference in mean CBMN frequency level in sample II as compared to sample I in group A and group D was compared; the difference was statistically not significant [Table 3].

Table 2: Comparison of micronuclei frequency before and after Ramadan fast among different groups

	Group	I	II	P
		Mean±SD		
Micronuclei frequency in 1000 cells	A	14.2±1.1	14.3±0.9	NS
	B	13.6±0.9	14.9±0.8	0.01
	C	9.3±2.8	9.5±0.8	NS
	D	9.6±2.1	9.7 ±1.7	NS
A= diabetic fasting group, B=diabetic non fasting group, C = nondiabetic fasting group, D= nondiabetic nonfasting , I= sample before fasting, II=sample after fasting NS= Nonsignificant				

Table 3: Post Hoc test – Multiple comparison of the difference in I & II of micronuclei frequency in test group Vs control groups

Dependent variable	Comparison among groups	P
Micronuclei frequency in 1000 cells	A Vs B	<0.05 (.002)
	A Vs C	>0.05
	A Vs D	>0.05
A= diabetic fasting group, B=diabetic non fasting group, C = nondiabetic fasting group, D= nondiabetic nonfasting		

DISCUSSION

The present study assessed the difference in the extent of somatic DNA damage in diabetic and healthy males before and after Ramadan fast which extended for 30 days. Average duration of fasting was 14±1 hrs per day. There were two meals per day and were no restrictions on the energy intake during non fasting hours. CBMN assay was used to measure the extent of somatic DNA damage in this study. The main question of inertest in this study was to analyze the alterations in the cellular oxidative functions as the Ramadan intermittent fasting completely alter circadian pattern of an individual abruptly which continue for a longer term. The present study observed that there was no significant change in DNA damage in diabetic patients after fasting and this coincide with other studies which support that intermediate fasting reduce oxidative stress in type 2 diabetes patients [12] and enhances the ability of nerve cells to repair DNA [13]. In healthy adults no alteration in the markers of oxidative stress was observed in many studies [14, 15] and alleviation in oxidative stress was noted by Faris et al., [16]. The present study is in agreement with these studies as it was also observed that no alteration in DNA damage occurred in healthy males. Fasting has beneficial role in the prevention of cancer as well by protecting the cells from DNA damage and promoting the programmed death of the damaged cells [17]. Previous studies also reported a reduction in markers of oxidative stress with a decrease in body weight [16]. This disparity may be due to the difference in sample size, or lifestyle difference in the study group. Various factors such as timing of sample collection and meals can affect the oxidant concentration [18, 19].

In the present study it was also noticed that diabetic patients without fasting has shown a marked increase in baseline DNA damage after 30 days. This result supports the findings of a previous observation in which significant increase in DNA strand breaks has found in diabetic patients than normal people [20]. Diabetes leads to the increased production of reactive oxygen species which intensifies the oxidative stress leading to DNA damage. The reactive oxygen species damage the cellular macromolecules and modify the DNA [20].

CONCLUSION

It is a well known fact that diabetes has an association with DNA damage and intensifying the diabetic complications [21]. Intermittent fasting enhances defense mechanism of antioxidant system and reduces proinflammatory cytokines [22]. The present study found that Ramadan fasting does not have a major role in augmenting DNA damage in diabetic patients.

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