Evaluation the phagocytosis activity in patients with type II diabetes mellitus

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Abstract

The study included 60 patients suffering from T2DM aged between 35 and 70 years and 30 healthy voluntaries as control attending Al-Hussain Teaching Hospital in Karbala province, from May to August 2013. The samples were arranged in two groups of fasting blood sugar (FBS) (200-400mg/dl) and (140-200mg/dl), group1, group2 respectively and group3 represent 30 apparently healthy individuals ages ranged between 35 -70 years.

The results appear significantly decrease in the percent of phagocytosis and killing index after 1.5 h and 24 h of incubation periods with bacteria in T2DM as compared with control.

Extracellular and intracellular superoxide dismutase (SOD), nitric oxide (NO) and malondialdehyde (MDA) was determined and the results were shown elevated extracellular free radicals , while the reverse occurs in the case of intracellular SOD and NO which shows a decrease in T2DM patients in compared with control.

C - reactive protein(CRP) was estimated and the results were shown positive relationship between FBS and CRP concentration also the results show high concentration of this component in T2DM patients than in control.

كفاءة فعالية عملية البلعمة في مرضى داء السكري النوع الثاني

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الخلاصة

شملت الدراسة 60 مريضا يعانون من مرض السكري النوع الثاني T2DM الذين تتراوح أعمارهم بين 35 و70 عاما و 30 فردا اصحاء (السيطرة) المترددين على مستشفى الحسين التعليمي في محافظة كربلاء، من مايو إلى أغسطس2013 . رتبت العينات في مجموعتين ، نسبة السكر لديهم (200-400 ملغم/ديسيلتر) ، (140-200 ملغم/ديسيلتر) على التوالي اما المجموعة الثالثة فشملت 30 فردا اصحاء ظاهريا تتراوح أعمارهم بين 35 -70 سنة. اظهرت النتائج انخفاض معنوي في النسبة المئوية لعملية البلعمة ومعدل القتل بعد فترة حضن ساعة ونصف و24 ساعة في مرضى السكري بالمقارنة مع السيطرة.

كما تم تقدير مستوى السوبر اوكسايد دسميوتيزSOD، وأوكسيد النيتريك (NO)خارج وداخل خلوي والمالوندايلديهاد (MDA). اظهرت النتائج ارتفاعا في مستوى الجذور الحرة خارج خلوية، في حين كان العكس يحدث في مستوى تلك العناصر داخل الخلايا حيث ظهر انخفاظا في المرضى مقارنة بالاصحاء.

تم تقدير بروتين الطور الحاد حيث اظهرت النتائج تراكيز عالية غير معنوية من هذا العنصر في المرضى مقارنة مع السيطرة. بالإضافة الى ذلك كانت هناك علاقة إيجابية بين تركيز كل من السكر الصيامي FBS وبروتين الطور الحاد.

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia (1). Which recognized more than 2000 years ago and is characterized by chronic hyperglycemia which is due to deficiency of insulin effect and results in abnormal metabolism of carbohydrate, protein and fat (2)? There are two main types of diabetes, type I insulin dependent diabetes mellitus (IDDM), which is characterized by pancreatic β-cell destruction mediated by immune mechanisms, severe insulinopenia and dependence on exogenous insulin to preserve life (3).

Whereas type II non-insulin dependent diabetes mellitus (NIDDM) , is caused by insulin resistance in the liver and skeletal muscle, increased glucose production in the liver, over production of free fatty acids by fat cells and relative insulin deficiency(4). Other type of diabetes could be latent, gestational, or secondary due to other diseases, on the other hand not all diabetic patients are at equal risk for complications (3). Type 2 diabetes which accounts for ~90–95% of those with diabetes, previously referred to as non–insulin dependent diabetes , type 2 diabetes, or adult-onset diabetes, include individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency and these individuals do not need insulin treatment to survive(5).

This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes (6). The immune system can be divided into innate and adaptive humoral systems. In innate immunity, neutrophils and monocytes play a crucial role in host defence by phagocytosing and killing invading microorganisms (7).

In diabetic patients, defects in the microbicidal function of neutrophils and monocytes are a major contributory factor for the development of bacterial infection and an increased rate of morbidity and mortality(8). The observed changes in neutrophils include impairment of the following: adhesion to endothelium and migration to the site of inflammation, chemotaxis, bactericidal activity , phagocytosis ,and production of reactive oxygen species (ROS) (9) .

