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RESEARCH ARTICLE

Biochemical and Molecular Study in Women with Diabetes Mellitus Type 1

Saad Mohammed Nida[1], Israa B. Raoof[2], Perry H. Saifullah[3]

1.Biotechnology research center ,University of AL-Nahrin
2.College of Pharmacy, University of Al-Mustansiriyah
3.College of Science for Women, University of Baghdad

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Corresponding Author

Israa B. Raoof

Abstract

Diabetes is a chronic metabolic syndrome resulting from defects in insulin secretion or action or both; The aim of this study was to detecting single strand antibodies against DNA in Type 1 Diabetes mellitus DM patients with nephropathy and myocardial vascular disease complication. The results showed that a significant increase in levels of Fasting blood glucose, Urea and BUN, lipid Prfile except HDL levels compared with control groups. Also, the study showed the purity of extracted DNA from blood and placenta in control groups and Placenta was higher than of D.M groups. Electrophoresis pattern showed that band of native DNA was more distinct and intense as compared with DM groups that migrated as short band of lower fluorescence intensity form indicating structural alterations in the DNA upon glycation. In this study, generation of single strand breaks has been confirmed by nuclease S1 digestion studies. The results showed a substantial digestion of modified DNA in DM groups by S1-nuclease while native DNA remained unaffected. The comet assay revealed higher percentage of DNA damage in diabetics groups when compared to control groups. The damage indicated by tail and head of comet, the study showed an increase in tail comet and a decrease in comet head in D.M patients with and without complications compared with control group.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetes mellitus results from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Symptoms of marked hyperglycemia include polyuria, polydipsia, sometimes with polyphagia, and blurred vision [(1)] Diabetes mellitus (DM) is linked to tissues and organ damage through several pathological mechanisms, such as an increased polyol pathway, increased intracellular formation and activity of advanced glycation end-products, activation of protein kinase C isoforms and finally over-activity of the hexosamine pathway. [(2)].S1 nuclease is an endonuclease that catalyzes the specific degradation of single-stranded DNA to cleavage mononucleotides. Comet assay is a commonly used method to detect oxidative damage in lymphocyte DNA and the degree of oxidative stress is related to the size of comet tail, [(3)] that showed that blockade of hyperglycemia-induced oxidative damage would reverse the pathways implicated in diabetic angiopathy in cultured endothelial cells. The Comet assay has been widely accepted and rapid tool for assessing DNA damage. [(4)].

Materials and Methods:

The Study was done of 80 samples of blood and serum of women were obtained under fasting conditions and they divided as: 20 samples of patients of Diabetes mellitus without complications ,20 samples of DM with cardiovascular (CV),20 samples of DM with Nephropathy and 20 samples of Subjects as control group in National Diabetes Center (NDC)

Determination of Serum Glucose level:

By using an enzymatic colorimetric method with a commercially available kit, the fasting Blood Glucose (FBG) was determined [5].

Determination of Serum Total Cholesterol (S.T.C.) level:

Serum cholesterol is measured by an enzymatic method using cholesterol kit [6]

Determination of Serum Triglyceride (S.TG) level:

Serum triacylglycerol is measured using an enzymatic method by TAG Kit [7]:

Determination of Serum High Density Lipoprotein(S.HDL) level:

Serum HDL-C is measured by HDL kit [8]

Determination of Serum Low Density Lipoprotein (S.LDL) level:

LDL-cholesterol is very difficult to isolate and measure .Hence, LDL level is most usually derived by the friedwalds formula as follows [9]:

LDL = Total cholesterol – [HDL + TG/5]

Determination of Serum Very Low Density Lipoprotein (S.VLDL) level:

Very low-density lipoprotein was estimated by using formula of friedwalds [10]:

VLDL-Ch = TG/5

Determination of Serum Urea level:

Enzymatic determination of urea level (urease –modified Berthelot eactionr)[11],

Determination of Serum Blood urea nitrogen (BUN) level:

The normal level of BUN is 7-20 mg/dl. [12]. Calculated by:

BUN (mg/dl) = urea (mg/dl) × 0.466

DNA extraction Kit:

The ReliaPrep™ gDNA Miniprep System (Promega, USA) provides a fast, simple technique for preparation of purified and intact DNA from mammalian. Samples are processed using a binding column in a micro centrifuge tube. The genomic DNA isolated is of high-quality and can be used in common applications such as Agarose gel analysis, restriction enzyme digestion and PCR analysis. [13].

