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Relationship of Interleukin 2 and 4 Levels in Moderate and Severe Hemophilia Patients with Arthropathy

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Abstract
Background: Hemophilia is a hereditary bleeding disease that can cause haemarthrosis, which can then progress to chronic arthropathy in those who have the most severe types of the disease. It can result in a significant degree of disability and negatively impact a patient's quality of life.
Aim: study the relationship of Interleukin 2 and 4 levels with development of chronic arthropathy in moderate and severe Hemophilia patients.
Method: has been carried out the assays on patients with hemophilia in the Center of Hematology at Al-Karama teaching hospital, Waist University. 50 patients were involved in this study with ages ranging from (3-45) years. 16 of them were diagnosed as hemophilia moderate, while 34 were hemophilia severe.25 healthy subjects with matched age were involved as a control group.
Results: revealed this study the interleukin (2 and 4) levels in Hemophilia patients are significantly higher (22.3 ± 7.9 and 11.4±3.2) pg/ml respectively at ($p < 0.05$) when they are compare with control values (1.8 ± 0.5 and 1.6±0.7) pg/ml**.**
Conclusions: values increasing of interleukin 2 or 4, can used as biomarker indications for development of chronic arthropathy in moderate and severe Hemophilia patients.

Keywords
Hemophilia, Interleukin 2, Interleukin 4, Arthropathy

INTRODUCTION

Hemophilia is a bleeding disorder that affects people from birth and is caused by a deficit or failure in one of the proteins responsible for blood coagulation. Hemophilia can be divided down into three categories: hemophilia **A** (Classic hemophilia), hemophilia **B** (also known as Christmas disease), and hemophilia **C** (also known as plasma thromboplastin antecedent (PTA) comprising 85%, 15% ,and <1% of cases respectively in which Factor VIII (FVIII), Factor IX (FIX), and Factor XI (FXI) deficiencies occur. While HC is an autosomal recessive disease, HA and HB are X-linked recessive syndromes that predominantly affect males [1, 2]. The rate of spontaneous mutations is 30% in people without a family history of the disorder. Females who carry this syndrome have a deficiency of the clotting factor in question, but the amount present remains adequate for normal clotting to occur. Carriers with less than 50% of the normal the amount of factor are at increased risk for bleeding, especially after undergoing surgery or experiencing trauma [3, 4].

Endothelial cells and liver cells, respectively, create factors VIII and IX, both of which circulate in the bloodstream in an inactive state. Von Willebrand factor (VWF) transports factor VIII until factor VIII is released from VWF in response to damage and interacts with factor IX. Both factor VIII and factor IX play a role in the coagulation cascade's intrinsic route. [5]

Acquired hemophilia is another type of the disease that does not run in families. Bleeding occurs, albeit much later in life, due to autoantibody formation against Factor VIII. Unlike the uncommon occurrence of haemarthrosis, skin bruising and soft tissue bleeding are common symptoms of acquired hemophilia. About half of all cases of hemophilia are considered acquired [6, 7].

METHODS

Subjects and Design Study

This study was done in the Department of Biology - college of Science / Wasit University in cooperation with Al-Karama teaching hospital affiliated With Wasit department of health, during the period from May 2022 to October 2022. The study involves 50 samples, verbal consent was obtained from subjects and all of them agreed to contribute to the study.
Two main subjects were included in the study:

Hemophilia patients

The present study includes 50 Iraqi patients with hemophilia disease, aged between 3 to 55 years. The patients were divided into two groups according to age the first age group was <10 and the second age group is ≥10.

The clinical diagnosis of hemophilia patients was carried out by an Hematologist specialist physician based on history, clinical examination; also this diagnosis was confirmed by using Factor VIII Patients were directly interviewed and the data were taken by using the questionnaire that includes age, gender, length, weight, and family history.

Healthy control subjects

The control subjects consisted of 30 apparently healthy individuals who had no pathological state at the time of this study; all of these individuals were matched to patients. Without any diseases during this duration were clinically considered as healthy in this study.

EIESA Kit Assay (according the company china (Beijing solar bio science and technology) the catalog number SEKH-0008

Human Interleukin 2 (IL-2)

Principle

The kit is for determining the quantifiable amount of human IL-2 that is present in the sample. In the kit, the plate is pre-coated with human IL-2 antibody standards. Samples are then pipetted into the wells, and any IL-2 that is present is detected by the antibody that is coated on the plate after it has been incubated.

After a thorough washing, a biotin-conjugated antibody that is specific for IL-2 is added to the sample so that it can be tested for the presence of the captured IL-2 protein. Following the addition of horseradish-conjugated Streptavidin (HRP) in order to produce the signal, tetramethyl-benzidine (TMB) reagent is next added. Following the washing step, enzyme conjugate was then applied to the wells in order to eliminate any unbound components. In order to halt the formation of the color, a solution containing sulfuric acid is utilized, Moreover, the intensity of the color may be evaluated at 450 nm to provide an idea of the quantity of bound protein.

Reagent Preparation

Before using any reagent, we made sure it was between 20 and 25 degrees Celsius in the room.

1. **Wash Buffer -** Thirty milliliters of wash buffer was diluted with 570 milliliters of deionized or distilled water to make 600 milliliters of wash buffer.
2. **Standard Specimen -** was made by mixing 1.4 milliliters of standard/sample diluent with the standard to be reconstituted. The result of the reconstitution was a stock solution with a concentration of 500 pg/ml. Before beginning the process of diluting the standard, it was allowed to rest for at least 15 minutes while it was gently stirred. After that, 500 milliliters of standard or specimen diluent was pipetted into a tube with a concentration of 250 mg/ml, as well as the remaining tubes. A two-fold dilution series was created from a stock solution with a concentration of 500 pg/ml. Pipette tips were changed between each transfer, and each tube was given a thorough mix. The standard with a concentration of 500 ng/ml was used as the high standard, and the diluent used for the standard and specimen was used as the zero standard (0 ng/ml).
3. **Working solution of biotin-conjugate** anti-human IL-2 antibody was prepared by making a biotin conjugate antibody diluent diluted to 1:100 with the concentrated biotin-conjugate solution in a clear plastic tube.
4. **Working solution of streptavidin-HRP** was prepared by making a 1:100 dilution of the concentrated streptavidin-HRP solution with the streptavidin-HRP diluent in a clear plastic tube.

Human Interleukin 4 (IL-4) according the company china (Beijing solar bio science and technology) the catalog number SEKH-0011

Principle

The kit is for detecting the measurable amount of human IL-4 that is present in the sample. The plate used in the experiment has been pre-coated with human IL-4 antibody standards. Samples are then pipetted into the wells, and any

IL-4 that is present is detected by the antibody that has been coated on the plate.After a thorough washing, a biotin-conjugated antibody that is specific for IL-4 is added to the sample so that it can be tested for the presence of the captured IL-4 protein. Following the addition of horseradish-conjugated Streptavidin (HRP) in order to produce the signal, tetramethyl-benzidine (TMB) reagent is next added. Following the washing step, enzyme conjugate was then applied to the wells in order to eliminate any unbound components. In order to halt the formation of the color, a solution containing sulfuric acid is utilized, and the color intensity, which is related to the amount of bound protein, may be detected at 450 nm.

Reagent Preparation

All reagents were brought to room temperature (20-25 C) before use.

1. **Wash Buffer** By adding 570 milliliters of deionized or distilled water to 30 milliliters of wash buffer, we were able to produce 600 milliliters of wash buffer.
2. **Standard Specimen** - was made by mixing 1.0 milliliter of standard/sample diluent with the standard to be reconstituted. The result of the reconstitution was a stock solution with a concentration of 1000 pg/ml. Before beginning the process of diluting the standard, it was allowed to rest for at least 15 minutes while it was gently stirred. The following step involved pipetting 200 ml of standard/specimen diluent into the 200 pg/ml tube as well as the remaining tubes. A two-fold dilution series was created from a stock solution with a concentration of 200 pg/ml. Pipette tips were changed between each transfer, and each tube was given a thorough mix. The standard with a concentration of 200 ng/ml was used as the high standard, and the diluent used for the standard and specimen was used as the zero standard (0 ng/ml).
3. **Working solution of biotin-conjugate anti-human IL-4** Using the biotin conjugate antibody diluent, the concentrated biotin-conjugate solution was diluted to a concentration of 1:100 in a clean plastic tube, producing the antibody.
4. **Working solution of streptavidin-HRP was prepared by making a 1:100 dilution** of the concentrated treptavidin-HRP solution with the streptavidin-HRP diluent in a clean plastic tube.