A similar profile has been observed in monocytes from diabetic patients with impaired chemotaxis and phagocytosis(10) Decreased bactericidal activity, impairment of phagocytosis and decreased release of lysosomal enzymes ,and reduced production of reactive oxygen species by neutrophils of diabetic patients have been described. Furthermore, reduction in leukocyte phagocytosis and bactericidal activity showed a significant correlation with increases in blood glucose levels. (11).

This generation of oxygen free radicals during cellular metabolism and due to certain environmental factors, including lifestyle, seems to play a critical role in the pathogenesis of type 2 diabetes mellitus. Free radicals are very reactive chemical species, can cause oxidation injury to the living beings by attacking the macromolecules like lipids, carbohydrates, proteins and nucleic acids. (12).

Proinflammatory cytokines and acute-phase reactants are correlated with clinical features of the metabolic syndrome, including measures of insulin resistance, body mass index (BMI), and circulating triglyceride and high density lipoprotein (HDL) cholesterol concentration(13).

The aim of study was to evaluate the effect of high sugar concentration in blood on phagocytic activities ; and also its relation with oxidative stress in patients with type II diabetes mellitus.

Materials and methods

The study included 60 patients suffering from T2DM aged between 35 and 70 years and 30 healthy voluntaries as control attending Al-Hussain Teaching Hospital in Kerbala province, from May to August 2013. Venous blood samples (5ml) obtained from all patients and controls after overnight fasting.

 2ml was collected in EDTA tube and the remaining 3ml collected in plain tubes for serum collection.

Determination of fasting blood sugar:

Fasting blood concentrations were measured depending on enzymatic colorimetric method (14)**.**

Phagocytosis Estimation:

1ml of blood was incubated with 1ml of 10^5 from *Staphylococcus epidermis* suspension for 1.5 h and 24 h (15) .

After periods of incubation smears of blood was prepared as below :

1- Drop of incubation blood was placed on a slide and pulled by another slide to get a thin smear.
2- The smear was lefted to dry for 3 minutes , then fixed by absolute ethanol.
3- The fixed smear was placed in hematoxylen stain for 10 minutes, the slide was washed with water and then placed in eosin stain for 30 second ,after that the slid was washed and became ready for examine under light microscope for phagocytosis estimation.

Estimation of SOD, NO, MDA

Super oxide dismutase (SOD) and nitric oxide NO was determined according to (16), (17) respectively.

Lipid peroxidation was measured as the amount of malondialdihyde (MDA) produced by the thiobarbituric acid (TBA) reaction, as described by (18).

Detection of C-Reactive protein

Quantitative determination of C-Reactive protein (CRP) by Vital diagnostics kit.

Statistical analysis

Data were analyzed by using the Sas 9.1(2012) for windows . Duncan multiple range test and Pearson correlation was used to detect the significant differences. The method which was used to measure the significances at level 0.05 was taken from . Results expressed as mean ± Standard deviation (SD).

Results and Discussion

The means of phagocytosis(P%) and killing index(KI) of phagocytic cells were assayed after 1.5 h & 24 h of incubation with bacterial suspension, these index were(62% , 2.1 , 71% , 3.8) in group 1, but in group2(69%, 2.6, 77%, 5.2) as compared with control (82%, 4.1, 88%,7.5) respectively as shown in table (1-1).

Table (1-1) Effect of FBS on percent of phagocytosis and killing index in type 2 diabetic .

| Group	FBS Mean ± SD	P% 1.5 h Mean ±SD	Killing index 1.5 h Mean ± SD	P% 24 h Mean ± SD	Killing index 24 h Mean ± SD
Patient FBS (200-400)	266.5± 62.65 A 1.000	62% ± 0.06 B -0.64	2.1 ± 0.54 B -0.42	71% ± 0.038 A -0.52	3.8 ± 0.38 **C** -0.65
Patient FBS (140-200)	159.2 ± 15.9 B 1.000	69% ± 0.44 B -0.47	2.6 ± 0.61 B -0.40	77% ± 0.21 A -0.40	5.2 ± 0.044 B -0.35
Control	96.8 ± 19.7 C 1.000	82% ± 0.24 A -0.2	4.1 ± 0.76 A -0.03	88% ± 0.032 B -0.13	7.5 ± 0.87 A 0.14-
P-value at 0.05	0.0001	0.0005	N.S	0.0031	0.0001

* Values expressed as mean ± standard deviation of mean and Pearson correlation.

