Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.8, 2014

Evaluation of Interleukin – 33 level in Iraqi children with Beta-thalassemia major

Afaf T. Marzook Msc.
Biochemistry Department of chemistry Ibn-Alhaitham/ College of Education for pure Sciences/University of Baghdad.
Email: tagreedaloom@gmail.com

Abstract

Background: Thalassemia and a bnormal hemoglobin are the most common genetic disorders and are considered health Prpblems in many developing countries. Beta-Thalassemia major is the most familiar type, in which the beta-globin chain synthesis is impaired.

Objective: The aim of this study is to evaluate a new recent member of the IL-1 super family of cytokines interleukin-33(IL-33) levels in serum that has a crucial rolein signaling cellular damage and infection diseases and in order to evaluate its utility as a clinical biochemical parameter in Beta-Thalassemia Major disease.

Methods: The present study was conducted on 40 subjects which divided in to 2 groups. First group includes 20 healthy individuals as control group (G_1). Second group includes 20 children with Beta-Thalassemia Major as patient group (G_2). All subjects attending Ibn-Al-Baladi hospital, and were (8-14) years old. Parameters measured in the sera of patient and healthy groups were interleukin-33 (IL-33), Iron and immunoglobulins (IgG,IgM,IgA) concentration, while (Hb) and fetus hemoglobin (HbF%) were determined in whold blood as diagnostic parameters in children with Beta-Thalassemia Major patient group and control group.

Results: A recent member of super family cytokines Interterleukin-33(IL-33) was determined in serum of Beta-Thalassemia Major Patients. Higher significant elevation was found when compared with healthy control.

Conclusion: From this study a conclusion was drawn, that evaluation of concentration of a new superfamily cytokines (IL-33) could be considered as clinical biochemical parameter in Beta-Thalassemia Major in Iraqi children patients. Also this study may demonstrated a relation between increased IL-33 levels and increased immunoglobulins and Iron overload.

Keywords: Beta-Thalassemia Major (BTM), cytokines, interleukin-33 (IL-33), immunoglobulins, Iron overload.

Introduction:

Beta-Thalassemias are a group of hereditary blood disorders characterized by a nomalies in the synthesis of the beta chain of hemoglobin molecule resulting in variable phenotypes ranging from sever anemia to clinically asymptomatic individuals (1). Depletion or impaired synthesis of Beta-globins chain causes an imbalanced production of alpha chains, which converts hemoglobin from a normal oxygen transporting function into toxic inclusion bodies causing peripheral erythrocyte hemolysis (2,3). Immunoglobulins are glycoprotein molecules which are synthesized in response to a foreigen substance called antigen and immunglobulins function as antibodies (4). Thalassemia Major entails arisk of Iron overload and multi-organ involvement (5). The goal of iron chelation therapy is to reduce the body burden of iron, especially iron with labile compartments in the plasma, nontransferrin-bound (NTBI) as wellas in varions cells within the body.

Removing iron in these pools will minimize the production of reactive oxygen species, thus reducing damage to internal organs such as the liver and heart resulting in reduced morbidity and impoved survival (6).

Interleukins are a subset of a larger group of cellular messenger molecules called cytokines which are modulators of celluar behavior. Interleukin-33 (IL-33) is anew member of IL-1 super family that is expressed by many cell types following pro-inflammatory stimulation and thought to be released on cell lysis (7). Interleukins are secreted rapidly and briefly, in response to astimulus, such as infectious agent. Once an interleukin has been produced, it transports to its target cell and binds to it via areceptor molecule on the cells' surface. This interaction triggers a cascade of signals within the target cell that finally change the cell' behavior (8). Interleukins regulate immune responses (9). IL-33 can function both as a traditional cytokine and as anuclear factor regulating gene transcription. IL-33 mediates its bioloyical effects via interaction with the receptors interleukin 1 receptor- like 1(Il-IR IL-1) ST2 and IL-1 receptor accessory protein (IL-1RACP), both of which are widely expressed, particularly by innate immune cells and T helper 2 (Th 2) cells. IL-33 strongly stimulatesTh 2 cytokine production from these cells and promte the pathogemsis of Th 2- related diseases such as asthma, Rheumatoid. Arthritis (10,11). Interleukin 33/ST 2 may be a dual function cytokine with both extracellular and intracellular signaling damage and infection diseases a property it shares IL-α. IL-33 influences the various cell types that express ST2 (12,13).

