

## THE ROLE OF CYTOKINES IN AUTOIMMUNE HEPATITIS PATIENTS IN BASRAH CITY SOUTH OF IRAQ

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### ABSTRACT

Autoimmune Hepatitis (AIH) is a chronic type of hepatitis, several cytokines have been implicated in the disease pathogenesis, these cytokines initially identified as products of immune cells that act as mediators and regulators of immune processes. The study aimed to explain the role of cytokines in AIH pathogenesis in Iraqi patients, Seven types of cytokines are measured (IL-4, IL-6, IL-10, IL-17, INF $\gamma$ , TNF, and TGF $\beta$ ) by ELISA technique for 25 diagnosed AIH patients and 15 healthy donors (control group). The result exhibited significant

elevation in both proinflammatory and anti-inflammatory cytokines among patients the results was (184. 413, 426, 4832, 279.91, 370.235, 291.13, 403.82 and 180.844) pg/ml for IL-4 , IL-6 , IL-10 ,IL-17 ,INF $\gamma$  ,TNF, and TGF $\beta$  respectively that reflex complex interplay of several cytokines in AIH pathogenesis, and there is a positive correlation between IL-6 and TGF $\beta$  with IL-17 that reflected the negative effect of TGF $\beta$  in AIH in present of IL-6.

### INTRODUCTION

Cytokines are a diverse group of non-antibody proteins that act as mediators between cells, They were initially identified as products of immune cells that act as mediators and regulators of immune processes but many cytokines are now known to be produced by cells other than immune cells and they can have effects on non-immune cells as well (Cannon,2000 ). The pathogenesis of autoimmune diseases was examined and analyzed largely in the context of the T helper 1 (Th1)/Th2 cytokine balance, with this cell subsets mutually cross-regulating each other (Abbas *et.al* , 1996; Coffman, 2006) , autoimmune diseases could be categorized

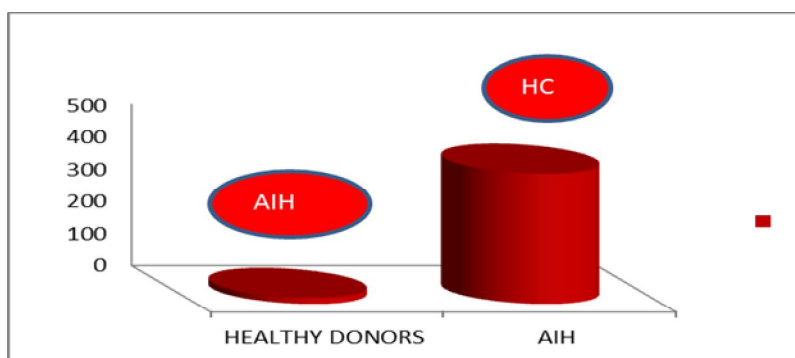
as predominantly Th1-driven if the major events were cell-mediated in nature, or predominantly Th2-driven if antibodies and/or immune complexes served as the main mediators. In view of the cross-regulation between Th1 and Th2, Autoimmune hepatitis is a Th1 predominant state (Kawashima, *et al* 2008). Various immunomodulatory regimens were developed that were aimed at restoring the cytokine balance, eg, by employing strategies to skew the cytokine response (immune deviation) to Th2 in the case of a Th1-mediated disease (Forsthuber *et.al* , 1996; Singh *et.al*, 1996; ) .Cytokines are currently being used clinically as biological response modifiers for the treatment of various disorders.

## MATERIAL AND METHODS

Twenty five AIH patients were obtained from Rodeen (2014) were diagnosed according to simplified score system from three main hospital in Basrah governorate during the period January 2012 to December 2012 and fifteen healthy donors are included in the study ,seven cytokines IL-4, IL-6, IL-10, IL-17,  $\text{INF}\gamma$ , TNF (Pepeotech -USA) and  $\text{TGF}\beta$  (Humman-USA) measured by ELISA technique ( Human reader , Germany ) for both group . Data was analyzed by statistical packages for social science (SPSS) version 21 using mean and standard deviation and the percentage for the study the effect between cytokines by using simple linear regression.