Determination of DNA Concentration and Purity by Spectrophotometer:

Five micro liters of each sample were added to 495 µl of Distilled water and mixed well to determine the DNA concentration and its purity by using spectrophotometer at optical density 260 nm and 280 nm

The concentration of DNA was calculated according to the formula:

DNA concentration (µg/ml) =O.D 260 nm x 50 x Dilution factor

The purity ratio of DNA was also estimated using this formula:

DNA purity ratio =O.D 260/ O.D 280

This ratio was used to detect DNA contamination in protein preparation. [3]

Determination of S1-Nuclease:

Using S1-Nuclease Kit (Promega, USA Company) The enzyme is used to remove single-stranded from double-stranded DNA, for selective cleavage of single-stranded DNA and for mapping RNA transcripts. S1-nuclease is active at low PH and required Zinc. [3].

Determination of Comet Assay:

Using (R&D Company, USA) The Comet Assay provides a simple and effective method for evaluating DNA damage to cells by combining the comet assay (also called single cell gel electrophoresis) [(14)].

Statistical Analysis

Results are expressed as Mean±SD. and significant differences between means were assessed by student t-test using the available statistical software packages (Microsoft Excel XP), statistical significance was set at $P<0.05$.

Results and Discussions:

Data demonstrated by Table-1 Fasting blood glucose, urea blood urea nitrogen was found to be significantly ($P \leq 0.05$) in DM patients with and without complications compared to control groups.

Data demonstrated in lipid profile levels that were significantly ($P \leq 0.05$) in DM patients with nephropathy and CV complications when compared with controls .while in DM without complications was showed no significant difference when compared with that of control Table (1).

Decreases in ß-cell function were modeled by changing the ß-cell response to plasma glucose concentrations. Insulin sensitivity was modeled by proportionately decreasing the effect of plasma insulin concentrations at both the liver and the periphery [(15)]

Also many factors play a role in the accelerated atherosclerosis observed in diabetes, lipoprotein abnormalities are key contributors [(16)]

Urea is a product of protein breakdown. [(17)] A test can be done to measure kidney disease, these substances (are not excreted normally, and so they accumulate in the body thus causing an increase in blood level. [(18)]

The *Blood urea nitrogen* test is one of the most commonly ordered tests to assess kidney function. The *Blood urea nitrogen* can also measure the amount of *urea nitrogen* found in the *blood.* Blood urea nitrogen (BUN) varies inversely with the rate of excretion of urea. [(219)] *Blood urea nitrogen* levels are elevated in Renal nephritis, renal failure (tubular necrosis), urinary tract obstruction. [(19)]

Electrophoresis pattern of native and glycosylated DNA was studied on a 1% Agarose gel. In Figures (1) to (4), it has been shown that the band of native DNA was found to be more distinct and intense as compared to that of diabetic groups.[(20)].

In this study, generation of single strand breaks has been confirmed by nuclease S1 digestion studies. The results showed substantial digestion of modified DNA by nuclease S1, while native DNA remained unaffected. Figures (5), (6). That genomic distribution of these lesions was investigated by DNA cleavage with the specific S1 nuclease. [(21)]

Table (1): Mean value of parameter levels in DM and control groups

Mean±SD	Control	DM without Comp.	DM with Neph	DM with CV
Valid N	20	20	20	20
FBG (mg/dl)	88.63±8.47	229.05±91.63	231.1±97.84	241.1±105.51
Cholesterol (mg/dl)	154 ± 30.3	157.05±28.4	182.9±45.6	238.5±44.9
Triglyceride (mg/dl)	96.88±21.3	106±41.5	142.6±58.2	198±102.8
HDL (mg/dl)	51.62±2.95	50.5±5.6	45.68±6.07	40.7±5.68
LDL (mg/dl)	77.29±24.72	82.6±28.3	100.7±50.7	153.7 ±44.3
VLDL (mg/dl)	20±5.09	24.8±15.2	28.15±11.8	44.50±7.86
urea (mg/dl)	23.25±4.03	26.25±3.84	41.9±6.07	26.6±2.92
BUN (mg/dl)	10.8±1.92	12.3±1.75	19.3±2.82	12.2±1.29

