



Determination of the alkaline phosphatase (ALP) in diabetic patients.

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ABSTRACT

Diabetes is a disease known since ancient times. The Greek physician Aretaeus noticed in early 200 BC that some patients had symptoms of frequent urination and extreme thirst, and he called this phenomenon "diabetes." In 1675 AD, the scientist Thomas Willis added the word Mellitus, which in Latin means "sweet as honey", after he noticed that the blood and urine of diabetics had a sweet taste, so the name of this disease became "Diabetes Mellitus" or diabetes. The researcher used the descriptive methodology, he got to me the most important conclusions and recommendations in the research:

Conclusions:-

- 1- During our examination of the blood serum of diabetic patients, it was found that the level of phosphatase activity level increased
- 2- The basal rate in the blood of infected patients is higher than that of the healthy ones.
- 3- It was found that the percentage of infected females is higher than males.

Where the symptoms were prominent on the injured through the following reasons:

A- Obesity.

b Exposure to psychological trauma.

C-heredity.

D-Deep thinking.

E - Decreased secretion of insulin by the pancreas

Recommendations:-

- 1- The injured must do sports.
- 2- Quit smoking.
- 3- Perform a liver test
- 4- Maintaining the normal level of glucose in the blood.
- 5- Regulating food quantity and quality
- 6.Helping to lose weight and get rid of obesity

Keywords:

D alkaline ,phosphatase (ALP) , diabetic , patients

Introduction:

Diabetes is one of the diseases known since ancient times. The Greek physician Aretaeus noticed in the early 200 BC that some patients show symptoms of frequent urination and extreme thirst, and he called this phenomenon "Diabetes". In 1675 AD, the scientist Thomas Willis added the word Mellitus, which means in Latin "sweet as honey," after noticing that the

blood and urine of diabetics had a sweet taste, so the name of this disease became "Diabetes Mellitus" or diabetes.

The two scientists, Joseph Von Mering and Oskar Minkowski in 1889 AD, discovered the role of the pancreas in diabetes mellitus. In 1910 AD, the scientist Schafer - Sir Edward Sharpey discovered that patients with diabetes suffer from a deficiency in one chemical

produced by the pancreas and he called it "insulin".

In the late 1920s, the scientist Bating and his colleagues from the University of Toronto, Canada, were able to isolate the hormone insulin for the first time from the pancreas of cows. This led to the availability of insulin injections, and they were first used on patients with diabetes in 1922

Diabetes is nowadays one of the most prevalent diseases in the world. ⁽¹⁾

Diabetes mellitus:

Diabetes mellitus is defined as a disorder in the sugar metabolism process that leads to an abnormally high level of sugar (glucose) in the blood for various reasons that may be

psychological, organic, due to excessive intake of sugars or due to genetic factors. It occurs as a result of a defect in the secretion of insulin from the pancreas. The amount of insulin that is secreted may be less than what is required or there may be a complete cessation of its production, and this case is called insulin deficiency, or the amount secreted is large in some cases, such as individuals with obesity, but there is resistance from tissues and cells in the body that impedes the function of insulin and this is called The condition is insulin resistance.

In both cases, glucose is unable to enter the cells, which leads to its accumulation in the blood and the possibility of It appears in urine ⁽²⁾.



Figure1 Diabetes

Glucose:

In addition to fructose and galactose, glucose is one of the monosaccharides with carbon atoms, but fructose and galactose quickly also convert in the body to glucose, which in turn is used as a main source of energy by many cells of the body such as brain cells and red and white blood cells. The normal concentration of glucose in the blood of a healthy person is in the range of 5 mmol/L (90 mg/dL), and this concentration can rise after a meal to reach 6 mmol/L, while for a diabetic patient, the concentration of glucose can rise to 20 mmol/liter. In normal conditions, the concentration of glucose in the blood after a

person has fasted for 12 hours should remain below the level of 100 mg/dL (5.55 mmol/L), and if it falls from 100 to less than 126 mg/dL, this is called a condition. Before diabetes (pre-diabetes), but if the concentration of glucose in the blood reaches or exceeds the level of 126 mg / offspring after fasting, the case is diagnosed as diabetes. ⁽³⁾

Glycogen:

Glycogen or glycogen is a multi-unit polymer, glucose constitutes the basic building unit in this molecule, which acts as an energy store in animals and fungi. Each glucose unit is linked

to the next unit by A-type bonds (1,4), while the branches consist of bonds (1,6).

When the level of glucose in the blood decreases, the process of breaking down glycogen into its basic units, such as glucose, begins. While the reverse process takes place to

convert glucose molecules into glycogen, when the level of glucose in the blood rises, insulin is the hormone responsible for the formation of glycogen in the human body, and the liver and muscles are the two organs responsible for storing it.

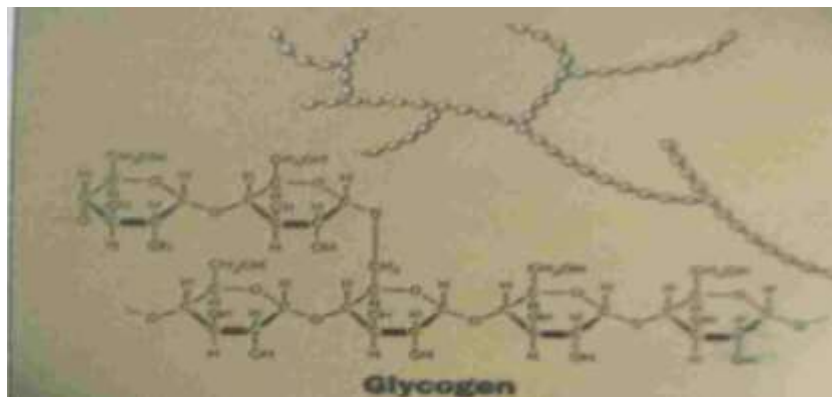


Figure2 Glycogen

The level of glucose in the blood is affected by a number of hormones that can be summarized as follows

1. Insulin?

Insulin is a hormone secreted by special cells in the pancreas called beta cells (i.e. cells No. 2), and they are among a group of cells spread in the form of islets within the pancreas, and

these islets are known as the "islets of Langerhans" in relation to their discoverer. The hormone insulin consists of two chains of amino acids linked by chemical bonds.

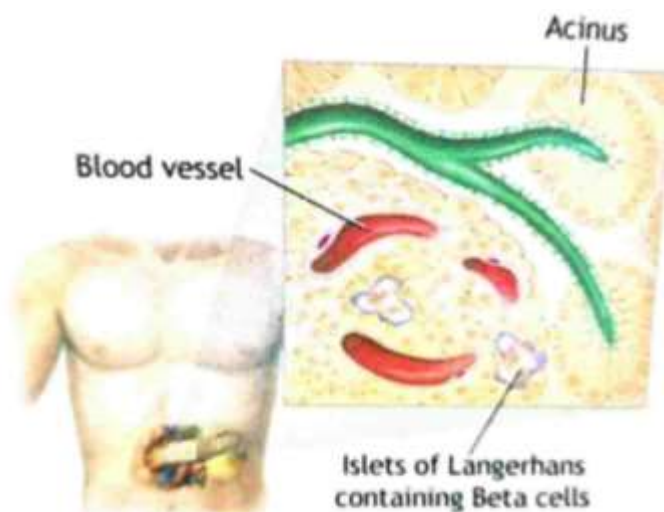


Figure3 Insulin

Insulin plays an important role in the chemical reactions inside most cells of the body, but its effect appears in the form of clear in:

Liver: Insulin stores sugar in excess of the body's need for energy generation in the liver

in the form of glycogen (animal starch); To reproduce sugar from it when needed. In the muscles, insulin stores excess sugar in the muscles in the form of animal starch. In fat cells, insulin helps store excess glucose in the

ability to store the liver and muscles in the form of fat in the fat cells in the body, and reduces the breakdown of these fats to take advantage of them when needed.

It is secreted by the bark of the adrenal gland in the adrenal cortex, and helps the process of gluconeogenesis from non-carbohydrate sources in a process known as gluconeogenesis.

Types of diabetes and its symptoms

1- The first type:

It is called Diabetes No. 1 and in the past this type was called Insulin Dependent Diabetes (IDDM). Mean It is used by diabetics who depend on insulin in their treatment.

Most people with type 1 diabetes are healthy and of normal weight when the disease occurs. It is characterized by lack of or severe insulin deficiency due to damage to most of the beta cells in the pancreas, which leads to a high level of sugar in the blood. Also, the symptoms of the disease may occur suddenly with labor, frequent urination, open appetite and weight loss, which take place within several days and increase the possibility of complications such as ketoacidosis and diabetic coma.

Before the discovery of insulin, the average lifespan of people diagnosed with type 1 diabetes was only two years. The development of insulin and its use in treatment has revolutionized the treatment of diabetes and transformed it from a rapidly killing disease into a chronic disease. With the extension of the life of patients who use insulin, long-term complications of diabetes began to appear on them, which include neuropathy, kidney failure, retinopathy, circulatory and heart diseases, which occur within 10-20 years from the beginning of the disease discovery.

The cause of the occurrence of the first type

1. A decrease in the efficiency of the body's immune system, which is accompanied by the emergence of antibodies that attack insulin proteins and Langerhans cells and cause damage to beta cells in the pancreas that secrete insulin.

2. Genetic factors

3. Viruses It may be a cause of type 1 diabetes. These viruses violently attack the beta cells of the pancreas secrete and destroy insulin.

2. The second type:

It is called diabetes mellitus No. 2 and in the past it was called non-insulin-dependent diabetes (NIDDM), which means diabetics who do not depend on insulin in their treatment. It was also called (adult sugar) because it usually begins after the age of forty. Symptoms of this disease appear gradually, and the chances of a diabetic coma and complications are less than the first type. This type of sugar is often discovered by chance when conducting routine medical tests. In this type, the pancreas secretes an amount of insulin, but it may be insufficient or there is resistance from tissues and cells in the body that impedes the function of insulin; Because of the lack of insulin receptors or the presence of antibodies to these receptors that prevent insulin and compete for it, which leads to a high blood sugar level;

In these patients, genetics and obesity play an important role in the occurrence of the disease.

Most patients are obese, especially people who have an increase in weight around the middle of the abdomen. Obesity stresses the pancreas. Serious complications can occur because the infection with this type of diabetes is not observed after several years, which include kidney failure and vascular disease (including vascular disease in the region).

This type represents the majority (90%) of diabetic patients. The patient with this type of diabetes responds mostly to the anti-diabetic tablets.

The cause of the second type

1. Obesity

It is estimated that 80% to 95% of the current increase in type 2 diabetes is due to obesity and increased abdominal fat, which may play an important role in insulin resistance. The increase in weight around the abdomen and the upper part of the body (the apple shape) is related to insulin resistance. To prevent the accumulation of fat in the middle of the abdomen, the factors that lead to this should be reduced, which include:

❖ alcoholic beverages;

- ❖ smoking.
- ❖ Daily stressors.
- ❖ Foods rich in fats or sugars.

2. Family history: About 25% to 33% of people with type 2 diabetes have family members sick
3. Low birth weight

Research has shown that low birth weight is a risk factor for type 2 infection Cause of sugar, and scientists attribute this to poor nutrition in pregnant women, which may lead to Impairment in metabolism in young children, and the possibility of developing diabetes ⁽⁵⁾.

The main differences between type 1 and type 2 diabete⁽⁶⁾

Properties	first type	second type
the age	Usually before the age of three	Usually after the age of forty
The appearance of symptoms	suddenly	gradually
physical condition	The patient is usually underweight or underweight	we called it
medical symptoms	Usually excessive thirst, strong desire to eat, frequent urination and weight loss	Often without symptoms
ketogenic acids	Often present	usually does not appear
Insulin inside the body	There is no	It exists, but the body is unable to use it
lipid abnormalities	Increased cholesterol and triglycerides in the blood	Increased cholesterol and triglycerides in the blood
Insulin therapy	wanted	Only required in 20-30% of patients
Pure medicine for diabetes	It should not be used	medically used
diet	Required with insulin	Required with or without medication

3. Type III: Secondary Diabetes

It occurs as a result of a pathological illness that affects the insulin-producing cells in the pancreas, the most important of which are:

4. Chronic inflammation of the pancreas.
5. Adenomas of the suprarenal gland (Pheochromocytoma).
6. Excision of the pancreas in case of cancerous tumors, for example.
7. Some endocrine diseases: such as acromegaly due to increased production of growth hormone, hyperthyroidism, as occurs in cases of thyrotoxicosis, and Cushing's Syndrome, which leads to an increase in cortisone levels.
- 8.As a result of taking some medications, such as thyroid hormone and cortisone

4 - Gestational Diabetes

Before the discovery of insulin and its successful treatment in diabetes, a diabetic woman was suffering from the disease's effect on ovarian function and fertility, and the frequency of miscarriage. Even if the pregnancy continued, this was accompanied by some complications and risks for the pregnant woman, such as:

- Significant increase in blood sugar level, especially in the last months of pregnancy.
- Increased risk of preeclampsia.
- Increased risk of urinary tract infections.
- Increased chance of eye complications.
- **General symptoms of diabetes**

- 1. Excessive thirst.
- 2. Increased frequency and amount of urination.
- 3. Increased appetite for food, especially sweets and sugars.
- 4. Dry throat and tongue.
- 5. In the first type of diabetes, a decrease in body weight can occur for no apparent reason.
- 6. The first type of diabetes can be accompanied by a feeling of laziness, weakness, lethargy and inability to tolerate muscular effort.
- 7 Bad vision, dizziness or headache and lack of focus.
- 8. Diabetic ketoacidosis may be the first indication of diabetes

- 6. Increased susceptibility to microbial infections.
- 10. Delayed healing of wounds and bruises when they occur.
- 11. Anxiety, mental disorder and unwillingness to work. ⁽⁴⁾

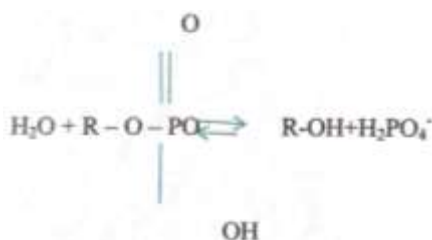
Diabetes treatment

- 1- Controlling glucose in the blood without using any medication
- 2- Treating diabetes with medication. ⁽⁵⁾

Alkaline phosphatase: Alkaline phosphatase (ALP)

This enzyme is called ortho phosphoric mono ester phosphohydrolase

(ALP) This enzyme works according to the following equation: -



Which has a pH of about 10 and the molecular weight varies according to the tissue source of the enzyme, as the rate for it is between (70,000 - 120,000) Daltons ⁽⁷⁾. It is found in several parts of the body, especially the liver, kidneys, intestines, placenta and bones, and the largest part of it is excreted into the blood circulation from the liver and bone tissue ^{(8), (9), (10), (11) and (12)}, while the level of ALP activity rises. By (2-3) times the normal level when infected with hepatitis ⁽²³⁾. The enzyme activity is inhibited by the presence of inorganic Ca^{+2} and phosphate ions.

((The proportion of the base enzyme phosphatrate increases in mechanical cases, and this increase is consistent with what was mentioned in the source ⁽¹³⁾. Since the rise in REM usually originates from the liver and bones))

Cases in which the upper total mandible rises:

- Diabetes (diabetes mellitus)
- Carbohydrate ingestion (in large quantities)

- Children (normally) due to bony growth
- cholestasis in patients with sickle cell anemia (cholelihines in)
- persons with sickle cell disease)
- Eating fat in large quantities
- ninth cell disease (histiocytosis)
- (hyperalimentation)
- Hyperparathyroidism, accompanied by orthopedic symptoms
- (hyperthyroidism)
- (hypophosphatemia)
- kidney rejection tissue)
- Liver disease
- Lung cancer
- myocardial infarction
- Western bone (osteosarcomi)
- Renal infarction
- rheumatoid arthritis
- rickets
- Hemolytic attacks in sickle cell anemia

It decreases in the following cases:

- pernicious anemia
- massive blood transfusions

- (hypophosphatasia)
- hypothyroidism)
- malnutrition
- chronic nephritis))
- Scurvy (the cause of vitamin C deficiency)
- Vitamin D intoxication

(Materials & methods)

The chemicals used in the research are supplied by Syrbio, in the form of a diagnostic kit.

They include:

RI(substrate buffer): Disodium phenylphosphate

Carbonate-bicarbonate buffer

R2(standard): Phenol

R3(inhibitor): Amino 4antipyine Sodium Aasenate

TOXIC.CORROSIVE REAGENT

Use an automatic pipette.

Avoid contact with the skin

R4(color reagent): Potassium Ferricyanide

Instrumentations: -

1- Spectrometer type (UV-visible specter photometer(uv-1650pc).

2- Centrifug (Beckman)\ USA

3- Water bath - Jalalho SW20

Sample collection and blood sample preparation:

(Collection and preparation of blood samples)

Forty samples were obtained during the research period 1-1-2013 until 2013-4-10, which included 20 samples for people with diabetes, their ages ranged from (30-80) years (12 females and 8 males), and 20 samples for healthy people (11 females). and 9 males), where samples were collected from Kirkuk General Hospital, in addition to Civil pathological analysis laboratories in Kirkuk.

Preparation of blood samples

Blood serum

Blood samples were collected in clean, dry tubes and placed in a German bath at 37 °C for 10 minutes, after which the coagulated fraction was separated from the clear solution using a centrifuge. quickly. 6000 For a period of 5 minutes, the clear solution is the blood serum. Determination of the activity of the alkaline phosphatase enzyme in the blood.

Determination of (ALP) in blood:

The activity of alkaline phosphatase (Alp) in the blood serum is measured using the colorimetric method, by means of a special analysis kit (kit) for several ready-made packages containing "the reagents and materials necessary to complete the test."

Principle:

It was used on the (Kind & King) method in the process of chromatographic estimation of the activity of basic phosphatase, depending on the following reaction:

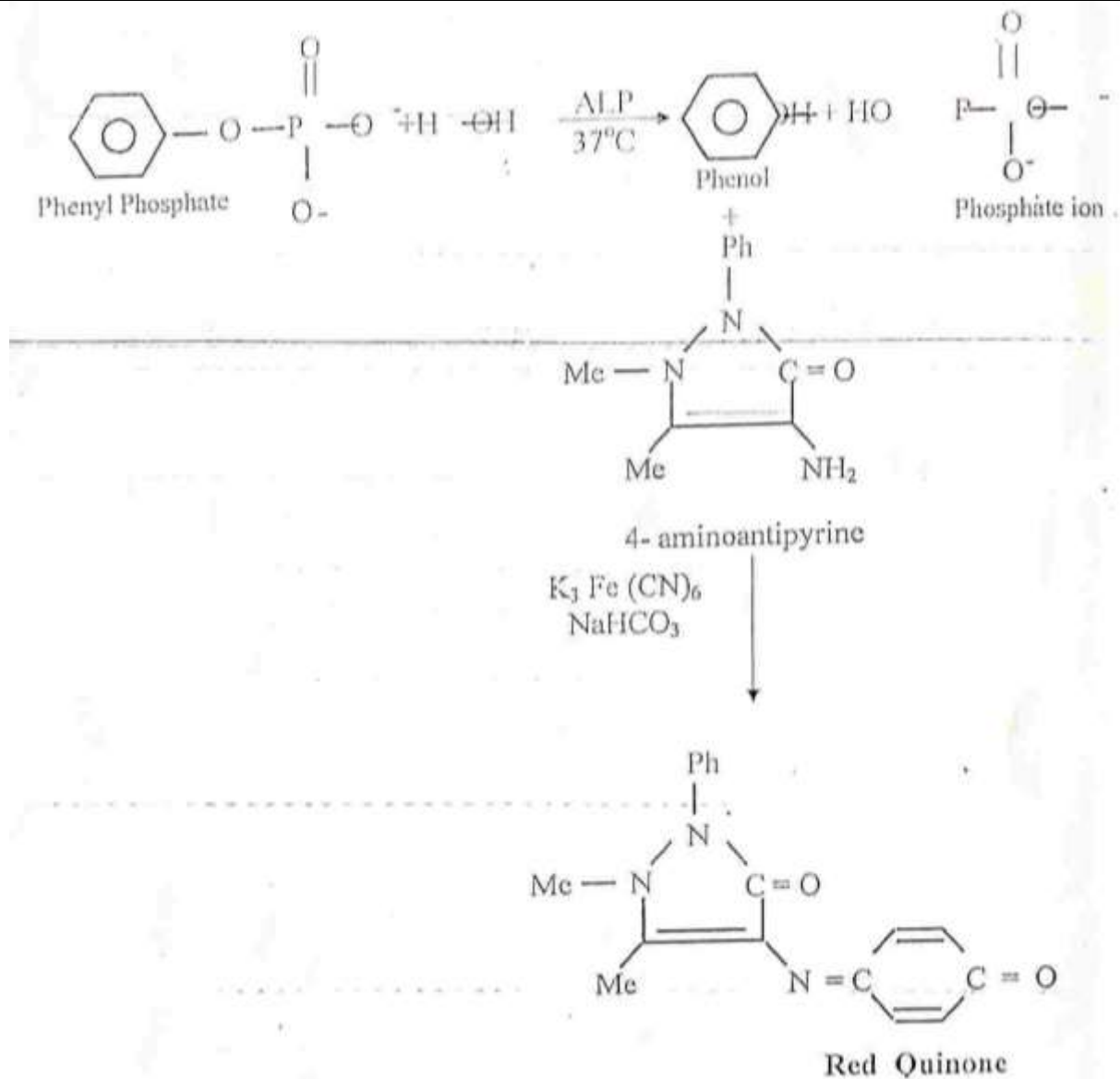


Figure (1:2) The activity of the enzyme ALP

Phenyl phosphate \xrightarrow{Alp} phenol+phosphate

The liberated phenol is estimated by the presence of 4 amine antipyrine and potassium iron cyanide, which is a red complex known as quinone, and the intensity is directly proportional to the activity of the enzyme. The presence of sodium arsenate in the reagents stops the enzymatic reaction.

(Method of work) Procedure: -

	Serum	Serum	Standard	Reagent
	Sample	Blank		Blank
Reagent 1	2ml	2ml	2ml	2ml

Incubation for 5minutes at 37C Waiting for 5 min at 37 °C in a water bath

Serum	50ml	-----	-----	-----
Reagent 2	-----	-----	50ml	-----

Incubation for 5minutes at 37C Waiting for 15 min at 37 °C in a water bath

Reagent 3	0.5ml	0.5ml	0.5ml	0.5ml
مزج جيد Mix well				
Reagent 4	0.5ml	0.5ml	0.5ml	0.5ml
Serum	-----	50ml	-----	-----
D-water	-----	-----	-----	50ml

The mixture is left for 10 minutes in a dark place. Absorbance measurement must be carried out within 15 minutes due to the change in color intensity

$$\text{Alp U/I} = \frac{(\text{A}) \text{ serum sample} - (\text{A}) \text{serum Blank}}{(\text{A}) \text{Stan}} \times 142$$

Results & Discussion

Through the study, the results showed a relative increase in the basal phosphatase level in the blood serum of the patients group of both sexes (0.3789±33,35436 K.A/100ML) compared with the healthy group (0,5903±24,29642) K.A/100ML. The increase

in enzyme is attributed to the fact that the liver tissue is one of the main sources of basal phosphatase, during which it is affected, leads to the leakage of this enzyme in a way Abnormal to blood (12), and this rise is consistent with what I mentioned (13).

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Referrals	basal fuftisz	the total number	Average +D	U\ L S
healthy The	20	20	00.5903 ±	24.29642
The patients	20	20	33.35436 ±	0.3789

Referrals	the total number	female	male
healthy	20	N=11 21.29351 ± 0.3516	N=9 5.946451±0.218
The patients	20	N=12 23.14847±0.3142	N=8 11.99595±0.2015

Conclusions: -

1- During our examination of the blood serum of diabetic patients, it was found that the level of phosphatase activity level increased The basal rate in the blood of infected patients is higher than that of the healthy ones.

2- It was found that the percentage of infected females is higher than males.
3- Where the symptoms were prominent on the injured through the following reasons:
A- Obesity.
b Exposure to psychological trauma.

C- heredity.
 D- Deep thinking.
 E - Decreased secretion of insulin by the pancreas.

Recommendations: -

- 1- The injured must do sports.
- 2- Quit smoking.
- 3- Perform a liver test
- 4- Maintaining the normal level of glucose in the blood.
- 5- Regulating food quantity and quality
- 6- Helping to lose weight and get rid of obesity

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