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Research Article

OXIDATIVE STRESS RESPONSIBLE FOR GENOTOXIC EFFECTS IN TYPE 2 DIABETES MELLITUS PATIENTS IN POPULATION OF WEST BENGAL

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Abstract- Type 2 Diabetes mellitus (T2DM) is a chronic, debilitating disease affecting a significant proportion of the population and becoming an epidemic worldwide. Increasing evidence in both, experimental and clinical studies suggest oxidative stress (OS) plays a major role in the pathogenesis of both types of diabetes mellitus and its complications. The OS may exhibit chromosomal instability leading to chromosomal aberration frequencies. A case-control study of sixty-two patients with T2DM (n=62) and ten healthy control subjects (n=10) were screened from different areas of West Bengal. All procedures were done with the informed consent of participants. Blood cultures were done for analyzing chromosome aberrations as per routine procedures In the present study, increased chromosomal aberration frequencies and mitotic index were observed in T2DM patients that may be due to increased oxidative stress leading to different mechanisms like oxidative damage, repair mechanisms and resulting in genomic instability, which in turn may contribute to an increased risk for cancer. Therefore, this study will explore the role of oxidative stress for genomic instability and causing cancer.

Keywords- Oxidative stress, Chromosomal aberrations, Oxidative damage, Genomic instability.

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Introduction

Non-insulin-dependent diabetes mellitus (NIDDM), commonly known as type 2 diabetes mellitus (T2DM), is a metabolic disorder characterized by high blood glucose in association with insulin resistance and relative insulin deficiency. Globally 285 million people are known to be suffering from T2DM, which is projected to increase to 438 million in 2030. India ranked first in the world for the prevalence of the disease, followed by China and USA [32]. There are currently approximately 40.9 million patients suffering from diabetes mellitus in India and it is expected to rise to about 69.9 million by the year 2025 [30]. Lifestyle and environmental factors, obesity and inherited genetic factors are associated with T2DM.

Increasing evidence in both, experimental and clinical studies suggest oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species (ROS) especially free radicals and a biological system's ability to detoxify the reactive intermediates or to repair the resulting damage. Production of reactive oxygen species (ROS) and lipid peroxidation increase in T2DM patients, particularly those having poor glycemic control and hypertriglyceridemia [27]. Previous studies have suggested that ROS, that includes O_2^-, $OH^•$, and H_2O_2, are highly reactive and capable of damaging cellular macromolecules, including proteins, lipids and DNA [26]. Oxidative stress leads to chromosomal instability, and amplification of these redox genes are responsible for higher chromosomal instability. Previous studies have shown that increased chromosomal aberration frequencies enhance higher risk of developing cancers through different mechanisms like DNA damages and repair mechanisms [10]. T2DM has been associated with elevated levels of DNA damage, increased susceptibility to mutagens, and a reduction in efficacy of DNA repair that causes genomic instability and consequently cancer. Chromosomal aberration study may be used as a biomarker for evaluation of genomic instability in diabetes patients. Chromosomal aberrations are numerical and structural disruptions of chromosomes that represents a major cause of genetic disorders. Numerical aberrations involving loss or gain of entire chromosome that gives rise to monosomy or trisomy. Structural aberrations affect parts of chromosomes, usually imposing damage in the chromosome and give rise to different rearrangements [2]. A large number of aberrations such as dicentrics, poly-centrics, rings, and numerous acentric fragments can be seen in some of the damaged cells [22]. DNA double stranded breaks are generally accepted to be the most biological significant lesion and ultimately lead to cancer [16]. Thus oxidative damage over time may cause genomic instability and may lead to cancers. Hence, the risk of various cancers may be higher in diabetic patients due to increased oxidative stress. Liver, pancreas, endometrium, breast, biliary tract, kidney, prostate, ovary, colon or rectum, bladder cancer, and hepatocellular carcinoma are the most common cancers occurring from diabetes [3, 8-9, 13-14, 17, 20, 31, 33-34] along with hyperglycaemia and obesity [31].

During the last decades, many in vitro assays have been developed for measurement of genotoxicity. The analyses of chromosomal aberrations (CA) are being used extensively to evaluate the presence and extent of chromosomal damage in human populations [15]. There are very few reports on the association between T2DM and the occurrence of genotoxic effects. Sudhaa Anand et. al. [31] reported that there was a significant increase in CA frequency in type-2 diabetic patients compared with healthy controls. Since free radical-induced DNA damage can be measured in vitro, we sought in the present study to evaluate T2DM associated genotoxicity with the use of the CA analysis assay. Thus, our study is an in vitro study and it aims to assess the genotoxic effects in cases of T2DM.

