Valuating of sister chromatid exchange (SCE) in patients with type 2 diabetes mellitus

Zafir Hassan Ghali and Noor Jamal Hussein* Department of Biology, College of Education University of Wasit- Iraq *Department of Biology, College of Science University of Wasit- Iraq تقييم التبادل الكروماتيدي الشقيقي في مرضى داء السكري النوع الثاني ظافر حسن غالي و نور جمال حسين*

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المستخلص

صممت هذه الدراسة لإختبار المعلمة الوراثية الخلوية تبادل الكروماتيدي الشقيق (SCE) في مرضى داء السكري النوع الثاني لإرتباطها الوثيق بالطفرات الوراثية أنجزت في الدراسة الحالية التحليلات الوراثية الخلوية على الخلايا اللمفاوية لعينات الدم المحيطي للمرضى المصابين بداء السكري النوع الثاني بإستعمال مزروع على الخلايا اللمفاوية لعينات الدم المحيطي للمرضى المصابين بداء السكري النوع الثاني بإستعمال مزروع الخلايا القصيرة الامد وأستعملت الخلايا اللمفاوية منذ مدة طويلة لتقييم الضرر الذي يحدث على مستوى الجنات والذي قد يحصل داخل وخارج الجسم الحي أظهرت النتائج ارتفاع معنوي في ترددات التبادل الكروماتيدي والذي قد يحصل داخل وخارج الجسم الحي أظهرت النتائج ارتفاع معنوي في ترددات التبادل الكروماتيدي الشقيقي في مرضى داء السكري الذي يحدث على مستوى الجينات والذي قد يحصل داخل وخارج الجسم الحي أظهرت النتائج ارتفاع معنوي في ترددات التبادل الكروماتيدي الشقيقي في مرضى داء معنوي في ترددات التبادل الكروماتيدي الشقيقي في مرضى داء وخارج الجسم الحي أظهرت النتائج ارتفاع معنوي في ترددات التبادل الكروماتيدي الشقيقي في مرضى داء السكري النوع الثاني [80.0+22] . تبادل كروماتيدي شقيق / خلية] مقارنة بعينة والذي قد يحمل داخل وخارج الجسم الحي أظهرت النتائج ارتفاع معنوي في ترددات التبادل الكروماتيدي الشقيقي في مرضى داء السكري النوع الثاني [80.0+22] . بالمالم وماتيدي شقيق / خلية] مقارنة بعينة الشقيقي في مرضى داء السكري يمتلكون السيطرة[40.0+22] . وحارج الوراثي نتيجة وجود التردد العالي من تبادل الكروماتيدي الشقيقي كما استنتجنا انه لا درجة عالية من الضرر الوراثي نتيجة وجود التردد العالي من تبادل الكروماتيدي الشقيقي كما استنتجنا انه لا درجة عالية من الضرر الوراثي نتيجة وجود التردد العالي من تبادل الكروماتيدي الشقيقي ألماسكري المور المالم المالي الموري المالم مراحي مالم من تبادل الكروماتيدي الشقيقي ألمان الموري الموري الموري المالم مراحي يالمالمر مالموري المالموني المالمون مالموري الموري الموري الموري الموري الموري الموري الموري المو

الكلمات المفتاحية:تبادل الكروماتيدات الشقيقة, داء السكري, الجهد التأكسدي

Abstract

This study was established to test the cytogenetic parameter of sister chromatid exchange (SCE) in type 2 diabetic patients for its close relationship with mutations. In this study, cytogenetic analyses were done on peripheral blood lymphocytes obtained from 25 type 2 DM patients using short-term lymphocyte culture .Human lymphocytes have long been used to assess the gene damage occurring in vivo as well as in vitro. Results showed significant high frequencies of SCEs were scored in type 2 DM patients [2.25 \pm 0.08SCE/cell] compared with control [1.28 \pm 0.04SCE/cell] (P<0.01). We concluded that the DM patients have significant differences in diabetic patients in relation with demographic characteristics (age and gender).

Key words: Sister chromatid exchanges, Diabetic mellitus, Oxidative stress

Introduction

Diabetes Mellitus (DM) is a metabolic defects of multiple etiologies characterized bv disturbances of protein, fat and carbohydrate metabolism resulting from disorders in insulin secretion, insulin action or both (1,2). It is a major worldwide health predisposing problem to markedly increased cardiovascular mortality and serious morbidity and mortality (3,4).The prevalence of diabetes mellitus is increasing globally, by 2030, the number of diabetes is expected to increase to 366 million (5,6). Oxidative stress has been involved in the pathogenic process of a diabetes mellitus. The products of an could oxidative stress play an important role diabetic in complications which involve micro macroangiopathic and processes through peroxidation of lipid (Low Density Lipoprotein oxidation) and the production of advanced glycosylation end-products (AGEs), which are responsible for producing fragmentation, cross-linking and damage of basic structures. carbohydrates, lipids, proteins and DNA. There are basically three types of diabetic mellitus recognized as type mellitus (T1DM) 1diabetes an autoimmune disease, type 2 diabetes mellitus and gestational (T2DM) diabetes (1). Sister chromatid (SCEs) refers exchanges to the exchange of certain homologous stretches of DNA sequence between two chromatids .It occurs normally in cells during mitosis. The rate of sister chromatid exchange is increased when genotoxic agents damage the cellular

