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## Controlled cell deletion of peripheral blood lymphocytes in type 2 diabetes mellitus

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### Abstract

**Background:** Controlled cell deletion (apoptosis) and cellular proliferation are the main mechanisms that are responsible for shaping tissues and organs in developing embryos. During adult life, apoptosis serves a protective mechanism which eliminates old, useless, and damaged cells. In healthy organisms apoptosis and cell proliferation are in balance.

**Aim of the study:** To detect apoptosis of peripheral blood lymphocytes in type 2 diabetes mellitus patients and its relation with the percentage of glycohemoglobin and the effect on the outcome of the disease.

**Method:** This study was carried out on 40 volunteers, twenty of them (9 males and 11 females) were patients with type 2 diabetes and twenty (5 males and 15 females) were normal healthy subjects and was served as controls of the study. From each patient a sample of blood was aspirated for detection of peripheral blood lymphocyte apoptosis and for the measurement of the percentage of glycated hemoglobin (HbA1c). For the control subjects, blood samples were aspirated to detect peripheral blood lymphocyte apoptosis.

**Results:** The percentage of peripheral blood lymphocytes apoptosis increased significantly ( $P<0.0001$ ) in diabetic patient ( $15.3\pm 50\%$ ) than the percentage in healthy subjects ( $4.9\pm 1.6\%$ ). There was a strong positive correlation between the peripheral blood lymphocytes apoptosis percentage and the percentage of glycated hemoglobin ( $r=0.56$ ,  $p<0.01$ ).

**Conclusion:** High blood glucose and in turn increasing blood level of glycated hemoglobin in poorly controlled type 2 diabetes mellitus disease is associated with increased rate of apoptosis of peripheral blood lymphocytes which can be considered as a marker of severity for this disease.

**Keywords:** lymphocyte apoptosis, glycated hemoglobin, diabetes mellitus

### Introduction

Apoptosis is also called cell suicide, differs from necrosis in the level of control of the process. Apoptosis is a controlled and regulated process and involves individual cells. Necrosis is an uncontrolled process of cell lysis leading to inflammation and destruction of tissue areas or even whole organs, which can cause serious health problems. Apoptosis and proliferation are responsible for shaping organs in developing embryos. During adult life, apoptosis is a protective mechanism which eliminates old and damaged cells. In healthy

Organism's apoptosis and cell proliferation are in balance. In diseases such as cancer there is an imbalance whereby cells have undergone certain mutations that prevent them from undergoing apoptosis <sup>[1]</sup>. There are several mechanisms inducing cellular apoptosis. There are extrinsic signals such as the binding of death inducing ligands to death receptors. Some of these ligands are expressed on the surface of cytotoxic T lymphocytes, for example, induction following cellular stress as that caused by oxidative stress through free radicals, deprivation of growth factor, or exposure to radiation <sup>[2]</sup>. Type 2 diabetes was often called non-insulin dependent diabetes; is the most common form of diabetes, affecting 90% - 95% of the people with diabetes. Glycated hemoglobin (HbA1c) is used to identify the average plasma glucose concentration over prolonged period of time. As the average amount of plasma glucose increases, the glucose stays attached to hemoglobin for the life of the red blood cell (normally about 120 days) and the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels, over the previous months prior to the measurement <sup>[3]</sup>.

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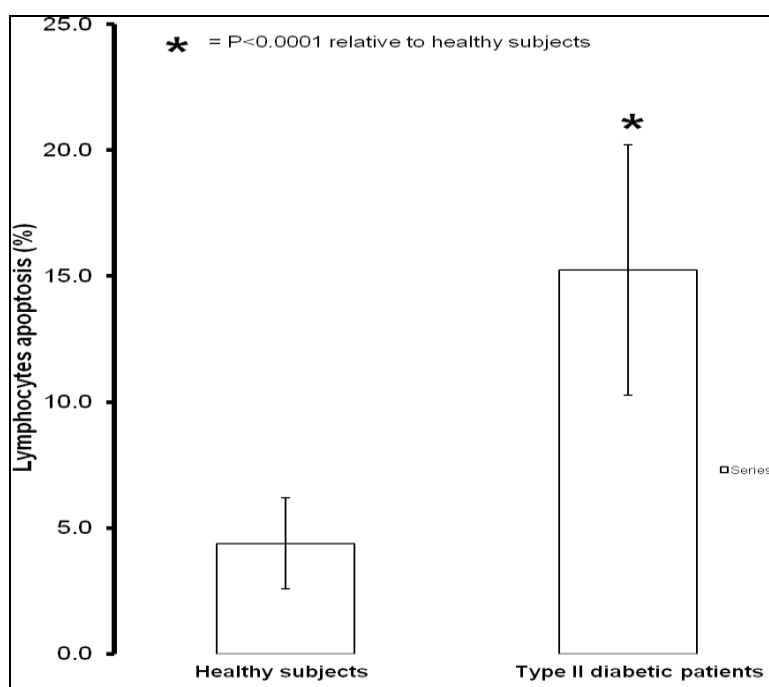
## Subjects and Methods

This study was a cross sectional study, carried out in the Physiology Department laboratory in Al-Mustansiriya College of medicine during the period from October 2010 to September 2011. It included 20 patients (9 males and 11 females) who were known cases of type 2 DM and 20 subjects (5 males and 15 females) who were normal healthy subjects depending on their past medical history and routine checkup investigations including blood sugar level testing. Four milliliters of venous blood were aspirated from each patient; the sample was anticoagulated and then divided into two aliquots: First aliquot (2 ml) was processed for peripheral blood lymphocytes (PBL) separation to count total and apoptotic lymphocytes. The second aliquot (2 ml) was used for detection of glycated hemoglobin level by HbA1c detection kit and was measured by

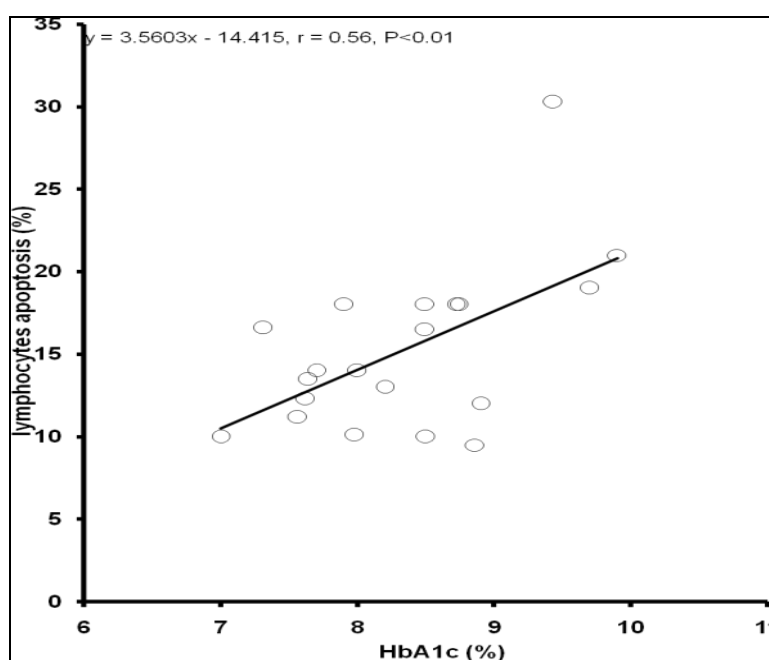
spectrophotometer [4]. For normal healthy subjects, only 2 ml of venous blood were aspirated and processed for PBL separation to count total and apoptotic lymphocytes. PBL separation was done according to the method of Goldrosen [5]. Total and apoptotic lymphocytes count was done according to Doyle and Griffiths [6] using Trypan blue exclusion test and expressed as cell/mm<sup>3</sup> by the haemocytometer counting chamber. The principle of this test is that the viable cells exclude Trypan blue dye (i.e. does not stained); while the dead cells accept the dye (stained blue).

## Statistical analysis

The data were analyzed by Microsoft Excel program and were presented as mean  $\pm$  standard deviation.  $P < 0.05$  was taken to indicate significant differences.



**Fig 1:** Peripheral blood lymphocyte apoptosis (%) in healthy subjects (n=10) and type II diabetic patients (n=20).



**Fig 2:** Correlation between HbA1c (%) and peripheral blood lymphocytes apoptosis (%) in patients with type II diabetes mellitus (n=20).

## Results

The percentage of peripheral blood lymphocytes apoptosis ( $15.3 \pm 5\%$ ) was higher significantly ( $P < 0.0001$ ) as compared to the percentage in healthy subjects ( $4.9 \pm 1.6\%$ ) as detected by trypan blue exclusion test (Fig 1). In addition, there was a strong positive correlation between the peripheral blood lymphocytes apoptosis percentage and the percentage of glycated

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