Materials and Methods

Study setting and subjects

A total of sixty two patients with T2DM (n=62) and ten healthy control subjects (n=10) were screened from different areas of West Bengal. The patients were confirmed T2DM by impaired fasting glucose test (>126 mg/dl) and oral glucose test tolerance (>200 mg/dl). The controls had normal glucose metabolism and none had a family history of diabetes. A detailed personal histories were collected from the participants with the help of a questionnaire. All procedures were done with the informed consent of participants.

Leucocyte culture and cell harvesting

Blood cultures were performed for analyzing chromosome aberrations according to routine procedures [25, 29]. Karyotyping was done on participants. Peripheral blood samples were aspirated by venipuncture into sodium-heparin tubes. Leucocytes rich plasma (0.5 ml) was added to 5 ml culture media accompanied with PHA M (0.04 ml/ml of culture media) and 20% fetal bovine serum and incubated at 37° C for 72 hrs. Colchicine was added at 70 h of culture. After 2 hrs, culture was centrifuged at 1000 rpm for 10 min and then treated with pre-warmed KCl (0.075M) for 15 min. It was fixed in methanol: acetic acid (3:1) for 10 mins. Fixatives were discarded after centrifugation and two more changes of fixative were done. The fixed cell suspension was laid on clean grease-free glass slide and air-dried and stained with aqueous Giemsa. 100 metaphase plates were scored randomly for analyzing chromosomal aberrations.

Results & Discussion

The general characteristics of the study participants are listed in [Table-1]. The mean age of the T2DM patients was 53.8±11.8 years, ranging from 30 - 77 years, with a mean duration of the diabetes of 8.7±6.4 years (range: 1–25 years). Fifty seven patients had a family history of T2DM in our study. Diabetic individuals had a higher BMI (basal metabolic rate) than the healthy controls. Impaired fasting glucose, Oral glucose tolerance test and HbA1C were significantly higher in T2DM patients than the healthy controls.

This study investigated the relationship between oxidative stress and genotoxic effects in T2DM patients. To the authors' knowledge, this is the first study to assess the genotoxic effects in cases of T2DM in West Bengal, India.

Table-1 *General characteristics of the study participants*

	Controls	T2DM patients
Number of subjects	10	62
Gender (males/females)	4/6	42/20
Age (years) (mean ± SD)	38.6±13.6	53.8±11.8
Duration of diabetes (years) (mean ± SD)	-	8.7±6.4
Impaired fasting glucose test(mean ± SD)	94.2±15.3	133.3±24.1
Oral glucose test tolerance(mean ± SD)	142.1±26.1	222.6±48.6
HbA1C(%)(mean ± SD)	4.9±0.42	7.34±1.2
BMI(kg/m^2) (mean ± SD)	24.6±3.86	29.6±3.6
Addiction(Tobacco/ Alcohol/ Betel Quid)	(4/2/5)	(38/19/45)
Habit(Exercise)	3	11

[N.B.: SD: standard deviation, BMI: basal metabolic rate].

ROS may play an important role in the pathogenesis of T2DM and its complications. The presence of an increased oxidative stress in diabetic patients is a well established fact. Under conditions of oxidative stress, cellular bio-molecules such as lipids, proteins and DNA get damaged. Oxygen-free radicals incorporate a variety of lesions in DNA that include oxidized bases, abasic sites, DNA strand breaks and the formation of cross-links between DNA and proteins [19]. Many of these lesions are cytotoxic and mutagenic.

It is also well documented in previous studies that diabetes enhances the risk of cancer, but till date the underlying mechanism is not clearly understood. Although there are many risk factors that are shared between cancers and diabetes patients, it is well established that an increased oxidative stress is responsible which contribute to genomic instability [18, 31] directly or indirectly. However, there were inconclusive results of several studies that reported an increased oxidative damage in diabetes patients using cytogenetic tests [7, 27, 5]. Several epidemiologic studies reported higher significant risk of cancer in diabetic patients and were 20% more in diabetic patients [35, 12]. Both males and females with T2DM are known to have an increased risk of renal cell carcinoma by 40%.