DNA (7). Observation of the frequency of SCE in short-term cultured peripheral blood lymphocytes is considered a beneficial technique for the assay the effects of chemical mutagens and/or carcinogens on eukaryotic chromosome (8). Type 2 diabetes is a condition with genomic characterized instability by an increased level of SCE (9).Oxidative stress due to chronic hyperglycemia reactive oxygen species leads to and loss generation (ROS) of chromosomal integrity (10). Many studies have shown that oxidative hyperglycemia stress induced by possibly contributes to the pathogenesis of diabetes and its (10-12).Dyslipidemia complications which characteristic of type 2 diabetes to mellitus leads an increased production of methylglyoxal aldehyde which induces ROS increase (13). This study was aimed to evaluate the sister chromatid exchange in type 2 diabetic patients.

Materials and methods

Selection of subject

Patients whom chosen for this study were men and women with diabetes mellitus disease. They have been chosen from various hospitals in wasit province which include: Al-Zahraa Hospital, Al- Karama Hospital and Wasit Blood Bank. 25 patients with T2DM from two genders (males & females) with ages 30-80 years (mean age: 45.3 years). Fifteen healthy control groups similar to type 2 diabetes mellitus patients in age and gender were comprised in this study.

Blood specimens' collection

Each patients and control groups were drawn about 2 ml of blood and putting into sterile vacutainer tube (10ml) containing 0.1ml heparin solution and was used for short-term cultures.

Cytogenetic technique

Short-term peripheral blood lymphocyte cultures were done under optimal conditions. The samples of blood from patients and controls were cultured in RPMI-1640 culture medium to carry out the cytogenetic analyses (14).

RPMI-1640 culture medium preparation

Preparation of RPMI-1640 culture medium was done under sterile conditions.2.5 gram of RPMI-1640 was dissolved in 50ml of deionized distilled water (DDW), 2.5ml of antibiotic solution (streptomycin+ penicillin), 3.75ml of sodium solution. 2.5ml bicarbonate of bromodeoxyuridine (BUdR) solution and 25ml of fetal calf serum were added. The final volume was broughtup to 250 ml with deionized distilled water .Cultural medium sterilization was done by Millipore filter $(0.22\mu m)$. The sterilized culture medium was kept at 4°C.

Blood culturing optimization

Cytogenetic analysis for culturing peripheral blood lymphocytes was done using blood specimens obtained from healthy individuals. In these experiments, various concentrations of colcemide, PHA, in addition to various specimens of blood cultured were used to detect the optimization of the culture media and to obtain clear results. In this study, the standard blood culturing involved inoculation of 0.1ml of heparinized peripheral blood in 3ml of RPMI-1640 culture medium which was manipulated in 10ml sterile vacutainer tube. Adding 0.2ml of PHA, then, incubated the culture medium at 37 °C for 72 hours (15).

Cell harvesting method

0.1ml of colcemide in a concentration of 10µg/ml was added to each culture tube for the final two hours of incubation time to harvest of mitogen stimulated cells (15).In the last of incubation time, tubes containing cells were centrifuged at 2000 rpm for 10 supernatant minutes. The was discarded and a little medium was remained over the cell pellet.8ml of warmed hypotonic solution (37°C) was treated the harvested cells with gentle mixing, then, the tubes were incubated in a water bath at 37°C for 25 minutes with shaking every 5 minutes (15).

The cells were centrifuged at 2000 rpm for 10 minutes, the supernatant was discarded, and the cells were fixed with 5ml of fixative solution which was added as drop-wise with well mixing .Washing the cells at least three times with the fixative solution .Eventually, the cells were suspended in a small volume of the fixative solution and kept at 4°C until slide preparation (15).

Preparation of slides

The slides were cleaned well by soaking them in chromic acid for 4 days, then, used in mounting the cell suspension. Slides were washed with continuous hot water, subsequently with continuous cold distilled water and kept at 4°C in a clean package containing distilled water (16).5 to 10 drops of cell suspension were mounted onto slides at a high of around (1.5) meter. Finally, the slides were left to air-dry in a dark room (15).

Working solution of giemsa stain solution

Giemsa stain solution was prepared freshly by mixing the following chemicals (15):

Vol	Material
1ml	Giemsa stock solution
1.25 ml	Methanol
0.5 ml	Sodium bicarbonate solution
40 ml	Distilled water

Working strength of hoechst stain solution

Hoechst stain working solution was prepared by diluting one part of Hoechst stain stock solution in 100 parts phosphate buffer saline (PBS) (15).

2XSSC Solution

The solution 2XSSC was involved of two solutions:

Solution A: Prepared by dissolving 0.735g of sodium citrate (Na3C6H5O7) up to a volume of 50 ml distilled water.

