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Comparative of hematological and biochemical values in hemophilia patients with arthropathy and healthy persons

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ABSTRACT

Background: Hemophilia is a genetic condition of bleeding that is more common in males. Although females are more likely to be carriers of hemophilia, they are not immune to the disease's severe bleeding tendencies and associated symptoms. Bleeding, especially those that reach the joints, have dire consequences if not treated properly.

Aim: study the relationship of some hematological and biochemical values in hemophilia patients with arthropathy and healthy persons, and what the differences in those values between the two cases.

The study: People suffering from hemophilia have undergone treatment at the Center of Hematology at Al-Karama teaching hospital, which is affiliated with Waist University. There were 50 patients engaged in this study, ranging in age from 3 years-45 years. It was determined that 16 of them had mild hemophilia, while the remaining 34 had severe hemophilia. While the healthy control group consisted of 25 individuals.

Results: The Hemoglobin (Hb), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) level in Hemophilia patients (13.2 ± 2.4), (4.52 ± 0.79), (76.4 ± 9), (24.7 ± 4) and (32.3 ± 2.1) respectively are significantly decrease at (p<0.05) when compare with control (14.4 ± 1.6), (4.78 + 0.46), (84.2 ± 3.8), (27.4 ± 2.5) and (33.3 ± 1.4) to same tests. While results revealed that the Liver enzyme Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), and Total Serum Bilirubin (TSB) levels in Hemophilia patients (6.1 ± 2.7), (154.9 ± 6.5), and (0.3 ± 0.2) respectively is significantly increase at (p<0.05) when compared with control group values (19.0 ± 4.7, 331.7 ± 1.5, and 0.6 ± 0.3).

Conclusions: Hepatotoxicity was intimately related with hemophilia patients with arthropathy clearly raised when increased of liver enzyme values. And decreasing in hematological values (Hb, RBC, MCV, MCH and MCHC in hemophilia patient with arthropathy.

Keywords: hemophilia, arthropathy, CBC, ALT, AST, ALP, TSB

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INTRODUCTION

Hemophilia occurs when one of the genes for blood clotting factors on the X chromosome does not work properly or is missing. Males have a higher risk of being impacted, while females have a higher risk of being carriers. Hemophilia A (HA), also known as typical hemophilia, is caused by a lack of clotting factor VIII and accounts for over 90% of all cases of hemophilia [1]. Hemophilia B (HB), on the other hand, is caused by a lack of clotting factor IX and is extremely rare. It is estimated that between 5,000 and 7,000 males are born with HA and 25,000 males are born with HB globally each year [2, 3]. The third type of hemophilia, Acquired Hemophilia (AHA), is distinguished by inhibitors of blood clotting factors. Possible causes include autoimmune diseases, hematological cancers, inflammatory conditions, and viruses [4]. The most noticeable symptom of hemophilia is excessive bleeding. The scenario of impairment and disfigurement may occur if the accident generates bleeding, and the blood affects some of those tissues, including joints, leading to intense pain and immobility due to swelling [5]. Some persons with hemophilia experience catastrophic brain hemorrhage as well as hemorrhage in other vital organs [6]. The development of compensatory therapy for hemophilia patients occurred in the middle of the 1960s, and thousands of healthy donors' plasma was collected and administered intravenously to patients without the necessary testing to ensure the donors were disease-free. Before the late 1980s, when effective treatments became available, most patients with hemophilia faced the risk of contracting potentially fatal but short-lived viruses like hepatitis B and C and HIV. The most effective method to lessen the likelihood of joint damage and make up for the shortage in patients with hemophilia is a preventive treatment, also known as the intravenous injection of the eighth factor and called the concentration factor regularly to prevent recurrent bleeding episodes [7].

MATERIALS AND METHODS

Subjects and design study

From May 2022 to October 2022, researchers from the Wasit University biology department and the Al-Karama teaching hospital in association with the Wasit department of Health conducted this study. All fifty participants who were asked to provide a sample gave their verbal agreement to do so, and their participation in the study was enthusiastically accepted.

Two main subjects were included in the study

Hemophilia patients:

The current study covers a total of fifty Iraqi patients diagnosed with hemophilia, ranging in age from three to fifty-five years old. The patients were separated into two groups, the first of which consisted of those younger than 10 years old, and the second of which included those older than 10 years old. The clinical diagnosis of hemophilia patients was performed by a hematologist specialized physician based on the patient's medical histories and the results of clinical examinations; this diagnosis was also validated by the administration of factor VIII. Patients were directly questioned, and data were collected by using a questionnaire that included questions about patients' ages, genders, lengths, weights, and family histories".