Exposure of human population to mutagenic agents and evaluation of genotoxicity can be monitored by assessment of CA [1]. Presently, several studies have investigated a number of parameters involving genotoxicity in relation to health and disease [28].

In the present study, the slides were analyzed blindfold for mitotic index and chromosomal aberrations. An increased random chromosomal aberrations and mitotic index were observed in the diabetic group. In our study, we had found chromosome breaks in most of the T2DM patients, as shown in [Fig-1a]. Among chromosome breaks, mostly chromatid breaks (2.8±0.98) and a few gaps in chromosomes (1.3±0.54) were analyzed in T2DM patients. A drastic increase in chromosomal aberration frequency was observed in T2DM patients (4.8±1.2) as compared to the healthy controls (0). Whereas there was also an increase in mitotic index in the diabetic group (5.8±2.8) when compared to the controls (1.2±0.9), as shown in [Table-2].

Table-2 *Indicates the mitotic index and chromosomal aberration frequencies in T2DM patients and controls*

Types	Diabetic cases(mean±SD)	Healthy Control(mean±SD)
Mitotic Index	**5.8±2.8**	**1.2±0.9**
Chromosomal Aberrations	**4.8±1.2**	**0**

N.B. SD: Standard Deviation

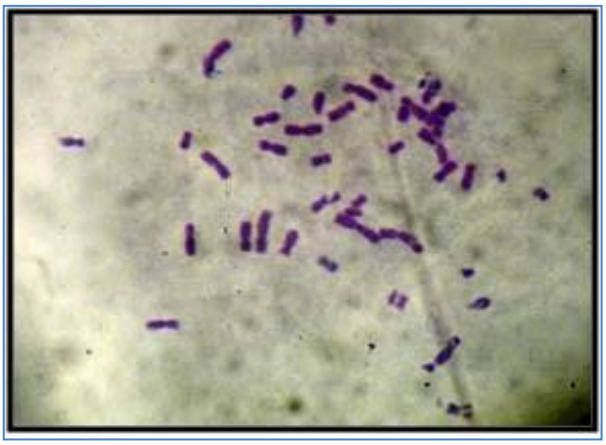

Fig-1 (a) -Indication of chromosomal break in T2DM patients [Giemsa stain, observed under compound microscope (Olympus, Model No. CH20Itr), 100x magnification].

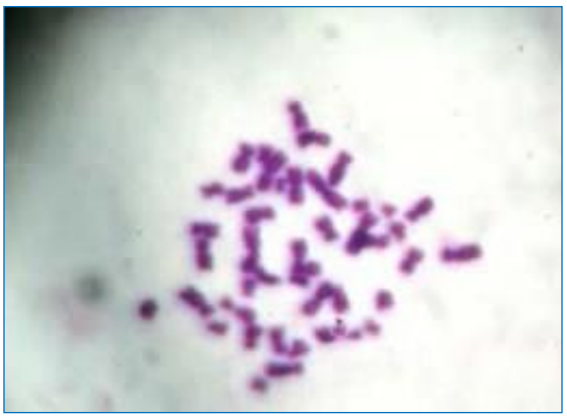

Fig-1 (b)–Indication of chromosome of normal healthy subject [Giemsa stain, observed under compound microscope (Olympus, Model No. CH20Itr), 100x magnification].

Increased chromosomal aberration frequencies and mitotic index observed in diabetic groups than the healthy controls may be due to increased oxidative stress that results in oxidative damage, ultimately leading to genomic instability, which in turn may enhance the risk for cancer. Sudhaa Anand et al [31], previously investigated the occurrence of genotoxic damage in T2DM patients as evidenced by CA assay. The authors reported a higher frequency of CA in patients with T2DM. According to the cytogenetic study held in Babylon by Maysaa et. al [21], it was observed that diabetic group showed a significant increase in mitotic index and chromosomal aberrations. Thus, our current data confirm and expand previous findings by showing that T2DM patients have increased frequency of CA. CA aberration assay may be used as a marker to evaluate the genomic instability in diabetes patients.

Conclusion

In conclusion, our present data assumes that an enhanced genomic instability with an increased frequency of CA can be a common biochemical feature of patients with T2DM.

Abbreviations: T2DM, CA, NIDDM, BMI, HbA1C.

Ethical approval: This study was approved by the institutional ethical committee on 10th May, 2016. Reg No. ECR/62/Inst/WB/2013 issued under Rule 122DD of the Drugs & Cosmetics Rules 1945.

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Conflict of Interest: None declared

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