*** Means with the same letter in column are not significantly different.**

In addition as shown in table above there is negative correlation between FBS and both P% and KI after1.5h and 24 h in group1(-0.64, -0.42, -0.52, -0.65) , group2(-0.47, -0.40, -0.40, -0.35) and control(-0.2, -0.03, -0.13, -0.14) respectively.

The results also revealed clearly that the level of FBS were affected largely on the phagocytosis process of phagocytic cells (neutrophil & monocyte)after both incubation times and these process were decline with elevated of FBS .

The phagocytosis process of neutrophil cells in both group1 and group 2 of T2DM patients after two incubation periods was less than in control as shown in figure (1-1 a,b) & (1-2 a,b) & (1-3 a,b) respectively.
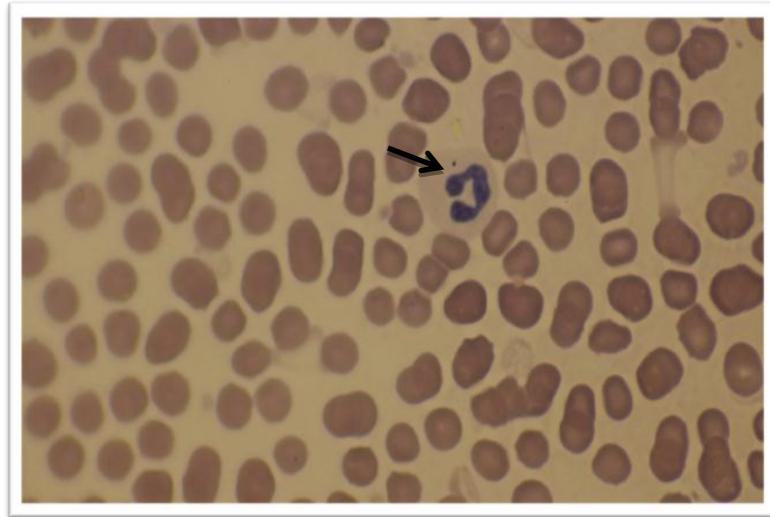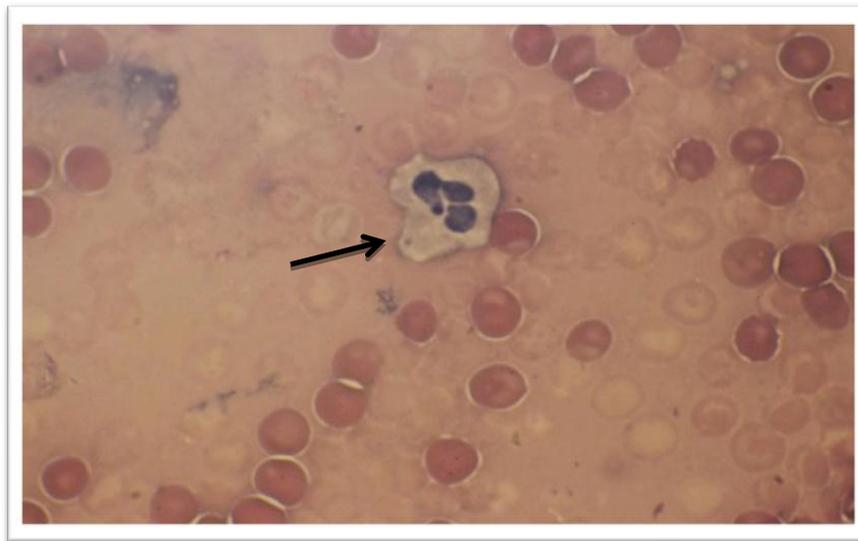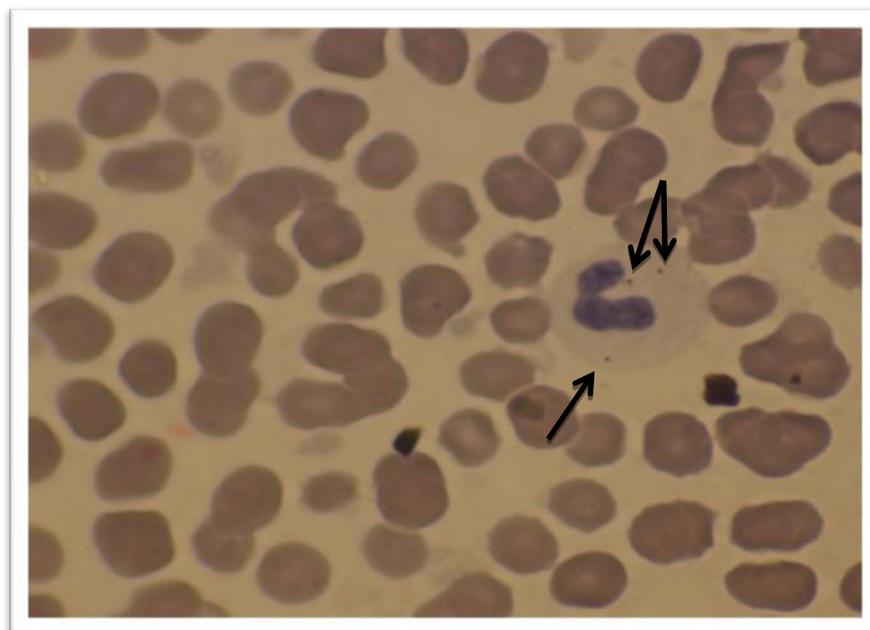
(a)
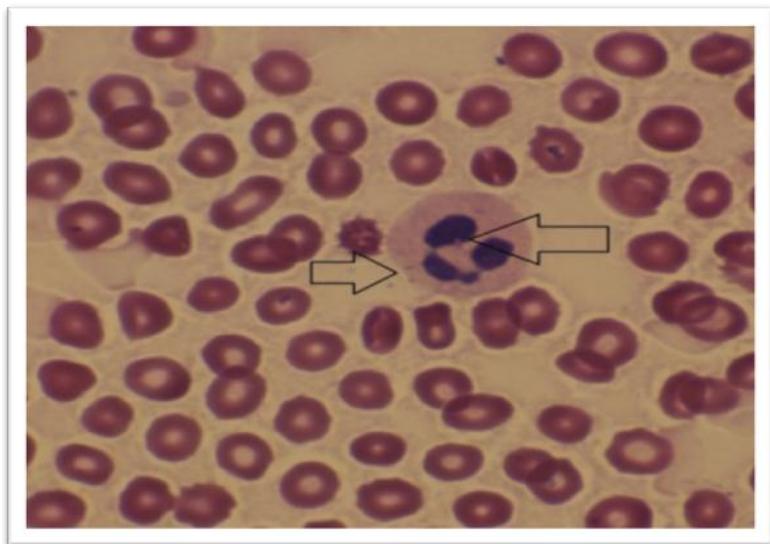
(b)