Healthy control subjects:

Thirty people who were initially deemed healthy and showed no symptoms of a pathological condition were partnered with patients to serve as "control subjects" in this study. Participants who did not experience any illness during the study were considered to be in "clinically good health".

Blood sample collection:

Three ml of venous blood were drawn from both patients and the control by using a 5 ml disposable syringe, each sample was immediately divided into three parts.

- The first part: 1 ml of blood was put in an anticoagulant EDTA K3 tube for haematological parameters, the sample was shaken gently and then directly used.
- The second part: 2 ml of blood was put in gel and clot activator tube for the Biochemical test, the blood was left to clot at room temperature (20°C-25 °C) for 15 minutes, then it was centrifuged for 10 minutes at 2500 run per minute-3000 run per minute, the serum was isolated and divided into several parts, each part of was kept in the Eppendorf tube, labelled and freeze at (-20°C) until use.

Hematological assay

Complete Blood Count (CBC):

The test was carried out using a complete blood cell picture measuring device CELL-DYN Ruby, after adding 1 ml of blood in an anticoagulant EDTA K3 tube, the sample was shaken gently and then directly placed in its designated place in the device and given the start command, and then the device automatically read the results. When the results appear, print instruction was given, all these steps according to German company Abbott Laboratories. The basic parameters obtained from full Blood Complete Count (CBC) were a total number of White Blood Cells (WBCs) and its five types: neutrophils, lymphocytes, monocytes, eosinophils, and basophils, Red Blood Cells (RBCs), Hemoglobin (Hb), Packed Cell Volume (PCV), and Platelets (PLT).

Biochemical tests

Alanine transaminase enzyme assay:

This test is performed in accordance with the IFCC recommendation; however, the performance and stability have been improved according to [8]. This is because the enzyme (ALT) catalyzes the reaction between L-alanine and 2-oxoglutarate to form a pyruvate compound, which is then reduced by NADH in the presence of Lactate Dehydrogenase (LDH) to form L-lactate and +NAD. The rate of oxidation of NADH is proportional to the activity of the ALT-catalyzed enzyme, which can be measured by the oligomerization of absorbance to provide an accurate reading. In the transaminase reaction, pyridoxal phosphate functions as a co-enzyme, ensuring that the enzyme reaction is carried out in its entirety.

Aspartate transaminase enzyme assay

This test is conducted in accordance with the suggestion provided by the IFCC; however, the performance and stability have been enhanced in accordance with [9]. The presence of an enzyme known as AST in the sample facilitates the transfer of the amine group from L-aspartate to 2-oxoglutarate, which ultimately results in the formation of oxaloacetate and L-glutamate. In the presence of Malate Dehydrogenase (MDH), the oxaloacetate molecule undergoes a reaction with NADH that results in the formation of NAD+. Measuring the degree to which there is an absence of absorbance enables one to calculate the percentage of NADH that has been oxidized; this percentage is proportional to the activity of the enzyme (AST) catalyst. Pyridoxal phosphate participates in the transaminase reaction as a coenzyme and helps to ensure that the enzyme reaction is carried out in its entirety.

Alkaline phosphatase enzyme assay

The method of chromatography was utilized in the manner described in accordance with a prescribed procedure. Phosphate and p-nitrophenol are the products that result from the breakdown of p-nitro phenyl phosphate by phosphatases in the presence of magnesium and zinc ions [10]. The amount of p-nitrophenol that is released is directly proportional to the catalytic activity of alkaline phosphatase ALP. The rise in absorbance can be used to assess this relationship.

Total serum bilirubin enzyme assay

The chromatography method described in which total bilirubin is mixed together with the presence of an appropriate dissolving agent and the diazonium ion in a media that is very acidic, was the one that was used. In addition, the amount of red azo dye that is produced has a color intensity that is directly proportional to the total bilirubin content in the sample, which may be determined by the use of light [11].

Statistical analysis

The results were statistically analyzed by using the Statistical Program for Social Science 13 (SPSS 13) by finding (mean + SD) and using the Least Significant Difference (LSD) test. Two-way ANOVA method was used to compare between results to identify significant differences between patients and healthy people, and the results are significant if the value of p-value is less than 0.05 ($p \leq 0.05$).