## RESULTS

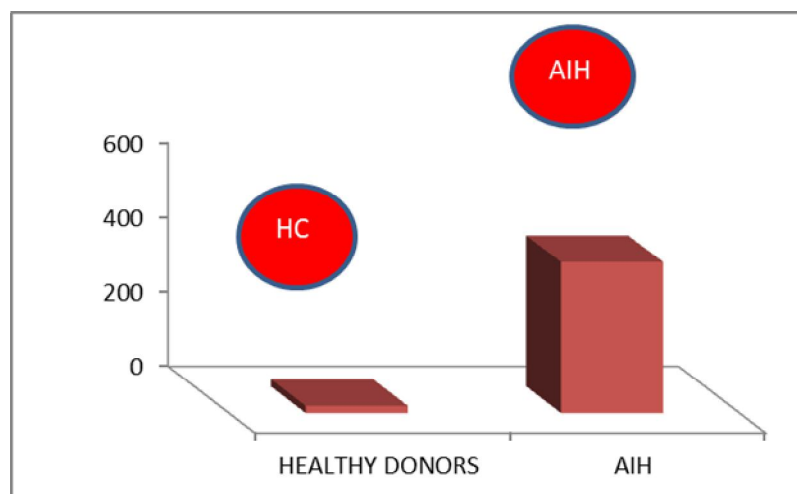
According to manufacture IL-4 concentration was calculated ,the result showed the high increase of IL-4 level among AIH patients (184.413 pg/ml ) ranged (35.06- 500.78) pg / ml as compared with healthy control who exhibit low level 4.49 pg / ml ranged ( 0.451-11.97 ) pg / ml with high significant different ( $p < 0.01$ ) figure (1 ).



**Figure 1: The Mean Level (Pg/ Ml) of IL-4 among Patients and Healthy Control**

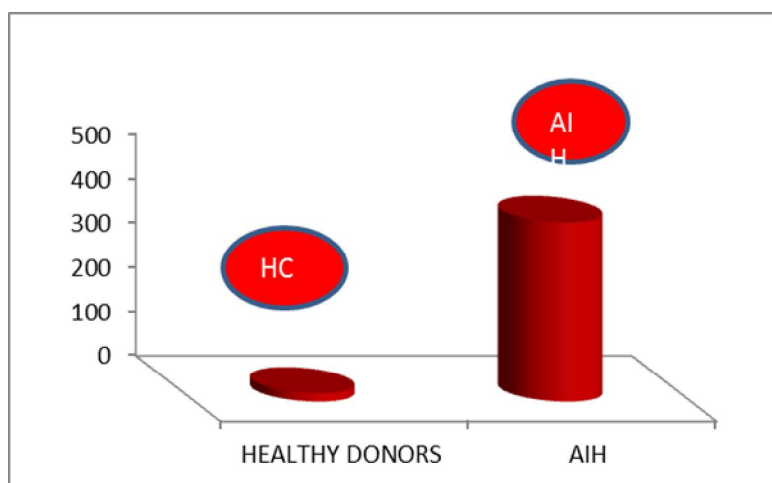
Pro Inflammatory cytokines IL-6 showed increase in level among patients it ranged (172.182- 928.932) pg/ml as compared with healthy control where the concentration was in range

(1.183 - 9.882) pg /ml the mean was (426.4832) pg/ml among the patients and (5.19)pg/ml among healthy control with high significant difference (  $p \leq 0.5$ ) figure (2)