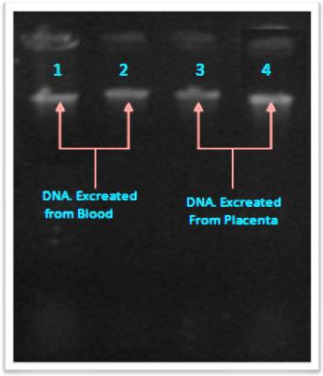

Fig (1): Agarose Gel Electrophoresis of DNA in Placenta and control groups

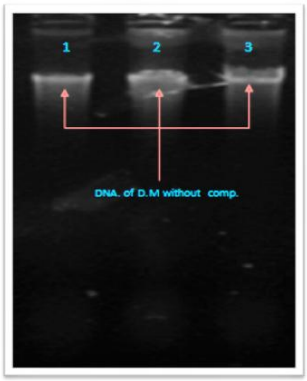

Fig (2) : Agarose Gel Electrophoresis in DNA of DM without comp. groups

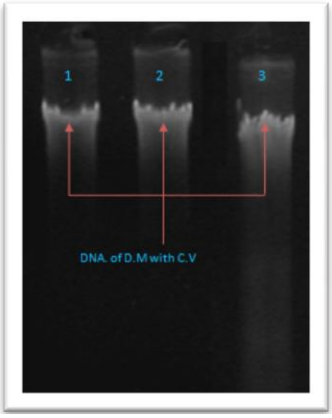

Fig (3): Agarose Gel Electrophoresis in DNA of D.M with CV groups

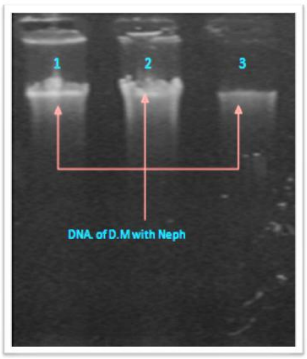

Fig (4): Agarose Gel Electrophoresis of D.M with Neph groups

Fig (5): Agarose Gel Electrophoresis of S1 Nuclease digestibility of DNA.excreted from blood and placenta groups

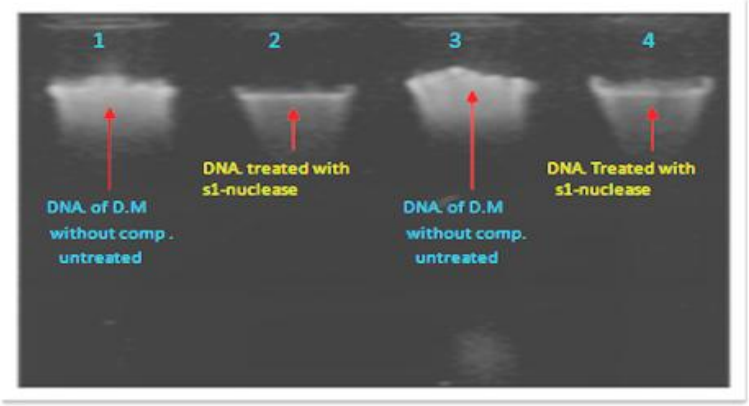

Fig (6): Agarose Gel Electrophoresis of S1 Nuclease digestibility of D.M with and without comp. groups

Table (2): Tail Parameters in Placenta, control and **D.M groups**

Parameters (Tail)	Control (Blood)	Placenta	D.M without comp.	DM with Neph	DM with C.V
Tail length (px)	0.0	0.0	66	108	110
Tail Area(px)	9.0	5.0	4277	5043	5149
Tail Intensity(px)	1	0.6	80746	292340	302579
Tail Mean Intensity (px)	0.11	0.1	18.87	56.09	58.76
DNA in Tail %	0.00	0.00	19.41	44.51	47.52
Tail moment	0.00	0.00	12.810	50.671	52.282
Olive moment	0.00	0.00	11.685	35.830	38.842

Table (3): Head Parameters in Placenta, Control and D.M groups

Parameters (Head)	Control	Placenta	D.M without comp.	DM with Neph	DM with C.V
Head Diameter (px)	120	133	100	98	92
Head Area (px)	9087	1076	6548	3406	2168
Head Intensity (px)	523490	56341	335254	336751	334028
Head Mean Intensity (px)	57.60	58.46	51.19	31.76	27.45
DNA in Head %	99.99	104.80	80.58	66.98	52.47

Table (4): Comet Parameters in Placenta, D.M and control groups

Parameters (Comet)	Control	Placenta	D.M without comp.	DM with Neph	DM with C.V
Comet Length (px)	120	133	158	186	210
Comet Height (px)	100	105	97	93	91
Comet Area (px)	9096	8154	10825	15705	17317
Comet Intensity (px)	523491	544620	416000	35806	336607
Comet Mean Intensity(px)	57.55	59.46	38.42	35.92	36.76