RESULTS

According to the findings shown in table 1, there was a discernible drop ($p \leq 0.05$) in the level of the blood parameter. The results (mean ± standard deviation) for hemophilia's Hb, RBC, MCV, and MCH went down like this: (13.2 ± 2.4), (4.52 ± 0.79), (76.4 ± 9), (24.7 ± 4), and (32.3 ± 2.1) respectively are all lower than the healthy persons (14.4 ± 1.6), (4.78 ± 0.46), (27.4 ± 2.5), (27.4 ± 2.5), and (33.3 ± 1.4).

In comparison to control participants, the levels of liver enzymes associated with hemophilia were found to have significantly increased ($p \leq 0.05$), as shown in table 2. The result of liver enzymes (ALT, ALP, and TSB T) (19.0 ± 4.7), (331.7 ± 1.5), (0.6 ± 0.3) respectively. While healthy results are (6.1 ± 2.7), (154.9 ± 6.5), and (0.3 ± 0.2).

Tab. 1. Associated between complete blood count between patients and healthy

Item	Patient and Control	N	Mean	p-value
Hb g/dl	Patients	50	13.2 ± 2.4	0.01
	Control	30	14.4 ± 1.6	
PCV%	Patients	50	40.3 ± 6.1	0.1
	Control	30	42.1 ± 4.4	
RBC 10^6/ul	Patients	50	4.52 ± 0.79	0.001
	Control	30	4.78 ± 0.46	
WBC × 10^9/ul	Patients	50	7.6 ± 2.3	0.4
	Control	30	8.1 ± 2.3	
Platelet × 10^9/ul	Patients	50	286.9 ± 8.5	0.001
	Control	30	168.9 ± 5.7	
MCV f/l	Patients	50	76.4 ± 9	0.001
	Control	30	84.2 ± 3.8	
MCH pg	Patients	50	24.7 ± 4	0.001
	Control	30	27.4 ± 2.5	
MCHC g/dl	Patients	50	32.3 ± 2.1	0.01
	Control	30	33.3 ± 1.4	

Tab. 2. Associated between liver enzyme between patients and health

Item	Patient and Control	N	Mean	p-value
ALT U/L	Patients	50	19.0 ± 4.7	0.001
	Control	30	6.1 ± 2.7	
AST U/L	Patients	50	21.1 ± 1.3	0.2
	Control	30	18.1 ± 0.5	
ALP U/L	Patients	50	331.7 ± 1.5	0.001
	Control	30	154.9 ± 6.5	
TSB mg/dl	Patients	50	0.6 ± 0.3	0.001
	Control	30	0.3 ± 0.2	

DISCUSSION

In the study, it was discovered that patients with hemophilia A have a lower average value of hemoglobin Hb compared to healthy people. This is because patients with hemophilia A have more frequent bleeding than healthy people do. Especially at younger ages because of the process of learning to walk or crawl, which results in frequent falls and blue bruises, as well as bleeding, particularly at the knees.

As for the decrease in MCV, MCH, and MCHC, the reason for this is due to the decrease in hemoglobin concentration and the size of the blood cells that are packed as a result of the frequent bleeding that hemophilia patients suffer from, or as a result of Microcytic anemia, in addition to patients who suffer from anemia as a result of chronic diseases such as arthritis or infection with parasites [12].

The data are presented in table 2, and they demonstrate a substantial rise ($p \leq 0.05$) in the average value of both ALT and AST, as well as ALP and TSB, as a result of liver illness and the decline in the function of the liver. When liver cells get contaminated, enzymes are released into the blood serum, which is what causes elevated levels. Alternatively, the cause may be due to viral hepatitis, which causes blockages either internally or externally in the liver. Additionally, it is possible that the cause is related to their infection with the Hepatitis C (HCV) and B (HBV) viruses that lead to cirrhosis of the liver, as well as their cancer, due to the fact that approximately 80% of hepatocellular carcinomas are connected with chronic viral infection [13, 14].

CONCLUSIONS

Changes in liver enzyme levels in patients with hemophilia represent hepatotoxicity, which is intimately tied to the function of the liver in these illnesses. Individuals with hemophilia have an increased risk of contracting HBV (B) and HCV (C). This is because Cryo or plasma derivatives, which are used to treat deficits in coagulation factors, contain these viruses.

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