**Figure (2): The Mean of IL-6 Concentration in Pg/Ml among Patients (N25) and Health Control (N15)**

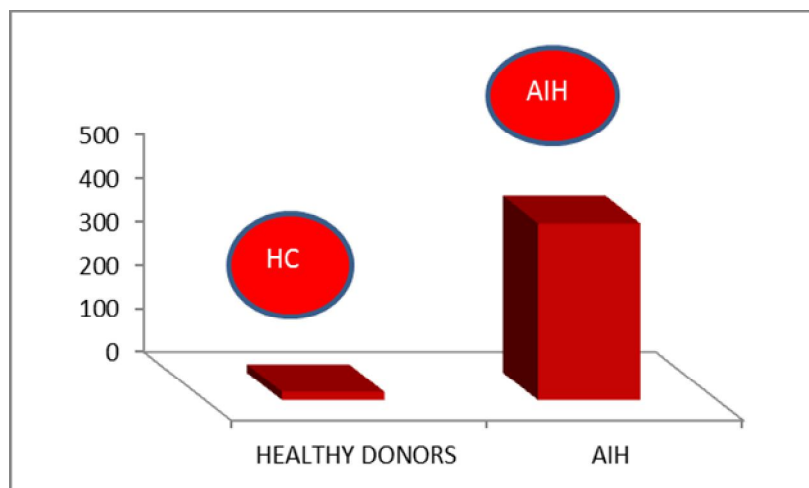
The study included evaluation of IL-10 level in both patients and healthy control where concentration are calculated according to the manufacture standard curve the results of IL-10 in sera showed in range (102.814 - 704.633) pg/ml among patients serum as compared with healthy control where the concentration was in range (0.665-10.048 ) pg /ml . the mean was (279.91  $\pm$  142.72 ) pg/ml among the patients and (4.3062 $\pm$  3.212 )pg/ml among healthy control with high significant difference (  $p \leq 0.05$  ) figure (3 )



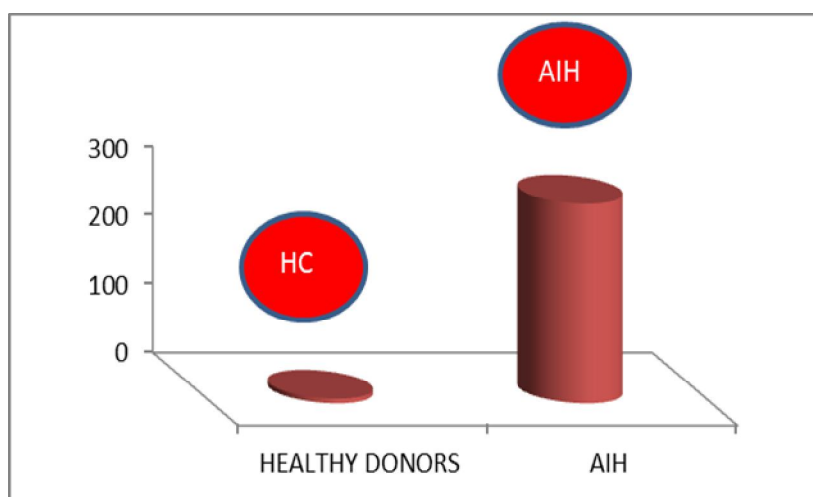
**Figure (3): The Mean Level of IL-10 Concentration in Pg/Ml among Patients and Health Control.**

IL-17 concentration were calculated like previous cytokines the result showed that IL-17 concentration was in range (110.9381- 521.941) pg/ml among patients serum as compared