Patients and Methods:

Forty subjects enrolled in this study which divided into 2 groups. First group consist of 20 healthy individuals as control group (G1) Second group consist of 20 subjects of Iraqi children with Beta-thalassemia Major as patient group (G2) treated by Iron chelators. The age of all studied groups were range from (8-14) years old.

Veinous blood samples of (6 ml) were obtained from all subjects enrolled in this study divided into two portions: The first portion of (2 ml) was transferred into plain tube containing (EDTA) to obtain whole blood. The second portion of (4 ml) was transferred into plain tubes left to clot at room temperature for 15 min. Then centrifuged at 3500 rpm for 10 min to separate the serum, and frozen till used .

Interleukin-33 (IL-33) determinations:-

Interleukin-33 (IL-33) has been determined using enzyme linked fluorescent assay (ELISA) technique using the manufacture instruction as supplied with kit from Ray Bio (R).

Serum Immunoglobulins determinations:-

Immunoglobulins (IgG, IgM, IgA) have been determined by a ready kit purchased from (parszmum company), Iran. The method depend on the turbidometric test which theimmunoglobulins form a complex with antibodies in solution which the absorbance read by spectrophotometer (14).

Serum iron determination:-

Iron concentration has been measured by Colorimetric method (15).

Whole Blood Hemoglobin (Hb) determinations:-

Hemoglobin level has been measured using the method of cyanomethemoglobin using Drabkin's reagent of commercially available kit (16).

Whole Blood Fetus Hemoglobin (HbF%) determinations:-

HbF has been detected on Hb electrophoresis on cellulose acetate membrane method (17).

Statistical analysis :-

The results were expressed as Mean $\pm$ SD.

Student-test was used to show the difference between group variation was considered significant when P-Values are ≤ 0.05.

Results:- Table (1) shows the levels of IL-33 concentration are (214.46$\pm$88.9 Pg/ml) , (40.47$\pm$58.7 Pg/ml) in sera of paticnts and control respectively.

This table shows significant increase in children patients compared with the healthy control was for IL-33 and also significant increase in immunoglobulins (IgG, IgM, IgA) and Iron concentration. Table (2) shows the levels of hemoglobin (Hb) and HbF% which revealed a significant decrease in Hb levels in patients (5.32$\pm$0.76) compared with control group (10.34$\pm$0.85), while this table shows a significant increase in fetus hemoglobin HbF% in patients (40.13$\pm$11.28) compared with healthy control group (0.18$\pm$0.01).

Discussion :

The results of the present study showed the serum of IL-33 level was significantly higher in children with Beta-Thalassemia major patients than in healthy control. No data in the literature was found concerning the level of lL-33 in such patients. Il-33 has been shown to signal through the ST2 (18,19). And to drive production of cytokines, both pro-inflammatory and T helper type 2 (Th 2) associated cytokines and chemokines in mast cells (20). Also there is strong evidence for a role of IL-33 and lymphocytes in regulating T helper type 2 (Th2) cytokines (21). Hence its ST2, which is a decoy receptor of IL-33, expressed strongly on Th2 cells (22,23). IL-33 stimulates the production of IL-5 and IL-1B in these cells when IL-33 is administered to mice, increased levels of IgE and Th2 cytokines ensure (23). Moreover IL-33 is the most recently discovered member of the IL-1family which exhibits structural similarity to IL-18 (24). The results of the present study showed the serum levels of immunoglobulins were significantly higher in children with Beta-Thalassemia Major (BTM) than in healthy control.

