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ABSTRACT

Background: Diabetes is a major public health problem and exposes people to serious complications

Objective: The objective of our work is to make a comparison of Biochemical and hematological parameters in type 1 and type 2 diabetic patients

Design: A retrospective clinical-laboratory study.

Setting: Laboratory biochemistry national institute of hygiene of Rabat, Morocco

Subjective: the studies were made at the biochemistry laboratory of the Hygiene institute of Rabat between June and August of the year 2019

Results: The study included 40 diabetic patients, including 8 patients with type 1 diabetes and 32 patients with type 2 diabetes. Our study found that the number of Type 2 diabetics (80%) is higher than that of Type 1 diabetics (20%), the number of women is greater than the number of men and the majority of Diabetic patients in our population are hypertensive (70%). Our results show that in type 2 diabetics the average levels of all the parameters are lower than those recorded in type 1 diabetics. While, total cholesterol and HDL found higher in type 2 diabetics than type 1 diabetics.

Conclusion: This study showed that total cholesterol, urea and creatinine increase with age, and people 60 to 75 are more at risk of bacterial infection, rheumatism, cardiovascular and kidney complications than other people. This study remains preliminary, it is therefore necessary to make an in-depth study on a larger population to detect the causes of diabetes for the purpose of raising awareness and reducing affected people all over the world.

INTRODUCTION

Diabetes is a chronic disease that cannot be cured but can be treated and controlled. It is caused by a lack or lack of use of a hormone called insulin. When there is a lack of insulin, or when it does not perform its function effectively, as is the case in a person with diabetes, glucose cannot be used as a fuel for cells. It then accumulates in the blood and causes an increase in sugar level (hyperglycemia). In the long run, high blood sugar causes complications, including eye, kidney, nerve, heart and blood vessel complications.

There are different types of diabetes: prediabetes, type 1 diabetes, type 2 diabetes, gestational diabetes and other rarer types. Type 1 diabetes, accounts for 10 to 15% of diabetes cases (1). It occurs most often in a non-obese subject before the age of 30 (2). Diabetes type 2 known as fatty or mature noninsulin-dependent diabetes, diabetes mellitus (NIDDM) is a metabolic disorder characterized by chronic excess of blood sugar (hyperglycemia) (3). The peripheral use of sugar in the cells: insulin, a hormone made by the pancreas, allows cells to collect and use glucose.

Gestational Diabetes affects 3 to 20% of pregnant women (4). It manifests itself as an increase in blood glucose towards the end of the 2nd and 3rd trimesters of pregnancy. In the majority of cases, it disappears after delivery.

Main objective of the study is concerned with three goals:

- To determine the distribution of diabetic patients by type of diabetes, sex, age and presence or absence of hypertension.
- A comparison of different biochemical parameters (fasting glucose, urea, total cholesterol, HDL, creatinine, uric acid) between type 1 diabetics and type 2 diabetics

• A comparison of the different cellular elements of blood which are white blood cells, red blood cells and platelets between type 1 diabetics and type 2 diabetics

MATERIAL AND METHODS

Sampling: This is a comparative study of some biochemical parameters in diabetic patients during a period of two months (June and August) of the year 2019 in the biochemistry department of the National Institute of Hygiene of Rabat (INH).). The study we have undertaken includes 40 diabetic patients from the Rabat region (Morocco). The selection of patients is based on a questionnaire on their gender, age, medical history and quality of life (Appendix 1).

Blood sampling: Blood samples are taken after at least 12 hours of fasting. Samples are made in two tubes: First, a dry tube to determine: Blood glucose, creatinine, uric acid, ASTL, total cholesterol and HDL. Second tube containing an EDTA anticoagulant for hematological analyzes.

The dry tube is centrifuged in a centrifuge at 4000 rpm for 10 minutes in order to obtain serum, the assay of all the parameters will be performed.

Methods of determination biochemical parameters:

Fasting blood glucose:

Enzyme reference method for hexokinase. Glucose is phosphorylated to glucose-6phosphate by the action of ATP and hexokinase (HK). Then, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the oxidation of glucose-6-phosphate by NADP⁺ to form NADPH (5).

glucose + ATP <u>HK</u> glucose-6phosphate + ADP

glucose-6-phosphate + NADP_____6phosphogluconate + NADPH + H +

The concentration of NADPH formed is directly proportional to the glucose

concentration. It is measured by increasing the absorbance at 340 nm. Reference interval: (0.65 - 1.10) (g / l)

Total cholesterol:

Total cholesterol find out via using colorimetric enzymatic method. Cholesterol esterase (CE) (6).

Esters of cholesterol + - H_2O \rightarrow CE cholesterol + RCOOH

Cholesterol + O_2 Chop Chlestene-4en-3-one + H_2O_2

2H₂O₂ + amino-4phenazone + phenol. ^{POD} red colored derivative (quinoneimine) + 4H₂O

The intensity of the staining developed is directly proportional to the cholesterol concentration. It is determined by the increase of the absorbance at 512 nm. Reference interval: (1.25 - 2.00) (g / l)

HDL cholesterol:

Enzymatic colorimetric assay was in homogeneous phase. In the presence of magnesium sulphate, dextran sulphate forms complex hydrosoluble with LDL, VLDL and chylomicrons (7).

HDL + H2O cholesterol ester cholesterol esterasemodified with PEG cholesterol HDL + ROOH

HDP + O_2 Cholesterol Oxidase Cholesterol Oxidase Modified by PEG Δ 4-Cholestenone + H_2O_2

2 H₂O₂ + 4-amino-antipyrine + HSDA * + H⁺ derivative colored blue-violet + 5H₂O

The intensity of the developed coloration is directly proportional to the HDL cholesterol concentration. It is determined by the increase of the absorbance at 583 nm.

Reference interval: (0.35 - 0.80) (g / l)

* HSDA: sodium N- (2-hydroxy-3-sulfopropyl) -3,5-dimethoxy aniline

Creatinine:

In Jaffé's reaction

An alkaline solution, creatinine reacts with the picrate to form a yellow-red product.

Creatinine + picric acid pH alkaline complex young orange The amount of dye formed (intensity of color) is directly proportional to the creatinine concentration of the sample. It is measured by the increase of the absorbance at 512 nm. Serum and plasma samples contain proteins that react nonspecifically in the Jaffé method (8).

Reference Interval: (5.0 - 12.0) (mg / l)

Uric acid:

Enzymatic colorimetric assay performed via using uricase and 4-amino phenazone.

In a first step, uric acid is oxidized in a reaction catalyzed by

uricase. The hydrogen peroxide formed reacts with [N-ethyl- (3-methylanilino)] (sodium 2-hydroxypropyl-3-sulfonate) (TOOS) and 4-amino phenazone in the presence of peroxidase (POD) to form a colored derivative quinoneimine (9).

Uric acid + 2 H₂O + O₂ $\xrightarrow{\text{Uricase}}$ allantoin + CO₂ + H₂O₂

2 H_2O_2 + H ++ TOOS + 4aminophosphonazone Peroxidase derivative quinone-diimine + 4 H_2O

The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is measured with the increase in absorbance at 520 nm.

The addition of ascorbate oxidase makes it possible to avoid the interference of ascorbic acid. Reference range: (26 - 74) (mg / l) *Urea*:

Urea can be measured by coupled enzyme reactions that are fast, have high specificity to ammonia and are commonly used. One of these reactions has been proposed as a permissible reference method (10).

Urea + $2H_{20}$ <u>urease</u> $2NH_{4^+} + CO_{3^{2^-}}$ NH₄⁺+ 2-oxoglutarate + NADH <u>GLDH</u> L-Glutamate + H_2O + NAD +

The parameters of the hemogram: Quantitative analyzes of red blood cells, quantitative analyzes of white blood cells and quantitative platelet analyzes (11).

Analysis devices: The biochemical parameters were analyzed on the Cobas

C311 controller, and the blood parameters on a Mindray semi-automatic device.

RESULTS

Distribution of patients: According to the type of diabetes the group of diabetic

DISTRIBUTION BY TYPE OF DIABETES

diabetics (Fig.1).



Figure 1: Distribution by type of diabetes

According to sex: Our study shows that the group of 40 diabetic patients are distributed in 92.5% women (37women) and 7.5% men (3 men) it is noted here that most women in our population with RAMED cards (90%)

are elderly and unemployed (the analyzes are done for free). Their main objective is to control their health, which explains the predominance of women (Fig 2)

Distribution of patients by gender



Figure 2. Distribution of patients by gender

diabetics (8 patients) and 80% of type 2 diabetics (32 patients) The results of this study reveal a higher number of type 2 diabetics than type 1

patients are divided into 20% of type 1

According to age: Our study shows that the group of 40 diabetic patients are divided

according to age the results are grouped in the following table1.

| Type of diabetic/Age | Type 1 | Type 2 | Total | Percentage% of patients by age |
|----------------------|--------|--------|-------|-----------------------------------|
| [40-50[| 1 | 4 | 5 | 12,5% |
| [50-60] | 2 | 15 | 17 | 42,5% |
| [60-70[| 3 | 10 | 13 | 32,5% |
| [70-80[| 2 | 2 | 4 | 10% |
| [80-90[| 0 | 1 | 1 | 2,5% |
| Total | 8 | 32 | 40 | 100% |

Table 1Distribution of diabetic patients by age

Our study shows that the group of 40 diabetic patients are distributed almost in all age groups from the age of 40, with a predominance among people aged 50 to 60 years (42.50%). However, for the 60 to 70 age group, we note a rate of 32.50%, people aged 40 to 50 the distribution rate is 12.50%. Our results also show that the rate of distribution of people aged 70 to 80 is 10% and for the elderly more than eighty is 2.50% (fig 3).

According to arterial hypertension: The distribution of patients according to the presence of arterial hypertension (Fig.3) shows that 70% of diabetic patients are hypertensive. This result is similar to that obtained in the study by article (12), which found that 66.7% of diabetic patients have high blood pressure at diagnosis of diabetes.

Distribution of patients according to the presence or absence of arterial hypertension



Figure 3. Distribution of patients according to the presence or absence of arterial hypertension

Comparison of the different parameters studied according to the type of diabetes: The comparison of some biochemical parameters between type 1 and type 2 diabetics is shown in Table 2.

| Parameter | Type 1 | Type 2 | Total | Standards |
|---------------------------|--------|--------|--------|-----------------|
| Percentage | 20% | 80% | 100% | - |
| Age (years) | 61,5 | 58,218 | 58,875 | - |
| Weight (kg) | 72,77 | 69,92 | 70,49 | - |
| Fasting blood glucose g/l | 1,930 | 1,4975 | 1,584 | 0,7-1,1 g/l |
| Urea g/l | 0,323 | 0,253 | 0,271 | 0,10-0,55 g/l |
| Cholesterol g/l | 1,613 | 1,932 | 1,879 | <u>≤</u> .2 g/l |
| HDL g/l | 0,36 | 0,50 | 0,48 | ≥0,4g/l |
| Creatinine mg/l | 8, 625 | 6,518 | 7,00 | 6-12 mg/l |
| Uric acid mg/l | 66,9 | 48,888 | 54,430 | g/l |

 Table 2

 Values of the various parameters analyzed

Fasting blood glucose: Type 2 diabetics have a slightly higher average fasting glucose

level of 1.93 versus 1.49 for type 1 diabetics (Tab.2 and figure 4)



Linéaire (Fasting blood glucose by type 1)

Figure 4. Fasting blood glucose by type 1 and 2 by age

Urea in diabetics: Our study shows that the average urea rate in type 1 diabetics (0.32) is higher than that in type 2 diabetics (0.25),

but both values remain normal (0.20-0.50), the high urea level in blood may be indicative of kidney damage (figure 5) (13).



Figure 5. Urea in type 1 and 2 diabetics by age

Creatinine: Our study shows that the two mean values of creatinine in both types of diabetic are normal (6-12 mg / l). Note that type 1 diabetics have a higher creatinine

average 8.62 against 6.51 for type 2 diabetics. The increase in serum creatinine indicates a decrease in glomerular filtration rate, and therefore renal failure (fig.6) (14).



Figure 6. Creatinine values by type of diabetics as a function of age

Total cholesterol: The total cholesterol results of our study show that the average total cholesterol in type 2 diabetics is 1.93, which seems higher than that of type 1

diabetics (1.61) (15). Our results show that, in type two diabetics, the average levels of all the parameters (fasting glucose, urea, creatinine and uric acid) are lower than those recorded in type 1 diabetics. On the other hand, mean levels of cholesterol total and HDL in type 2 diabetics are higher than those seen in type 1 diabetics. In our population, we have noticed that cholesterol levels increase with age, and people aged 65 to 80 have a higher cholesterol level than others. (Fig 7), we note here that we recorded that the result of total cholesterol in diabetic patients is normal (16).



Figure 7. Total cholesterol by type of diabetes by age

HDL cholesterol: HDL cholesterol is composed of lipoproteins that carry cholesterol from the arteries to the liver. It is often called "good cholesterol" because it helps prevent the buildup of cholesterol in the blood vessels. HDL results show that the average HDL value in type 2 diabetics (50.83) is higher than the average HDL value in type 1 diabetics (36.83).



Figure 8. Mean HDL values by type of diabetes

Uric acid: The result of uric acid shows that type 1 diabetics have a mean uric acid (66.91) higher than that of type 2 diabetics (48.88). It is noted here that both values are normal (26 - 74) (mg / l). It should be noted that the

analysis of the uric acid concentration will make it possible to detect the risk of gout disease or evaluate the functioning of the kidneys (17).



Figure 9. The average values of uric acid

It is concluded that in type 2 diabetics the average levels of all the parameters (fasting glucose, urea, creatinine and uric acid) are lower than those recorded in type 1 diabetics. On the other hand, the average levels of total cholesterol and HDL in type 2 diabetics are higher than those in type 1 diabetics, which probably accounted for the absence of cardiovascular complications in

these patients and age is considered a risk factor for cardiovascular and renal diseases. *Comparison of the different cellular elements of blood that are white blood cells, red blood cells and platelets between type 1 diabetics and type 2 diabetics:*

Hematological analyzes between type 1 and type 2 diabetics are shown in Table 3

Table 3The rate of white blood cells, red and platelets

| | Type of diabetes 1 | Type of diabetes 2 | Normal value |
|--|--------------------|--------------------|---|
| white blood cells x 10 ⁹ /l | 5,5 | 6,96 | between 4x10 ⁹ and 10 x 10 ⁹ /l |
| Red blood cells x 10 ¹² /l | 4,72 | 4,39 | between 4 x 10^{12} and 5,3x $10^{12}/l$ |
| HGB g/dl | 14 | 13,36 | The normal value is 13.5 to 17.5 g / dL in men and 12.5 to 15.5 g / dL in women |
| Platelets x10 ⁹ /l | 238 | 250 | between 150 x 10 ⁹ and 400x10 ⁹ /l |

The white blood cell count: Our study of hematological analyzes shows that the two average values of white blood cell count in both types of diabetic are normal $(4-10 \times 109 / 1)$. Note that type 1 diabetics have an average white blood cell count that is higher 6.96 vs. 5.5 for type 2 diabetics. The white blood cell result indicates whether there is a bacterial infection or inflammatory syndrome, such as

rheumatism. This study shows that our patients probably do not have these complications (18).

The rate of red blood cells:

Type 1 diabetics have a slightly higher mean red blood cell count of 4.72 versus 4.39 for type 2 diabetics (Tab.3), but both values are normal (4-5.3 \times 109 / l). This result shows that our patients do not have anemia problem.



Figure 10 The average values of GR (Red blood cells), GB (White blood cells), HGB (Hemoglobin).

The platelet count: The platelet rate results show that the average platelet count value in type 2 diabetics (250.66) is higher than the average platelet count value in type 1

diabetics (238). Generally, if the platelet count is low, it can upset the process of blood clotting in case of haemorrhage. These values are normal.

PLQ x109/I



Figure 11. Average platelet values

DISCUSSION

In the work, we have mentioned 8 cases of type 1 diabetes and 32 type 2 diabetic cases. The results of this study reveal a higher number of type 2 diabetics than type 1 diabetics (Fig.1). . So our sample included 40 diabetic patients: 92.5% women (37 women) and 7.5% men (3 men) with an average age of 59 (years). They are divided into two groups 20% of type 1 diabetics with an average age of 62 years and 80% of type 2 diabetics with an average age of 59 (years).

Our results show that the percentage of type 2 diabetics and the average age of this population are higher compared to the percentage of people with type 1 diabetes because the number of people with type 2 diabetes increases rapidly across the population. In the world, this increase is associated with economic development, aging populations, increased urbanization, changes in diet and reduced physical activity, and other lifestyle changes (2).

Internationally, the increase in the prevalence of diabetes is observed worldwide, in both industrialized and developing countries (13). The number of people aged 20 to 79 with diabetes in the world is estimated at 425 million in 2017 and is projected to reach 552 million in 2030 (International Diabetes Federation, 2011) (14).

In Morocco, more than 2 million people aged 18 and over are diabetic, 50% of whom are unaware of their disease and the number of children with diabetes is estimated at more than 15,000 (15).

Contrary to some data in the literature which indicates that the number of people with diabetes in the world by sex, men with diabetes are about 14 million more than women (2).

The results of fasting blood glucose results indicate that type 2 diabetics have a higher fasting glucose level (1.93) however the study shows that the glucose level in type 1 diabetics is 1.49 (Tab.2). These two values are not normal (0.65 - 1.10) (g / l), according to who these fasting glucose values remain fluctuating and unstable at some patients but no significant differences between one type of diabetes and another (Fig4) (16).

The urea analyzes show that there is a proportional relationship between the age of the patients in diabetics and the urea value, so we noticed that the urea rate increases with age so our patients might have a kidney and cardiovascular problem in the future. So age in patients with diabetes seems like a cardiovascular and renal risk factor.

From this result we have noticed that creatinine concentrations increase with age and creatinine concentrations in people over 60 are more than those recorded in people under 60, so we can conclude that age is a risk factor for kidney infection in diabetic.

From this result we noticed that the creatinine concentrations increase with age and the creatinine concentrations in people over 60 years are more than that recorded in people under 60 years, our study also shows that creatinine values in type 1 diabetics are higher than creatinine values in type 2 diabetics. So it can be concluded that age and type 1 diabetes are a risk factor for kidney infection

Our study shows that the two mean values of creatinine in both types of diabetic are normal The increase in serum creatinine indicates a decrease in glomerular filtration rate, and therefore a renal failure (14).

HDL results show that the average HDL value in type 2 diabetics (50.83) is higher than the average HDL value in type 1 diabetics (36.83), high HDL cholesterol levels are considered to promote protection against cardiovascular complications, including coronary heart disease (16).

Our results show that, in type two diabetics, the average levels of all the parameters (fasting glucose, urea, creatinine and uric acid) are lower than those recorded in type 1 diabetics. On the other hand, mean levels of cholesterol total and HDL in type 2 diabetics are higher than those seen in type 1 diabetics, which probably accounted for the absence of cardiovascular complications in these patients, and age is considered a risk factor for cardiovascular and renal diseases. From these cholesterol total and HDL results, we can deduce that our patients are aware of the disadvantages of cholesterol on health specifically cardiovascular diseases and kidney failure and the second thing may be that our patients respect their diet, so it's a conscious and educated society.

The result of white blood cell counts indicates that our patients do not have these complications. Red blood cell levels Type 1 diabetic have a slightly higher mean red blood cell count for type 2 diabetics (Tab.3).

CONCLUSION

Our work was performed on a population of 40 diabetics. We observed a slight difference in mean values of each parameter tested between the two types of diabetes. Our results show that in type 2 diabetics the average levels of all the parameters (fasting glucose, urea, creatinine and uric acid) are lower than those recorded in type 1 diabetics. On the other hand, the average levels of cholesterol total and HDL in type 2 diabetics are higher than those recorded in type 1 diabetics, which probably accounted for the absence of cardiovascular complications in these patients. Our results show that age appears to be a risk factor for cardiovascular and renal diseases, our results is consistent with previous results which indicates that age in diabetics is a factor of cardiovascular disease (19).

The results of white blood cell, red blood cell and platelet count show that our patients do not have a problem of bacterial infection, rheumatism or blood clotting (20). These results prove diabetic is a painful disease for the patient, sometimes causing dramatic repercussions on the physiological balance. People with diabetes must analyze many biochemical parameters (blood glucose levels, lipid status, kidney status, etc.) regularly and several times a year in order to diagnose and follow their disease properly. The diabetic patient must follow a healthy lifestyle mainly by adapting the diet and increasing physical activity to better live with diabetes.

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