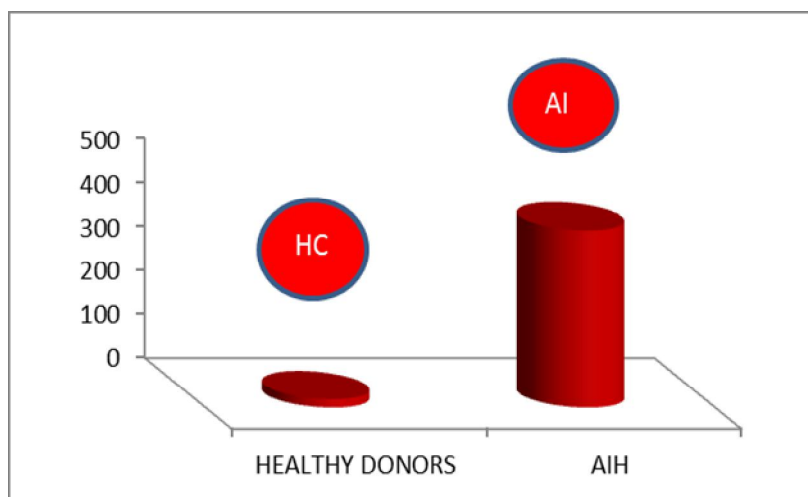
with healthy control where the concentration was in range (0- 35. 122)pg /ml. the mean was (370.235) pg/ml among the patients and (7.757) pg/ml among healthy control with high significant difference ( $p \leq 0.05$ ) figure (4 ).



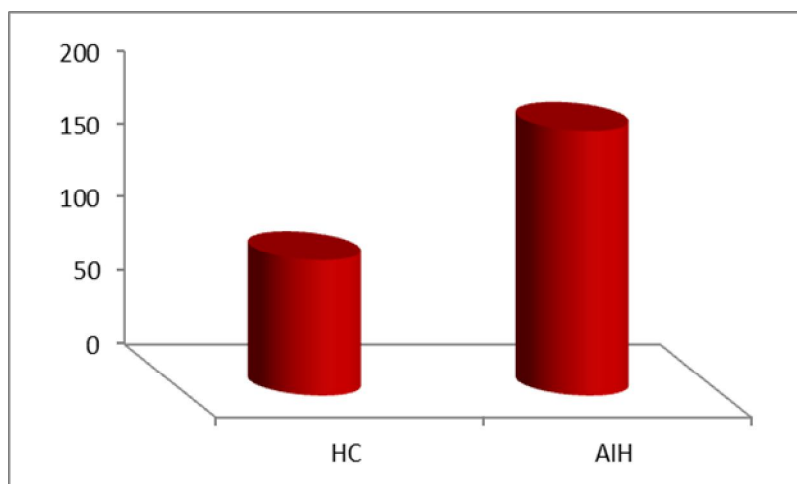
**Figure (4):** the mean of IL -17 level Pg/ml among AIH patients and healthy control (HC)As previous way TNF cytokine concentration calculated according to the manufacture standard curve .TNF showed the range (92.046- 490.3) pg/ml among patients serum as compared with healthy control where the concentration was in range (0.359 - 13.592)pg /ml . The mean was (291.13) pg/ml among the patients and (6.713)pg/ml among healthy control with high significant difference ( $p \leq 0.05$ ) figure (5 )



**Figure (5):** mean of TNF level Pg/ml among AIH patients and healthy control (HC)According to the manufacture standard the results of INF  $\gamma$  showed that AIH patients exhibited increase INF  $\gamma$  level as compared with healthy control with high significant difference ( $p < 0.01$ ) The mean was (403.82) pg/ml ranged (207.37- 708.04) pg/ml where it was( 19.116) pg/ml ranged (1.984- 37.93) pg/ml among healthy control figure (6).



**Figure (6):** mean of INF $\gamma$  level Pg/ml among AIH patients and healthy control (HC) According to the manufacture standard curve TGF- $\beta$  level are calculated, The result showed that AIH patients exhibited increase TGF- $\beta$  level as compared with healthy control with significant difference ( $P < 0.05$ ). The mean was (180.844) pg/ml ranged (95.332- 285. 876) pg/ml where it was (92.243) pg/ml ranged (11.091- 210.108) pg/ml among healthy control figure (7).