Fig (7): Comet image of peripheral blood lymphocytes in healthy control groups

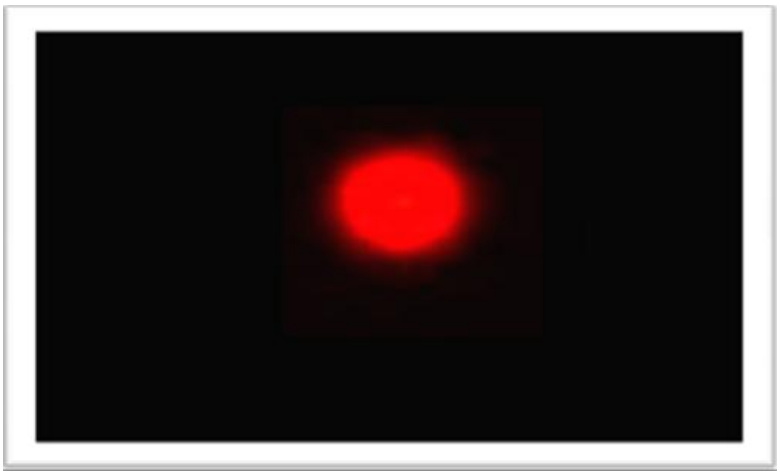

Fig (8): Comet image of peripheral blood lymphocytes in Placenta

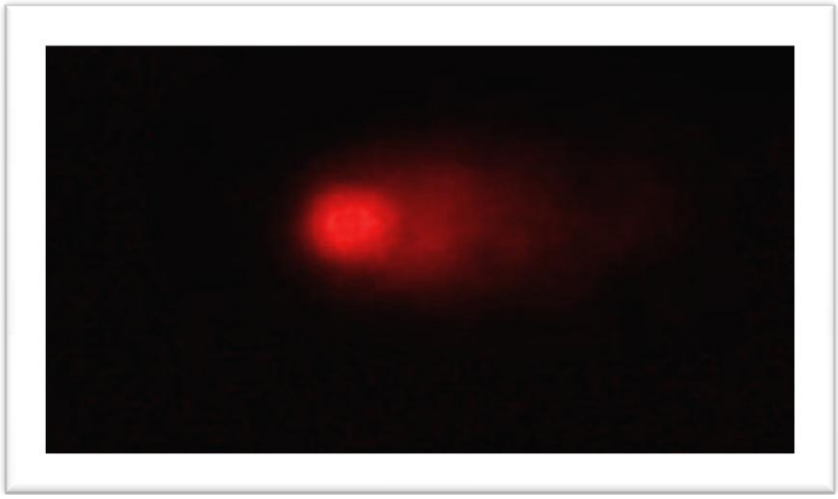

Fig (9): Comet image of peripheral blood lymphocytes in D.M without comp. groups

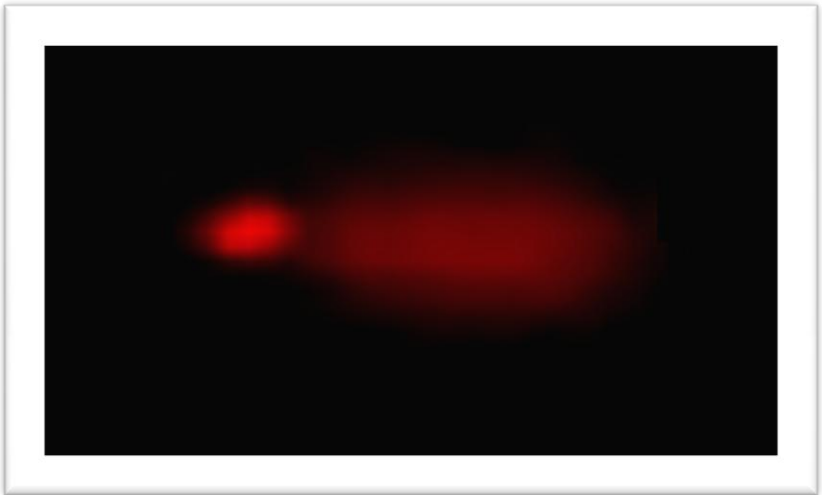

Fig (10): Comet image of peripheral blood lymphocytes in D.M with CV groups

Fig (11): Comet image of peripheral blood lymphocytes in D.M with Neph groups

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