Figure (1-1) phagocytosis by neutrophil in group1(a)after 1.5 h of incubation with bacteria. (b) after 24 h of incubation with bacteria (under 1000x).

(a)

(b)

Figure (1-2) phagocytosis by neutrophil in group2(a)after 1.5 h of incubation with bacteria. (b) after 24 h of incubation with bacteria (under 1000x)
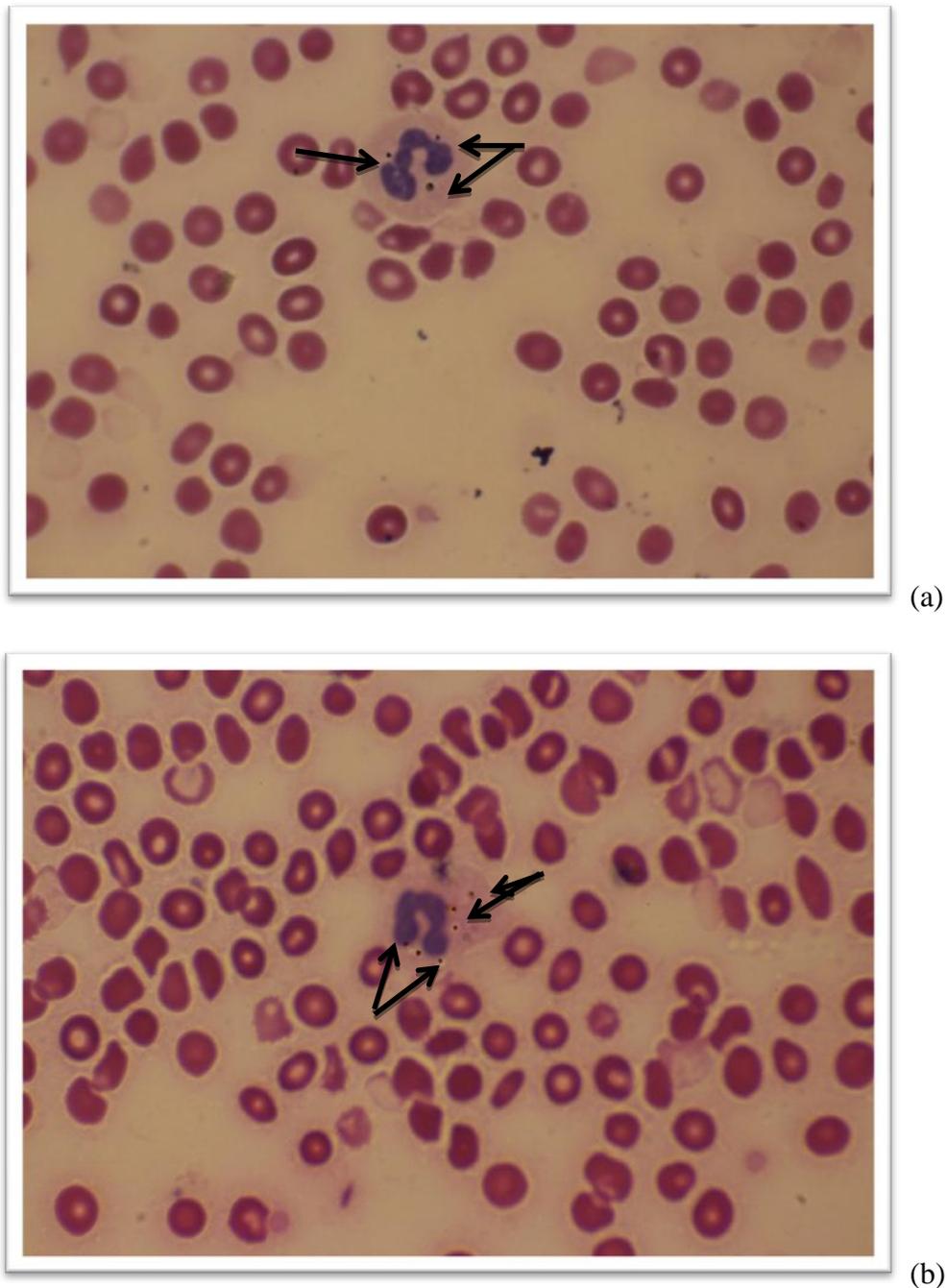
(a)

(b)

Figure (1-3) phagocytosis by neutrophil in control(a)after 1.5 h of incubation with bacteria. (b) after 24 h of incubation with bacteria (under 1000x).

The results in this study which appeared decreasing percent of phagocytosis in patient than control. The depresses phagocytosis and cell viability due to DM lead to oxidative stress which affects the neutrophils functions, causes lipid peroxidation and alters the structure of cell membrane lipids(19)(20).

In addition , phagocytosis and superoxide production are important for neutrophils to kill bacteria. These neutrophil functions require ATP-involved energy, which is produced mainly by the metabolism of glucose to lactate. Since neutrophils from diabetic hosts represent impaired glucose metabolism, excessive glucose level exerted inhibitory effect on superoxide production by neutrophils. Activation of nicotinamide adenine dinucleotide phosphate NADP (NADPH) oxidase is closely related to superoxide production by neutrophils. In the presence of excessive glucose levels, the hexokinase pathway (glucose is converted to glucose-6-phosphatase) is saturated and glucose is converted to sorbitol using the polyol pathway in neutrophils. Activation of the polyol pathway decreases the availability of NADPH, leading to reductions of NADPH oxidase activity and superoxide production (21).

NADPH oxidase generates superoxide by transferring electrons from NADPH inside the cell across the membrane and coupling these to molecular oxygen to produce superoxide anion, a reactive free-radical. Superoxide can be produced in phagosomes, which contain ingested bacteria and fungi, or it can be produced outside of the cell. In a phagosome, superoxide can spontaneously form hydrogen peroxide that will undergo further reactions to generate reactive oxygen species (ROS) (22). Therefore the reductions of NADPH oxidase activity and superoxide production in diabetic patients may lead to decrease phagocytic and killing activities by neutrophil .

In some instance when the bacterial cells invasion the phagocytic cells may be causes death it(apoptosis)as shown in figure (1-4a,b).

The invasion of bacterial cells to phagocytic cells usually causes defect in phagocytic cell activity and impaired the clearance of neutrophils and their toxic substance by macrophage that lead to deleterious death cellto surrounding tissue neutrophil uptake by macrophages and their subsequent degradation is an important means of limiting tissue injury associated with inflammation that usually occur in health human (1).
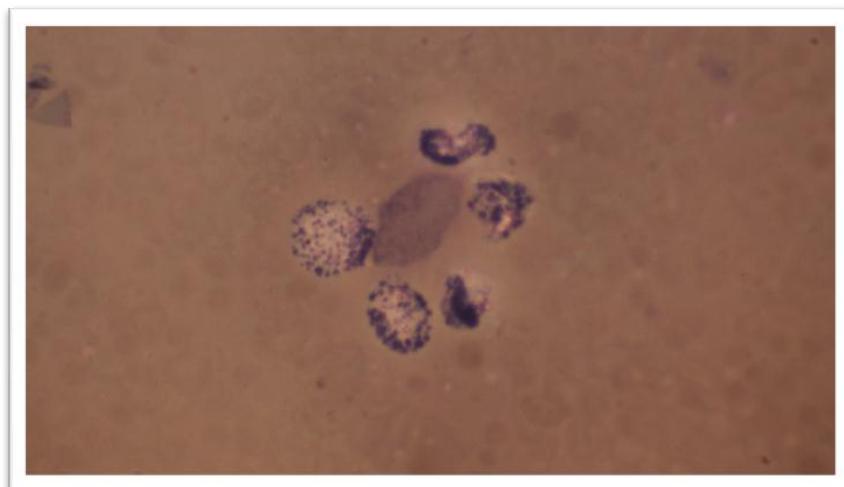
(a)
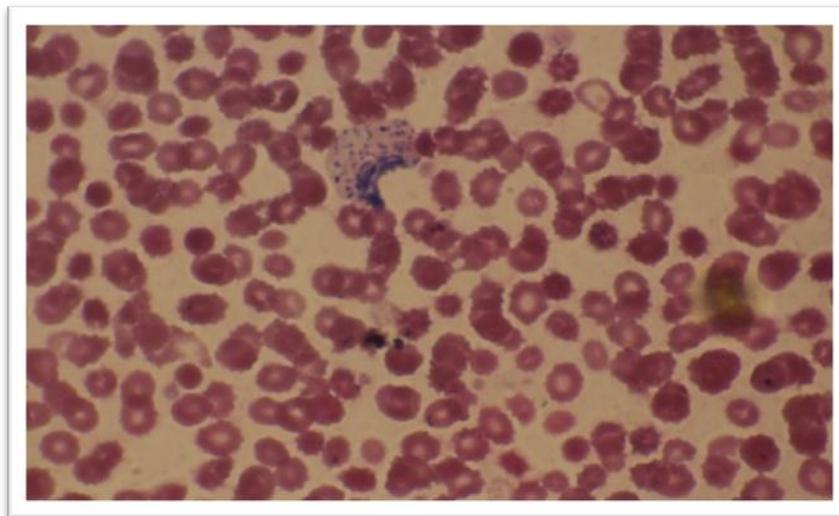
(b)

Figure (1-4) Explain apoptosis in phagocytic cells(a) monocyte, (b) neutrophil after 24 h incubation with bacteria in group1(under 1000x).

Fasting blood sugar (FBS) and oxidative stress and CRP in type 2 diabetic patients

According to the results appear in table 1-2 there were increase in mean concentration of extracelullar superoxide dismutase (Ex.SOD) , nitric oxide (Ex.NO) and malonaldyhide (MDA) in group 1 (0.019, 0.44, 10.6) and group2 (0.012, 0.34, 9.2) respectively as compared with control(0.011, 0.33, 6.8), but decrease in mean concentration Intracelullar SOD and NO mean concentration in group1 (0.017 ,0.055 mmol/L), group2 (0.02, 0.024 mmol/L) as compared with control (0.021, 0.080 mmol/L).

The results also show positive correlation between the level of FBS and the concentration of (Ex.SOD, NO and MDA) in group1(0.29, 0.07, 0.25), group2(0.09,0.02, 0.09) as compared with control(0.06, 0.03, 0.01).

The results also shown the relationship between hyperglycemia and oxygen free radicals so the increase in blood glucose level lead to elevated level of extracellular SOD, NO, and MDA in T2DM patients than control group. MDA were higher in diabetics patients compared to controls. Increased levels of lipid peroxidation may cause oxidative injury to blood cells. Elevated levels of NO promote the peroxidation of the lipid moiety and induce immune responses and inflammatory reactions that cause cell damage. Furthermore, the correlation between endothelial dysfunction and oxidative stress in patients with type 2 diabetes support the hypothesis that the impairment of intracellular antioxidant system and endothelial dysfunction are frequently associated in diabetes mellitus. (22).

Hyperglycemia may lead to elevation of free radical included reactive oxygen species and reactive nitrogen species which cause the increase in lipid peroxidation which expressed as MDA(23).

While the opposite occur in the case of intracellular SOD & NO which was negatively correlate with FBS in group1(-0.11, -0.03) , group2 (-0.27, -0.06) as compared with control (-0.30, -0.10).

The results also revealed decreasing in the level of free radical in patients when compared with the control this indicated the impairment of neutrophil function in both phagocytosis and killing the pathogen. When intracellular killing capacity of the neutrophils was assayed, it was seen that the diabetic neutrophil had a definite low intracellular microbicidal function(19).

Also this table shows positive correlation of CRP with the level of FBS in group1(0.21) , group 2 (0.16)and control (0.08) .

The level of CRP was increased in T2DM patients as compared to control, the elevated levels of CRP refer to the development of type 2 diabetes, supporting a possible role for inflammation in diabetogenesis. Oxidative stress might be implicated in promoting a state of low-grade inflammation indicated by markers such as CRP with type 2 diabetes(24)(25).

Table 1-2 Fasting blood sugar and oxidative stress in type 2 diabetic patients .

Group	FBS Mean ± SD mg/dl	Ex-SOD Mean ± SD	In-SOD Mean ± SD	Ex-Nitric Oxide Mean ± SD mmol/L	In-Nitric oxide Mean ± SD	MDA Mean ± SD mmol /L	CRP Mean ± SD <6 mg/dl
Patient FBS (200-400)	266.± 62.6 A 1.000	0.019 ± 0.008 A 0.29	0.017 ± 0.02 A -0.11	0.44 ± 0.15 A 0.07	0.055 ± 0.10 A -0.03	10.6 ± 6.1 A 0.25	6.45 ± 2.16 A 0.21
Patient FBS (140-200)	159.± 15.9B 1.000	0.021 ± 0.03 A 0.09	0.020 ± 0.02 A -0.27	0.34± 0.12 A 0.02	0.024± 0.024 A -0.06	9.2± 10.3 A 0.09	5.9 ± 2.3AB 0.16
Control	96.8± 19.7 B 1.000	0.011± 0.007 A 0.06	0.021± 0.02 A -0.30	0.33± 0.17 A 0.01	0.08 ± 0.08 A -0.1	6.8 ± 4.5 A 0.03	4.5 ± 1.4 B 0.08
P-value at 0.05	0.0001	N.S	N.S	N.S	N.S	N.S	N.S

* Values expressed as mean ± standard deviation of mean andPearson correlation.

* Means with the same letter in column are not significantly different.

Conclusion: There is an immune suppression in the innate immune response during study the efficiency of phagocytosis in patients with type II diabetes. Impairment in neutrophil function to kill the pathogen that depends on producing intracelullar SOD and NO.

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