**Figure (7):** Mean Of TGF-B Level Pg/Ml among AIH Patients And Healthy Control (HC)

To study the effect of IL 6 and TGF $\beta$  on the IL 17 level statistic analysis used SPSS system and regression analysis applied, the analysis exhibit that there is a positive relationship between IL6 and TGF $\beta$  with IL 17 level and the statistical analysis gave predictability that the increase of IL 6 in one unit in the presence of TGF $\beta$  will lead to increase IL 17 about 0.351pg/ml where the increase of TGF $\beta$  in the presence of IL6 will lead to increase IL 17 level about 0.497 pg/ml, the analysis showed there is strong correlation between IL 6 and TGF $\beta$  with IL 17 where the correlation coefficient was 0.75 and it is significant ( $p < 0.000$ ) Table (1)

**Table (1): Regression Analysis Explain the Correlation between IL-17, IL-6 and Tgf $\beta$** 

S.E	R <sup>2</sup>	R	SIG	T test	B's	SIG	F value	Depended ariable	.Independent variable
SE		Carrollton							
0.74	0.581	0.762	0.00	3.88 5.83	0.351 0.497 130.632	0.000	15.274	IL-17	IL-6 TGF $\beta$ Constant

$$IL-17 = 130.632 + 0.35IL6 + 0.49 TGF\beta$$

## DISCUSSION

Measurement of cytokines for patients are selected from autoimmune Liver disease were diagnosed as Autoimmune hepatitis patients according to simplified score system done by Rodeen (2014).

The study exhibited increase of all measured cytokines that might a result of liver cells damage and disease prognosis the study exhibited increase in the IL-4 cytokines level among AIH patients .The exact role of IL-4 in the disease is debatable , some authors believed is a protective effect (Yshidome *et.al*,1999; Kato *et.al*,2000) whereas other believed it is play as a key role in the several aspect of lymphocyte differentiation and function and has described as being involved in many autoimmune and Inflammatory disease (Choi & Reiser, 1998) , it has been reported that high IL-4 concentration in the liver is causing hepatocyte apoptosis (Jaruga *et.al*, 2003), IL-4 exerts its action by binding its receptor (CD124), which is present on many cell types, including hepatocytes and activating specific signaling cascades (Aoudjehane *et.al*, 2008). there is suggestion that IL-4 is detrimental in AIH is thought to be render Th2 cells resistant to T<sub>Reg</sub> cells mediate suppression (Pillemer *et.al*, 2009 ), and the neutralizing anti-CD124 antibodies reflects an attempt of immune system to reduce a negative role of IL-4. (Jaruga *et.al*, 2003; Aoudjehane *et.al* ,2008 ).Auto antibodies that neutralized IL-4 activity might favor liver Inflammation indirectly by favoring development in lymphoid tissue and recruitment to the liver of T cells subsets, such as Th1 and Th17 involved in autoimmunity and Inflammation (Zhao *et.al*, 2011). Recently Goh *et .al* (2013) found that IL-4 may play role in hepatocyte regeneration after injury that may explain the persistence of the chroic AIH by rapier the damaged liver cells with persistent of AIH trigger. From other hand increase level of IL-6 is reflex the role of this cytokines in the disease progress were Increase IL-6 plays roles in the host defense by stimulating various cells and when it acting on hapatocytes IL-6 strongly induce abroad spectrum of acute phase protein such as C Reactive protein CRP (Siewert *et.al*,2000) . from other hand IL-6 play important