Solution B: Prepared by dissolving 0.441g of sodium chloride up to a volume of 50 ml distilled water. The final solution of 2XSSC solution was prepared by mixing equal volumes of A and B solutions

The staining technique of fluorescence plus giemsa dye

The technique of fluorescence plus Giemsa (FPG) staining was used to stain 4 to 5 slides for each sample to account sister chromatid exchanges and for detection of cell replication kinetics. The following FPG modified from a procedure which was reposed from Benn and Perle (17):

1-The slides were immersed in phosphate buffer saline for 5 minutes.

2- The slides were stained in Hoechst stain for 15 to 25 minutes, then rinsed well with distilled water.

3- The slides were mounted in an appropriate tray using 2XSSC solution.

4- The mounted slides with 2XSSC solution were irradiated approximately 5 to 15 cm using ultraviolet (UV) lamp for two hours , then washed with distilled water and left to air-dry in a dark room.

5- Giemsa stain solution was used to stain the slides for 25 minutes or until differentiation.

Cytogenetic analysis

Sister chromatid exchanges (SCEs)

Sister chromatid exchanges were calculated in 25 well-spread second metaphases (14).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) program was used to results statistically. analyze the According to Snedecor and Cochran (18), the statistical analysis included descriptive statistics (means and standard errors) and inferential statistics (T-test) The Sudent t-test was used to compare two groups. This was followed by the least significant difference multiple comparison test (LSD) at the 0.05 % and 0.01% levels of significance to reveal the position of difference.

Results and Discussion

The results of metaphase test confirm significant high frequencies of SCEs in DM patients. The mean value indicates the number of SCE observed in minimum 25 cell of second cell division (M2). There is a significant difference of SCE/metaphase between $[2.25\pm0.08]$ T2DM patients and control group $[1.28\pm0.04]$ (P < 0.01) as shown in (table1). High frequency of SCEs in those patients with type 2 DM indicate that diabetic mellitus induce lesions leading to SCE formation. It may be due to altered DNA repair systems(19). High levels of SCEs

demonstrates chromosome breaks that may lead to mutations (20). These results indicate that high frequencies of SCEs in DM patients could be a predisposing factor for some complications related with diabetes and an early marker of DNA damage (21).Oxidative stress due to chronic hyperglycemia leads to reactive oxygen species generation (ROS) and loss of chromosomal integrity (10). Many studies have shown that oxidative stress induced by hyperglycaemia possibly contributes to the pathogenesis of diabetes and its complications (10-12). This theory is supported by evidence that many biochemical pathways accurately associated with hyperglycemia can increase the free radicals production. They can intervene with several functions and the cell mitosis (22).Our results are agreement with results obtained from previous animal studies (23,24).Dyslipidemia which characteristic of type 2 diabetes mellitus leads to an increase of production of methylglyoxal aldehyde which induces ROS increase (14).Methylglyoxal causes stable modification of DNA bases, which in induces SCE human turn in lymphocytes treated in vitro (25). Somatic DNA damage has also been linked to several disease processes including coronary artery disease (CAD) particularly those who also had diabetes as shown in Table 1.

Table (1): Cytogenetic analyses ofperipheral blood lymphocytes fromcontrol and patients group.

Cytogenetic parameters	Control	Diabetic mellitus	P-value	
Sister	1.28 ± 0.04	2.25 ± 0.08	0.01**	
chromatid				
exchange				
(SCE)				
** Highly significant different p-value 0.01				

There are no significant differences in frequencies of SCE between genders (males and females) and this agreement with Frenny study (21), but contrast with study of Hedner as he found females had significantly more SCE frequencies than males (26).

Table 3: Cytogenetic parameters ofperipheralbloodlymphocytesbetweenmaleandfemaleuntreateddiabetic group

SCE		
Male	Female	
2.02±0.12	2.28±0.11	
P>0.05		

Bender observed a statistically higher mean frequency of SCE in females than in males with significant positive regression with age (27). Hedner studied SCE in relation to age and sex and did not find correlation between the frequency of SCE and age, but older individuals had significantly more structural aberrations than younger (26). The highest percentage of SCE frequencies was in age group (30-40) as shown in (Table 4).

Table (4): Cytogenetic parameters of peripheral blood lymphocytes from age (years) of untreated diabetic

Age (years)	SCE	
30-40	$2.35{\pm}0.13^{a}$	
40-50	2.25±0.16 ^a	
50-60	$2.27{\pm}0.13^{a}$	
60-70	$2{\pm}0.5^{\mathrm{a}}$	
70-80	1.9±0 ^a	
The same latter refers to no significant difference between means.		

Conclusions

Data conclude that cytotoxic index sister chromatid exchange (SCE) is a sensitive tool to achieve the cytogenetic abnormalities in diabetic mellitus. Our result demonstrated that 2 diabetes patients have type significantly more genetic damage (SCE frequency). This indicates that SCE may be a useful constituent in a panel of biomarkers for the risk of diabetes.

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