This finding suggest that the defence strategies of the body are collectively known as immunity, which mediated by a specialized group of proteins known as immunoglobulins or antibodies that have protective function and mediate immunity (25). Iron over load was suggested by some investigators as an important contributing factor in altering the immune parameters in thalassemia patients (26). And that study agreed with the result of the present study which showed the serum level of Iron was significantly higher in children with (BTM) patients than in healthy control. These findings also agreed with a no tier study which has been suggested that iron overload causes migration of T helper type 2 (Th2) cells to the gut and lymph nodes and results in an elevated immunoglobulins levels in Beta-Thalassemia major patients compared with healthy control (27). Also the results of the present study showed the levels of diagnostic parameters of (BTM) which were hemoglobin (Hb) and fetus hemoglobin (HbF%). The results revealed a significant decrease in Hb level in patients group compared with control group and this result suggest that in (BTM) patients there is an imbalance of globin chain synthesis

leads to red blood cells damage resulting in destruction of R.B.C in the bone marrow and peripheral circulation (hemolysis) (28).

But the result of this study revealed a significant increase in fetus hemoglobin (HbF%) in children with (BTM) patients compared with control group. That is due to (HbF%) distribution rather than the occasional occurrence seen with other diseases or in specific fetal cells (28). Elevated (HbF%) level causes membrane damage of the fetal cells leading to premature R.B.C destruction and bone marrow production of abnormal cells (29).

Conclusions:

From the present study, a conclusion can be drawn, that evaluation of concentration of a recent super family cytokines IL-33 could be considered as a clinical biochemical parameter in Beta-Thalassemic major (BTM) children patients in Iraq. The increasing IL-33 levels may reflect the relation with the increased serum immunoglobulins levels and iron overload. These findings may indicate that IL-33 plays an important role in natural immunity and molecular cytogentic diseases such as Beta-Thalassemia Major (BTM), which may influence on immunoglobulins production and iron overload. This finding support that IL-33 stimulates the production of other interleukins such as IL-5 and IL-1B in T helper type 2 (Th2) cells in Iraqi children patients with Beta-Thalassemia Major.

References :

1-Galanello R, Origa R: "Beta-thalassemia" :Orphanet Journal of Rare Diseases"; (2010), 5:11, P:1-15http://www.ojrd.com/ content/5/1/11.

2-Benz, E.J: " Harrison's principles of Internal Medicine" in "Disorders of hemoglobin's" : Fuci As, Braun Weld, E, kasper, et al. (2008). P: 640-641.Mc. Graw-Hill, USA.

3-Hassan, M., Yaish, MD, MAX J., Coppers MD, phD, MBA: " Pediatric Thalassemia "Webscape professional". (2010).

4-Murry, RK, Granner, DK, Mays, PA and Rodwell, VW: (Plasma proteins and immunglobulins) In: Harper IIustrated, (2003), P:580-597, 26^{th}-ed lane Medical books, Mc-Graw-Hill companies.

5- Kohne, E: "Hemoglobinopathies" : " Clinical manifestion, and diagnosis", Hemoglobinopathos. DitchArzteb, (2011), 108 (31-32): 532-540.

6-Fucharoen.s Winichagoon: " New updating into hemoglobinopathies", IntnI. J. Lab. Hem; (2012), 34:559-565. Blackwell publishing ltd.

7-Sanda,s. et al: " IL-33 and ST2 comprise acritical biomechanically induced and Cardioprotective signaling system," Clin. Invest; (2007), 17:1538-1549.

8-IVSL: Interleukin (IL) (Protein)- Britannica Online, Encyclopedia; (2013).

9- IVSLNational Cancer Institute at the national institute of health from online 1-800-4- Cancer. Live help online chat.

10- IVSLFrom Wikipedia , the free encyclopedia, Cytokines and cells online Pathfinder ,Encyclopedia; (2012).

11- Hong, YK; Jooy,B. Jeon,CH, CHOMZ, gaJ.H, Oh HJ, Heo YJ, Park SH, kim HY and Min JK: " Measurement of Interleukin-33 (IL-33) and IL-33 Receptors (s ST2 and ST2L) in Patients with Rheumatoid Arthritis" J. Korean Med. Sci; (2009), 26: 1132-1139.

12- Ashley, M. Miller : " Role of IL-33 in inflammation and disease " . Journal of inflammation, (2011), 8:22.

13- Haraldsen G., Baloh J, Polleimer J, Sponheim J, kusher, AM: " Interleukin-33- cytokine of dual function or novel alarmin? Trends Immunol., (2009), 30:227-233.