role in activating B lymphocyte differentiation and increase the autoantibodies secretion (Thakey *et. al*, 2004) in addition , IL-6 is important to the transition between acute and chronic Inflammation by recruitment of monocyte to the area of Inflammation (Kaplanski *et.al* ,2003 ) that explain the high level of IL-6 cytokine among AIH patient in current study, were in normal host IL-6 have protective role by it is function to suppress of the level of pro-Inflammatory cytokines during acute response without compromising the level of anti – Inflammatory cytokines , the increase of IL-10 level among AIH patients in the current study may explain the complex action of IL -10 or it may have role in the production of autoantibodies among the AIH patients. studies show that IL-10 secreted from NK cells has been linked to increases in Th2 related autoimmune disorder ( Dai *et.al*, 2009, Yu & Wang, 2011), The broad effect of IL-17<sup>+</sup> cells in the pathogenesis of liver disease is complex and remains to be fully explored. However, in liver disease the role of IL-17<sup>+</sup> cells is known to be mediated via production of several cytokines including IL-17 and it targets a number of cell types via binding to IL-17R that is expressed such as Kupfer cells, stellate cells, biliary epithelial cells and endothelial cells are all known to express IL-17R and making them possible targets for autoimmunity (Lafdil *et.al*, 2009). this interleukin is produced from subset of CD4 cells that differ from Th1 and Th2 cells it is termed Th 17 (Harrington *et.al* ,2005; Park *et.al*, 2005), and it has also been demonstrated to induce IL-6 expression via the mitogen-activated protein kinase pathway in hepatocytes, thus indicating that Th17 cell proliferation is the key trigger in the pathogenesis of AIH and that the positive feedback loop between Th17 cells and hepatocytes elicit the Inflammatory process (Zhao *et.al* ,2011), IL-17 stimulates IL-6 expression, which favors Th17 proliferation while reducing the development of T<sub>Reg</sub> cells. Therefore, IL-17 may orientate the balance between Th17 and T<sub>Reg</sub> cells towards Th17, which play critical role in the development of autoimmune hepatitis. The study exhibited increase in TNF- $\alpha$  and INF $\gamma$  level among patients, recently study by Iwamoto *et.al*, (2013) exhibited TNF- $\alpha$  stimulation enhanced CCL20 expression in hepatocytes these findings suggest that TNF- $\alpha$  is essential in the induction of AIH through upregulation of hepatic CCL20 expression, which allows migration of dysregulated splenic T cells that reflect the important role of TNF in AIH disease.

Interferon IFN $\gamma$  acts as a critical pro-Inflammatory mediator in autoimmune processes, whereas it exerts regulatory functions to limit tissue damage associated with Inflammation. However, a detailed understanding of the complex roles of IFN- $\gamma$  in the development of organ-specific autoimmunity is still lacking, IFN- $\gamma$  may indirectly cause hepato cellular



damage through the induction of other hepatotoxic substances, (Diehl, 2000), elevation of TGF $\beta$  level in patients serum reflex the complex role of cytokines in the AIH pathogenesis , level statistic analysis used SPSS system and regression analysis applied , the analysis exhibit that there is a positive relationship between IL6 and TGF $\beta$  with IL 17 level and the statistical analysis gave predictability that the increase of IL 6 in one unit in the presence of TGF $\beta$  will lead to increase IL 17 about 0.351pg/ml where the increase of TGF $\beta$  in the presence of IL6 will lead to increase IL 17 level about 0.497 pg/ml, the analysis showed there is strong correlation between IL 6 and TGF $\beta$  with IL 17 where the correlation coefficient was 0.75 and it is significant ( $p < 0.000$ ) Table (1) from other hand the value of F-test is high and it is significant that give the conclusion of the role of IL 6 concentration in the present of TGF $\beta$  to expectation the level of IL 17 and the analysis is able to explain 58% of IL 17 source in the patients serum . A study done by Longhi *et al* (2012) found that the blocking of IL 17 will led to increase the immunosuppressive of T<sub>Reg</sub> cells so the work on block IL 6 may play prime role in future therapeutic of AIH disease. So the study recommended Study other different types of cytokines for Th1, Th2 to predict of ability to producing proinflammatory and anti-inflammatory mediators and find ways to ensure blocking some cytokines that contribute to differentiation T helper cells to Th17 that play role in AIH pathogenesis such as IL-6.

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