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Cytogenetic and Immunogenic Study of Type -2 Diabetes Mellitus Patients

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Abstract The current study which lasted from January to March (2010), the samples were collected from 50 untreated type-2 DM patients in different ages 18-65 years and sex-matched healthy control subjects were collected from specialized center for endocrinology and diabetes. The somatic cell chromosomal instability was assessed in peripheral blood lymphocytes. Chromosomal analyses (Mitotic index, Blastogenic index, Chromosomal aberration) were measured as well as phgocytosis index and percent. The study results showed that chromosomal state effected significantly in the DM patients by reducing both of mitotic index and blastogenic index while the chromosomal aberrations increased gradually according to the glucose level in blood and age. The immunogenic state effected by decreasing both phagocytosis percent and phagocytosis index significantly in the DM patients according to age.

Keyword Cytogenetic, Mitotic Index, Phagocytosis, Diabetic Mellitus II, DNA Damage, Chromosomal Aberrations

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1] Diabetes mellitus represent a serious health problem affecting millions of individuals worldwide, by the year 2025; the World Health Organization (WHO) predicts that 300 million people will have diabetes mellitus[2]. Oxidative stress is thought to play a significant role in the aetiopathology of type -2 diabetes mellitus[3, 4]. Previous studies have clearly shown that ROS, including O_2^-, OH^-, and H_2O_2, are highly reactive and capable of damaging cellular macromolecules, including proteins, lipids and DNA[4, 5] Moreover, individuals with diabetes mellitus have reduced antioxidant defense capacity[5]. It has been demonstrated that diabetes mellitus is associated with elevated level of oxidative DNA damage and with the increased susceptibility to mutagens and the decreased efficacy of DNA repair[6, 7]. This can contribute to the chromosomal instability in diabetics. Several *in vitro* assays have been developed for measurement of genotoxicity, blastogenic index, mitotic index, chromosomal aberration assay in lymphocytes are being used extensively to evaluate the presence and extent of chromosomal damage in human populations. There are limited reports on the association between diabetes and the occurrence of genotoxic effects[8] reported that there is a significant increasing in sister chromated exchange and micronuclei in type -1 diabetes mellitus. The mitotic index assay good indicator to study the chemical and physical agent's effects on genetic material. The recent studies refer to ionic rays, some diseases, chemical agents and some viruses can be causes modulating in the mitotic index (inhibition or stimulation). From the study of [9].

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2. Aims of the Study

The present study aimed to:

1. Investigate of chromosomal analysis of type- 2 DM patients by using some parameters (MI, BI, and CA).
2. Study of immunogenic state of type- 2 DM patients by measuring phagocytosis index and phagocytosis percent.

3. Materials and Methods

3.1. Samples Collection

Between January to March 2010, 50 diagnosed cases of type-2 DM and 50 age- and sex-matched healthy control subjects were collected from specialized center for endocrinology and diabetes.

3.2. Lymphocyte Cultures and Cell Harvesting

Peripheral blood samples were drawn by venipuncture into sodium-heparinized sterile syringe. For each donor, three blood cultures were set up. A 0.5-ml whole blood sample was added to a culture medium (5 ml) containing

RPMI 1640 medium (pH 6.8–7.2), 15% fetal calf serum, 10 µg/ml phytohemagglutinin L (PHA-L), 0.5 mg/ml l-glutamine, and antibiotics (100 IU/ml penicillin, 100µg/ml streptomycin) for 24 h at 37℃.

3.3. Chromosomal Aberration Assay

CA assay was performed for the entire incubation period of 72 h. Colcemid (10µg/ml) was added 70 h after initiation of all cultures. The cells were harvested and processed through treatments with a hypotonic solution (0.075M KCl) and fixative (3:1 methanol: glacial acetic acid). Chromosome slides were stained with 5% Giemsa solution (pH 6.8) for 2.5min, identified by the light microscope (Olympus, Japan) at oil emersion was used[10].

3.4. Phagocytosis Assay

2 ml of heparinized blood were centrifuged 3000 rpm (Boeco centrifuge U-320, Germany), A puffy layer (WBC) was gently isolated by glass Pasture pipette and suspended with 1 ml of (RPMI-1640), 100 µl of WBC solution (98% viability) have been incubated for one hour with 100 µl of heat killed *Candida albicans* suspension (1×10^3 cell/ml) at 37℃. 100 µl of the mixture (WBC and *C. albicans*) was spread on slide after completed dry fixed with absolute methanol and stained with 5% Giemsa solution (pH 6.8) for 2.5min, washed with PBS and slides were examined on 40X (Olympus, Japan) the percentage of phagocytosis was calculated using the following equation.

$$\text{Phagocytosis percent \%} = \frac{\text{NO. Of phagocytes}}{\text{Total NO. Of phagocytes and non phagocytes}} \times 100\%$$

3.5. Blood Sugar Determination

Blood sugar was determined according to manufacturer's instructions for diagnostic kit (Biolab, UK), the determination of serum glucose was conducted as the following steps.