14- Shivanada, Nayak B: " Manipal Manual of Clinical Biochemistry " (For Medical Laboratory and Msc. Students), (2008), 3^{rd}.ed " plasma proteins ": p:106, JAYPEE BROTHERS, New Delhi, India.

15- Garcic, A. etal: " Photometric colorimetric test for Iron with Lipid Clearing Factor (ICF), J. Clin. Chem . Acta (1979); (94): 115-119.

16- Lewis, SL and Dacie, IV: " Practical hematology" (1975), 5^{th}.ed, Edinbury. Churchill- Livingstone company.

17- Rochette,J., Graig, J. and Theins, S; Blood Rev; (1994); 8(4): 213-224.

18- Thomas M. Devlin: " Text book of Biochemistry with Clinical Correlation"; (2011), P: 520-521, 7^{th}.ed, John Willey Sons, Inc.

19- Schmitz, J. et al,: " IL-33, aninterleukin-1-like cytokine that signals Via the IL-receptor- related protein ST2 and induces T helper type2- associated cytokines," Immunity; (2005), (23): 479-490.

20- Nile CJ, Barksby E, Jitpraserwong P, Preshaw, PM and Taylor, J J: " Expression and regulation of interleukin-33 in human monocytes ", Immunology, (2010), (130): 172-180, Blackwell publishing Ltd.

21- Humphreys, NE, Xu D, Hepworth MR, Liew, FY, Grencis RK: " Interleukin-33 (IL-33), a potent inducer of adaptive immunity to intestinal nematodes," J. Immunol, (2008); (180): 2443- 2449.

22- Ali S, et al: " IL-1-receptor accessory protein is essential for IL-33 induced activation of T lymphocytes and mast cells ", Proc. NatIAcadSci, (2007), (104): 18660-18665.
23- Ho LH, et al: " IL-33 induces IL-13 production by mouse mast cells independently of IgE-Fcepsilon RI signals ", J. leukoc. Biol; (2007), (82): 1481-1490.
24- Dianarello CA, " Interleukin 18, a pro-inflammatory cytokines ", Eur. Cytokine Newtw; (2000), (11): 483-486.
25- Hope RA, Longmore JM, Maunus, SK and wood Album, CA: " Oxford hand book of Clinical Medicine "; (2010), p:109-125, 8^{th}.ed, Oxford University Press, UK.
26- Weather land DJ ; Clegg, JB; Higgs, D., Wood, W: " The hemoglobinopathies " In: " The metabolic basis of inherited diseases," (2000), p: 4000-4046,8^{th},ed. Mc-Graw –Hill.
27- Weatherall, DJ: " Towards an understanding of molecular biology of some inherited anemia " The story of Thalassemia. In: Blood, pure and eloquent; (1980), 5^{th}.ed, p: 373-380, Mc-Graw Hill publishing.
28- Ahmed A., susan, J., Reza A., Sohelia, A., Nima, J and Mehran,K; British Journal of Hematology; (2005), (214): 220-223.
29- Lissaure, T and Coluyden, G: " IIustation text book of Pediatrics," (2001), 2^{nd}.ed, p: 305. International Ltd.

تقدير مستوى الانترلوكين - 33 في الأطفال العراقيين المصابين بمرض بيتا ثلاسيميا الكبرى

عفاف ذياب مرزوك – ماجستير كيمياء حياتية
قسم الكيمياء – كلية التربية للعلوم الصرفة أبن الهيثم / جامعة بغداد

الخلاصـــــة :
الثلاسيميا والهيموغلوبين غير الطبيعي هي أكثر أمراض الدم الوراثية والتي تعتبر من المشاكل الصحية في عدة بلدان متطورة. بيتا- ثلاسيميا الكبرى هي أكثر أنواعأمراض الدم المعروفة والتي تتميز بوجود خللاً في تخليق سلسلة بيتا كلوبين.

الهـــدف:
تقدير مستوى العضو الحديث في الانترلوكين-1 من السايتوكيناتالانترلوكين-33 في مصل الدم وإمكانية استخدامه كعامل كيموحيوي سريري في أمراض عديدة منها مرض بيتا-ثلاسيميا الكبرى.