STATISTICAL ANALYSIS

Finding the (mean + SD) and applying the LSD (least significant difference) test were both part of the statistical analysis that was performed on the results using the statistical program for social science version 13 (SPSS 13). The two-way analysis of variance (ANOVA) approach was used to compare the data in order to identify significant differences between patients and healthy people. The results are considered significant if the value of P-value is lower than 0.05 ($P \leq 0.05$) .

RESULTS

The results in Table (1) showed a significant increase ($P<0.05$) in the level of Interleukin including : Interleukin 2 and Interleukin 4 for Hemophilia and compared to healthy subjects. The results (mean± SD) of the Interleukin 2 (22.3 ± 7.9) (hemophilia compared to healthy (1.8 ± 0.5) and Interleukin 4 (11.4±3.2) of Hemophilia and compared with healthy (1.6±0.7). But no signification values of interleukin 2 and 4 of hemophilia patients in (moderate and sever) as showed in table (2).

Table 1 Interleukin 2 and 4 levels of hemophilia patients and healthy control

Test		Parameter	Value	P-Value
Hemophilia	n=50	IL2 pg/ml	A 22.3 ± 7.9	0.04
Moderate n=16	Sever n=34	IL4 pg/ml	A 11.4±3.2	
Control	n=25	IL2 pg/ml	B 1.8 ± 0.5	0.03
		IL4 pg/ml	B 1.6±0.7	

A&B letters show significant difference ($P \leq 0.05$) between patients and control.
Human Interleukin 2 (IL-2) Human Interleukin 4 (IL-4)

Table 2 Interleukin 2 and 4 levels in patients of moderate and sever hemophilia

Parameter	Hemophilia severity	Number	Mean ± SD	P value
IL2 pg/m	Moderate	16	44.3±1.2	*NS* 0.1
	sever	34	11.9±4.3	
IL4 pg/m	Moderate	16	22.3±5	*NS* 0.1
	sever	34	6.3±1.7	

NS=Not significant

DISCUSSION

The results in Table (1) show a significant increase ($P \leq 0.05$) in the average value of both interleukin 2 and interleukin 4 patients with hemophilia compared to healthy due Synoviocytes and tissue macrophages (M) in the joint area absorb erythrocytes and store the iron they release as hemosiderin. Synovial hypertrophy is induced by iron via the expression of the oncoproteins C-MYC and MDM2. Increased expression of pro-angiogenic mediators such as vascular endothelial

growth factor-A (VEGF-A), stromal-cell derived factor 1 (SDF-1), and pro-matrix metalloproteinases (pro-MMP) are triggered by the hypoxic environment created by synovial hypertrophy[8] [9].Glycosaminoglycan release from the cartilage matrix and degradation of cartilage and subchondral bone is caused by VEGF-A-stimulated synovial neoangiogenesis and plasmin-mediated conversion to MMPs, respectively. Tumor necrosis factor alpha (TNF-), interferon-, interleukin (IL)-1, IL-6, monocyte chemoattractant protein-1 (MCP-1), and IL-1 are only some of the pro-inflammatory mediators released by synoviocytes and tissue macrophages that help drive these processes.2 A key player in the biology of regulatory T-cells (Treg cells) and immune response homeostasis [10] [11], IL-2 is a pro-inflammatory cytokine released by activated CD4+ and CD8+ T-cells. When chondrocytes are exposed to IL-1, they produce more hydrogen peroxide (H2O2), which reacts with iron (Fe2) to form harmful hydroxyl radicals (OH) and triggers apoptosis. Hemophilic arthropathy is marked by synovial enlargement, cartilage and bone degeneration, and joint abnormalities, and is thought to proceed due to a vicious cycle of re-bleeding maintained byneoangiogenesis and vascular remodeling [12].

But elevated IL-4 is capable of preventing damage to cartilage that is caused by blood, and it does so more effectively than what has been documented in the past for IL-10 [13]. Following exposure to blood at higher concentrations of IL-4, cartilage matrix turnover returns to its normal state. It has been suggested that blood exposure induces an upregulation of the IL-4 and IL-10 receptors on chondrocytes, which has a direct chondroprotective effect. Both IL-4 and IL-10 are capable of inhibiting the production of the proinflammatory cytokines IL-1b and TNF-a; however, IL-4 is more effective at doing so. Additionally, IL-4 prevents chondrocyte apoptosis.

These receptors were found to be increased after blood was allowed to come into contact with cartilage, suggesting the possibility of a secondary direct action on cartilage[14]. Because chondrocytes have such a low expression of the IL-4 receptor, researchers were unable to discover any direct long-term effects of IL-4 on healthy cartilage [15].

CONCLUSIONS

One of the most serious complications that hemophilia patients face is arthropathy, which occurs as a result of recurrent joint bleeding. The interleukin 2 and interleukin 4 increased with hemophilia patients with arthropathy, can used as crucial biomarker indications of this type of disease.

REFERENCES

1. Castaman G, Matino D. Hemophilia A and B: molecular and clinical similarities and differences. Haematologica. 2019;104 (9):1702–1709.
2. Benjamin J. Samelson-Jones1,2 and Lindsey A. George (2023): Adeno-Associated Virus Gene Therapy for Hemophilia Annual Review of Medicine 2023. 74:231–47
3. Lambert C., Meité N. D., Sanogo I., et al. Hemophilia carrier's awareness, diagnosis, and management in emerging countries: a cross-sectional study in Côte d'Ivoire (Ivory Coast) Orphanet Journal of Rare Diseases . 2019;14(1):2–7. doi: 10.1186/s13023-019-1005-9
4. Calvo-Guirado, J. L., Romanos, G. E and Delgado-Ruiz, R. A (2019). Infected tooth extraction, bone grafting, immediate implant placement and immediate temporary crown insertion in a patient with severe type-B hemophilia. BMJ Case Reports CP; 12(3): e229204.
5. Manzoor S, Ganie MA, Majid S, Shabir I, Kawa IA, Fatima Q, et al. Analysis of Intrinsic and Extrinsic Coagulation Pathway Factors in OCP Treated PCOS Women. Indian Journal of Clinical Biochemistry. 2020 Jun 17:1-0.
6. Charlebois J, G-étienne R, St-louis J. Management of acquired hemophilia A: review of current evidence Janie. Transfus Apher Sci. 2018;57(6):717-720
7. Mehta P, Reddivari AKR. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Sep 30, 2022. Hemophilia
8. Roosendaal G, Mauser-Bunschoten EP, De Kleijn P, et al. Synovium in haemophilic arthropathy. Haemophilia 1998;4:502–5.
9. Nieuwenhuizen L, Schutgens RE, van Asbeck BS, et al. Identification and expression of iron regulators in human synovium: evidence for upregulation in haemophilic arthropathy compared to rheumatoid arthritis, osteoarthritis, and healthy controls. Haemophilia 2013;19:e218–27.
10. Sen D, Chapla A, Walter N, Daniel V, Srivastava A, Jayandharan GR. Nuclear factor (NF)-kappaB and its associated pathways are major molecular regulators of blood-induced joint damage in a murine model of hemophilia. J Thromb Haemost 2013;11:293–306.
11. Ovlisen K, Kristensen AT, Jensen AL, Tranholm M. IL-1 beta, IL-6, KC and MCP-1 are elevated in synovial fluid from haemophilic mice with experimentally induced haemarthrosis. Haemophilia 2009;15:802–10
12. Sen D, Chapla A, Walter N, Daniel V, Srivastava A, Jayandharan GR. Nuclear factor (NF)-kappaB and its associated pathways are major molecular regulators of blood-induced joint damage in a murine model of hemophilia. J Thromb Haemost 2013;11:293–306.
13. Jansen NW, Roosendaal G, Hooiveld MJ, Bijlsma JW, van Roon JA, Theobald M, et al. Interleukin-10 protects against blood-induced joint damage. Br J Haematol 2008;142:953e61
14. van Meegeren MER, Roosendaal G, Jansen NWD, Wenting MJ, van Wesel AC, van Roon JAG, Lafeber FPJG. IL-4 alone and in combination with IL-10 protects against bloodinduced cartilage damage. Osteoarthritis Cartilage. 2012; 20: 764-72.
15. Lubberts E, Joosten LA, van Den Bersselaar L, Helsen MM, Bakker AC, van Meurs JB, et al. Adenoviral vector-mediated overexpression of IL-4 in the knee joint of mice with collagen-induced arthritis prevents cartilage destruction. J Immunol 1999;163:4546e56.