Pipette into 3 Test tubes

	Blank mL	Standard mL	Test mL
Working Reagent	3.00	3.00	3.00
Standard	-	0.02	-
Sample	-	-	0.02

Mix well; incubate for 15 minutes at 37°C.

Read the absorbency of the test and standard against blank at 500 nm wavelength.

Calculation

Glucose in mgs/dL= Absorbency of test / Absorbency of standard x 100.

3.6. Statistical Analyses

SPSS program was used to analyze the results statistically, ANOVA I and multiple comparisons by using the least significant difference LSD was used at p≤005.

4. Result and Discussion

Table 1. Shows the general characteristics of the study participants, the mean age of type-2DM patients was 36.12±8.11 years with body weight mean 67.43±5.45 and blood sugar level mean 223. 21± 15.13 mg/dl, 23 (46%) had a family history of type-2 diabetes and 5(10%) of patients had a family history of type-2 diabetes.

Table 2 and figure 1 showed the frequencies of total CA, types of aberrations BI and MI in the two study groups there is a significant differences between patients and control group. Whilst table 3 refers some cytogenetic characteristics of two groups according to age, the MI, BI and CA were suffered from graduated reduced when increasing age.

Table (1). General characteristics of the study volunteers

	Control	*Type-2 DM*
Number of subjects	50	50
Gender (males/females)	27/23	27/23
Age (years) (M±SD)	35.34±10.23	36.12±8.11
Duration of diabetes (years)	-	Less than 1 year
Body white (M±SD)	78.15±7.12	67.43±5.45
Fasting blood sugar (mg/dl) (M±SD)	91.76±10.32	223. 21±15.13
Smoke habit (n)		
Smoker	15	11
Non-smoker	35	39

Table (2). cytogenetic characteristic and chromosomal aberration of the study participant

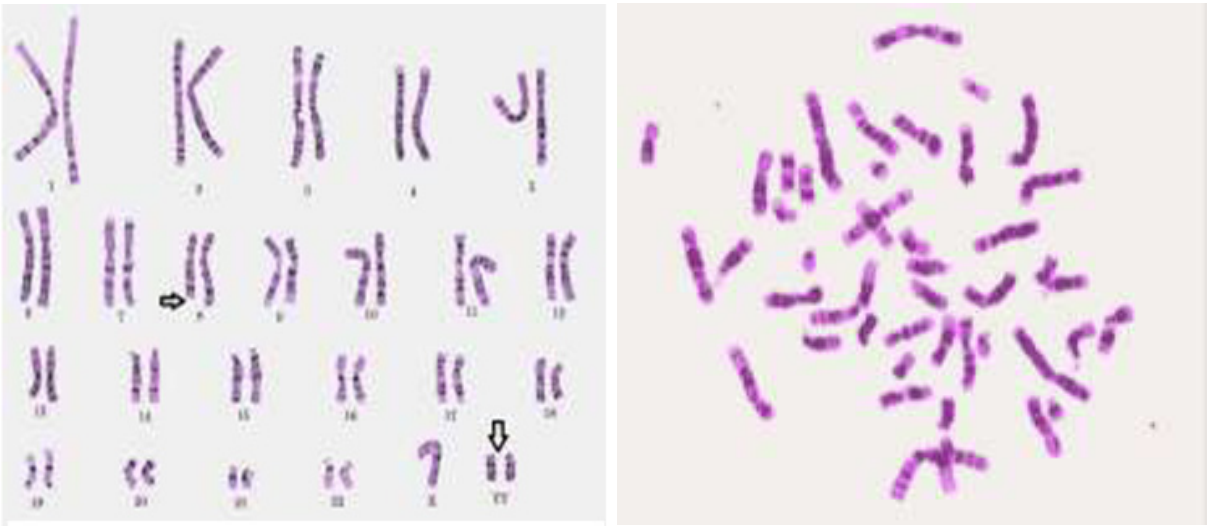
	Control	*Type-2 DM*
Mitotic index	1.84 ±0.33 (50)	1.63±0.145*(50)
Blastogenic index	41.09±0.019 (50)	38.65±0.21*(50)
Chromatid break	0.00	0.002±0.002*
Isochromatid break	0.00	0.006±0.002*
Chromatid acentric fragment	0.001±0.003	0.007±0.003*
Total chromosome aberration	0.001±0.002	0.015±0.005*

*significant at $p > 0.05$
Each number represents M±SD for 100 lymphocytes

Table (3). Cytogenetic characteristic and chromosomal aberration of the study participant per age group

Age group	characteristic	Control	Type-2 DM
≥25	MI	1.9±0.115	1.63±0.21*
	BI	42.3±4.77	40.56±2.11*
	CA	0.00	0.006±0.002*
25-35	MI	1.87 ±0.113	1.55±0.45*
	BI	41.45±0.34	39.32±0.12*
	CA	0.00	0.002±0.001*
35-45	MI	1.77±0.23	1.34±0.67*
	BI	39.67±0.56	37.56±0.54*
	CA	0.00	0.001±0.001*
≤45	MI	1.56±0.65	1.11±0.45*
	BI	35.67±0.34	33.45±0.67*
	CA	0.001	0.006±0.003*

significant at $p \leq 0.05$
Each number represents M±SD for 100 lymphocytes per age group



A B

Figure (1). A chromatide brakes in peripheral blood lymphocytes of type-2 DM, B metaphase of healthy one

The same reducing style was seen in patients group with significant differences at P≤0.05. It is evident that the generation of reactive oxygen species (oxidative stress) may play an important role in the etiology of some of the diabetic complications. This hypothesis is supported by evidence that many biochemical pathways strictly associated with hyperglycaemia can increase the production of free radicals. They can interfere with several functions and mitosis of the cell. The genotoxic effects observed in type-2

DM patients in the current study are in agreement with results obtained from previous animal studies[11,12] and chromosomal analyses of type II diabetic women[13] as well as the study of [14]. This damage may trigger inflammatory processes, as well as hyperglycemia-induced oxidative stress [15] that may be sufficient to increase the chromosomal damage. Free radical can cause oxidative damage to DNA .CA involves breakage of both DNA strands followed by an exchange of whole DNA duplexes. It occurs normally in cells during mitosis. Whenever, genotoxic agents damage the cellular DNA, the rate of CA increases. Many studies

have shown that oxidative stress induced by hyperglycaemia possibly contributes to the pathogenesis of diabetes and its complications[16, 17, and 18].

In-vitro genotoxic effects have shown increased plasma level of Malondialdehydrate (MLD) and total thiols in type II diabetic patients as compared to control subjects. Study has further shown that diabetic patients harbouring 16189 T variant of mtDNA impair the ability of a cell to respond properly to oxidative stress and oxidative damage. This suggests that diabetic complications may be related to oxidative stress-induced DNA damage[19, 20].

4.1. Phagocytosis Assay

The results in table 4 show the effects of type-2 DM on both phagocytoses index and percent according to age, we can see a clear reduction in both parameters with significant differences at P≤0.05, the reduction in PP in patients group reduced from 48.87 in age group ≥25 year to 43.76 in age group ≤45 the both effects (age and typ-2 DM) have the same effects on PMN leukocytes activity .

Table (4). Phagocytosis percent and phagocytosis index of PMN per age group

Age group	*characteristic*	*Control*	*Type-2 DM*
≥25	PP	0.8 ± 52.23	48.87±4.5*
	PI	0.15 ± 8.6	7.33±0.56 *
25-35	PP	51. 8 ± 0.5 9	46.78±6.8*
	PI	8.2 ± 0.0 8	6.45±0.34*
35-45	PP	47.89 ± 0.18	46.23 ± 2.34*
	PI	7.6 ± 0.54	5.34±0.23*
≤45	PP	38.45 ± 0.56	43.76±2.45*
	PI	5.65±0.34	4.89±0.45*

Significant at $p \leq 0.05$
Each number represents M±SD

Table (5). Phagocytosis percent and phagocytosis index of PMN per blood sugar levele

Fasting blood sugar (M±SD)	PI	PP
91.76± 10.32 (50) control	7. 5±0.34	49. 6±2.45
223. 21± 15.13 (50) Type-2TD	5.8±1.67*	46.41±1.89*

Significant at $p \leq 0.05$
Each number represents M±SD

As we know the phagocytosis is non specific defiance as result of intracellular signal transduction pathways regulated by reactive oxygen species (ROS), effects of ROS stem from interactions with various ion transport proteins such as ion channels and pumps, primarily altering Ca^{2+} homeostasis and inducing cell dysfunction, the Ca^{2+} transport system in PMN of patients with type II diabetes, evaluating the possible correlation between cell modifications and the existence of specific oxidative stress damage. PMN from type II diabetes patients displayed oxidative stress features (accumulation of some ROS species, membrane peroxidation, increase in protein carbonyls, increase in SOD and Catalase activity) and Ca^{2+} dyshomeostasis (modified voltage-dependent and inositol 1, 4, 5-triphosphate-mediated Ca^{2+} channel activities, decrease in Ca^{2+} pumps activity). The data support a correlation between oxidative damage and alterations in intracellular Ca^{2+} homeostasis, possibly due to modification of the ionic control in PMN of type II diabetes patients[21, 19, and 22].

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