الطريقـــة:
تم تصميم الدراسة الحالية على 40 فرداً والذين تم تقسيمهم إلى مجموعتين. المجموعة الأولى تضمنت 20 فرداً من الأصحاء كمجموعة سيطرة (G_1). المجموعة الثانية تضمنت 20 فرداً من الأطفال المصابين بمرض بيتا ثلاسيميا الكبرى (G_2)أخذت من مستشفى ابن البلدي. تراوحت أعمار المجموعتين بين (14-8) سنة. تم قياس العوامل : الانترلوكين-33 وتراكيز الكلوبيولينات المناعية. (IgG, IgM, IgA) والحديد في مصل دم كل من مجموعة الأصحاء ومجموعة الأطفال المصابين بمرض بيتا ثلاسيميا الكبرى وكذلك تم تقدير كل من الهيموغلوبين (Hb) والهيموغلوبين الجنيني (HbF) في الدم كعوامل تشخيصية لمرض بيتا- ثلاسيميا الكبرى .

النتائج :
لوحظ وجود فرقاً معنوياً عالياً عند تقدير مستوى العضو الحديث في مجموعة الانترلوكين-1- من السايتوكينات (الانترلوكين-33) في الأطفال المصابين بمرض بيتا ثلاسيميا الكبرى عند مقارنتهم مع مجموعة الأصحاء.

الاستنتاجات :
من هذه الدراسة الحالية تم تقدير العضو الحديث في مجموعة الانترلوكين-1 من السايتوكينات وهو الانترلوكين-33 واعتباره كعامل كيموحيوي سريري في الأطفال المصابين بمرض بيتا-ثلاسيميا الكبرى في العراق. المستويات العالية للانترلوكين-33 يمكن أن توضح العلاقة مع المستويات العالية للكلوبيولينات المناعية والحديد المتراكم. هذه الاستنتاجات تشير إلىأنالانترلوكين-33 يلعب دوراً حاسماً في أمراض المناعة الطبيعية وأمراض الوراثة الخلوية الجزيئية مثل مرض بيتا-ثلاسيميا الكبرى والتي يمكن أن يؤثر على إنتاجالكلوبيولينات المناعية والحديد المتراكم. هذا الاستنتاج يؤيد بأن الانترلوكين-33 يحفز إنتاجانترلوكيناتأخرى مثل انترلوكين5-ILوانترلوكينIL-1B في الخلايا التائيةT helper type 2 (Th2) cells عند الأطفال العراقيين المصابين بمرض بيتا- ثلاسيميا الكبرى .

الكلمات المفتاحية : بيتا –ثلاسيميا الكبرى ، السايتوكينات، الانترلوكين– 33 ، الكلوبيولينات المناعية والحديد المتراكم .

Table (1):- The Concentration of (Mean±SD) for IL-33, Immunoglobulins and Iron level in patient and control groups

Groups parameters	Controls(G1) n= 20 Mean ± SD	Patients(G2) n=20 Mean ± SD	 P value
IL-33 Pg/ml	40.47 ± 58.7	214.46 ± 88.9	P ≤ 0.05
IgG mg/dl	401.6 ± 7.6	607.6 ± 15.04	P ≤ 0.05
IgM mg/dl	48.24 ± 1.22	88 ± 2.99	P ≤ 0.05
IgA mg/dl	67.44 ± 2.02	96.64 ± 2.99	P ≤ 0.05
Iron M mde/dl	10.38 ± 3.10	22.49 ± 5.80	P ≤ 0.05

Table (2):- The Concentration of (Mean±SD) for Hemoglobin& fetus hemoglobin levels in patient and control groups

Groups Diagnostic parameters	Controls n= 20 Mean ± SD	Patients(G_2) n=20 Mean ± SD	 P value
Hemoglobin (Hb) g/dl	10.34 ± 0.85	5-32± 0.76	P ≤ 0.05
Fetus hemoglobin (HbF%)	0.18 ± 0.01	40.13 ± 11.28	P ≤ 0.05

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Recent conferences: http://www.iiste.org/conference/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar