WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 281-288. Research Article ISSN 2277– 7105

EVALUATING LEVELS OF INTERLEUKIN-1B AND INTERLEUKIN-17 IN RHUMATOID ARTHRITIS PATIENTS IN THI-QAR PROVINCE

Ali Naeem Salman* and Ahlam Muhsen Kudair

Biology Dept., Collage of Education for Pure Science – Thi-Qar Univ.

Article Received on
8 Aug 2015,

Revised on 28 Aug 2015,
Accepted on 17 Sep 2015

***Correspondence for Author**
Ali Naeem Salman
Biology Dept., Collage of Education for Pure Science – Thi-Qar Univ.

ABSTRACT

The current study was conducted at College of Education for pure science Department of Biology /Thi – Qar University in Thi – Qar province, during the period from October 2014 to May2015. The study aimed to evaluate immune status of rheumatoid arthritis patients by measuring the levels of interleukins (IL-1β, IL-17) in the serum using a technique enzyme-linked immune sorbent adsorptive (ELISA), the study included a total of 70 patients with RA they were (20males and 50 females) and they were aged between (20-65) years. When compared with 10 apparently healthy people as control. The results showed a significant increase ($P \leq 0.01$) in the levels of interleukins (IL-1β, IL-17) in the serum of all patients with RA compared to the control group.

KEY WORDS: IL-1β, IL-17, Thi-Qar. University.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of thesynovial tissue. RA affects approximately 0.5-1% of the world population (Hambright*et al*., 2011; Martínez*et al*., 2011) and being more common in women than in men, and in ages between 40 and 60 years (Ebringer*et al*., 2010). It causes functional disability and premature death. Approximately 70% of patients have irreversible joint destruction and 80% of active young adults in the labor market are affected by stiffness and devastating pain (Filipovic*et al*., 2011). The etiology of RA is unknown, the exposure for RA probably varies between individuals as result of genetic variation (Weyand and Goronzy., 1997) and hormonal factors (Wilder, 1996). Mechanisms underlying RA are complex and depend on a number of internal and external factors. However research has demonstrated both genetic and environmental factors for the susceptibility and the development of the disease (Tobon*et al*., 2010). Among

environmentalfactors, smoking,alcohols, coffee drinking, and possibly obesity (Lahiri*et al*., 2012).

Cytokines

are soluble molecules, short-lived proteins or glycoproteins produced constitutively or under proper stimulation (Janeway and Bottomly 1994), cytokines secreted by several cell types such white blood cells and various other cells in the body. These proteins assist in regulatingthe development of immune effecter cells, and some cytokines possess directeffecter functions of their own (Flynn and Chan., 2001). The release of specific cytokines into the systemic circulation had beenobserved in RA .Concentration levels usually reflect disease severity and prognosis (Goronzy and Weyand, 2009). Cytokines are divided into pro-inflamtory cytokines (TNF-α, IFN-γ, IL-1, IL-2, IL-6, IL-8, IL-12, IL18 and IL-17) and anti- inflamtory cytokines (IL-4, IL-10, and TGF-β),and that an imbalance between pro- and anti-inflammatory cytokine activities leads chronic inflammation and joint damage (McInnes and Schett, 2007), cytokines among those that play a role in parthenogenesis of RA are(IL-1β, IL-17).

Interleukin-17 (IL-17)

There are six members in the interleukin 17 (IL-17) cytokine family, including IL-17A (commonly referred to as IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25) and IL-17F. Specialized T cells, called Th17 cells, are themajor source of IL-17A and can also be produced by several other innate immunecell types, such as lymphoid tissue inducer cells, natural killer andnatural killer T cells, macrophages and Paneth cells. IL-17 familycytokines have been linked to many autoimmune diseases, includingmultiple sclerosis (MS), rheumatoid arthritis (RA), inflammatorybowel disease and psoriasis (Jin., 2013 *et al*). IL-17 is an important link between T cell–mediatedadaptive immunity and the innate immune system, especially the inflammatory component of innate responses. In humans, expressionof IL-17A in RA synovium and synovial fluid was first describedin 1999 (Cabhaud*etal* 1999), it has pleiotropic effects on leucocytes and stromalcells (Miossec., 2003). For example, it induces IL-6 and IL-8 production by fibroblasts (Fossiez., *et al* 1996) and stimulates macrophageIL-1 and TNF-α production (Jovanovic*etal*., 1998).

Interleukin -1 β (IL-1β)

IL-1β is a member of the interleukin1 family produce by activated macrophages as a pro protein. IL-1β is a key mediator in the pathogenesis of inflammatory syndromes such as RA

(Cantagrel*etal*., 1999), for example overexpression in rabbit knee joints causes arthritis with clinical and histological features characteristic of RA (Kay, 2004). It cause damage to the joints and systemic effects by stimulating the synthesis of IL–6 and the C-reactive protein (Schiff., 2000). There is correlation found between each of IL-17, IL1β as IL-17stimulates macrophages to produce various inflammatory cytokines, such as IL-1β and TNF (Jovanovic*et al*., 1998) in the same time IL-1β induce Th17 to produce IL-17. Figure (1)

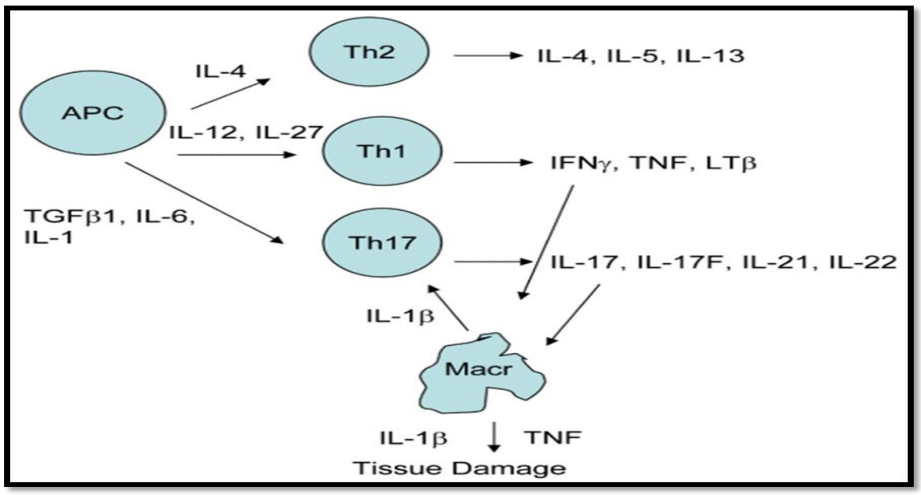

Fig (1): Mechanisms by which IL-1and IL-17 reciprocally activate function. (Alison and Paul, 2008).

MATERIALS AND METHODS

Study desigen

This study was performed on (70) Iraqi patients with RA patients, who attended AL-Husain teaching hospital, AL-Shatra general hospital and AL-Rfai in the period from beginning of October (2014) to the end of May (2015). Also, this study included (10) person apparently healthy individuals as a control group., who have no history or clinical evidence of RA or any other chronic disease, and no obvious abnormalities.

Blood Samples Collection

Blood samples were collected by venipuncture from 70 patients and 10 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. The blood samples were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -10 C°. These sera (70RA patients and 10 controls) were used for estimating the concentration of interleukin (IL- 1β, and IL-17).

Kits

Kits of (IL-1β &IL-17) provided by (Elabscience company, China). The sera of patients and controls were assessed for the level of two cytokines, which were IL-1β, IL-17, by means of ELISA that were based on similar principles.

METHODS

A - Principles of Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-1β, IL-17 has been pre-coated onto a micro plate. Standards and samples were pipetted into the wells and any IL-1β, IL-17 present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-1β, IL-17 was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of IL-1β, IL-17 bound in the initial step. The color development was stopped and the intensity of the color is measured.

B – Assay procedure

All reagents were brought and samples at room temperature before use. The samples were centrifuged again. It is recommended that all samples and standards be assayed in duplicate.

1. All reagents were prepared, standards were made, and samples.
2. The number of wells were determined according to the assay layout sheet.
3. One hundred µl of standards and samples were added to per well. Then the wells were covered with the adhesive strip and incubated for 2 hours at 37°C.
4 The liquid of each well was removed, without wash.
5. One hundred µl of biotin-antibody (1x) were added to each well and covered with a new adhesive strip ,after that incubated for 1 hour at 37°C.
6. Each well was removed and washed, repeating this process two times for a total of three washes. Each well was washed by filling with wash buffer (200µl) by using auto washer, and allowed it stand for 2 minutes, Next the last wash, any remaining wash Buffer was removed by aspirating or decanting, then the plate inverted and blotted it against clean paper to wells.
7. One hundred µl of HRP-avidin (1x) were added to each well and covered the micro titer plate with a new adhesive strip then incubated for 1 hour at 37°C.
8. The wash process was repeated for five times as in step 6.

9. Ninety µl of TMB Substrate were added to each well and incubated for 15-30 minutes at 37°C, protected from light.

10. Fifty µl of stop Solution were added to each well.

11. Later the optical density was determined of each well within 5 minutes, by using a micro plate reader set to 450 nm and the result was printed on paper by printer.

Statistical analysis

The analysis of data was expressed as mean ± SD. The comparisons between each RA patient group with matched healthy control were performed with T-test by using computerized Minitab 14 program. P<0.01 was considered to be the least limit of significance, the statistical analysis was done by using Pentium-4 computer through the (SPSS program) Statistical Package For Social Sciences (version-20).

RESULTS

The present study showed the presence of a significant increase in the rate of concentrations of IL-1β, IL-17 in sera of patients with RA, compared with the average concentration in the sera of healthy control group, as was the rate of concentration of IL-1β in patients (41.51±13.51) pg/ml compared to the control group (18.61±9.21) pg/ml, while IL-17 concentration (70.34+32.34) pg/ml for patients compared to the healthy control (30.91± 6.1) pg/ml. Table (1).

Table (1) Comparison of serum (IL-1β, IL-17) concentrations (pg/ml) of the patient groups with healthy controls group HS; High significant.

Parameter	Subject	No of cases	Mean± SD	T-value	Df	P-value
IL –1β	Pateints	70	41.51±13.51	5.17	78	0.000 HS
	Control	10	18.61±9.21			
IL – 17	Pateints	70	70.34+32.34	3.64	78	0.000HS
	Control	10	30.91± 6.19			

DISCUSSION

The results of the current study showed a high level of concentration of IL17-in the patient group compared to the healthy control group. These results agree with the results studies of all (Ziolkowska*etal*., 2000; Metawi., *et al* 2011; Moran 2009; Muhammed., *et al* 2014; Al-Saadany., *etal*, 2015) who indicated to an increase in the concentration of IL17-in the sera of patients with RA. The reasons of observation high levels of IL-17 in current study perhaps that patients had a higher.

Percentage of Th17 cells, which is the main source of IL-17and probablythe synovium of patients is expanded and contains large numbers of fibroblasts, macrophages and highly differentiated T cells theseconsidered the important source of IL-17.

The results of the current study showed too, a high level of concentration of IL1β-in the patient group compared to the healthy control group. The result of this study is agree with the results of each (Paramalingam., *et al*, 2007; Altomonte., *et-al*, 1992) whose have indicated the high level of concentration of IL-1β in the sera of patients with RA versus healthy individuals. The reason for the increase in the concentration of IL-1β is associated with elevated concentration of IL-17as the IL-17exerts various biologic activities, which potentially cause tissue destruction and degeneration during chronic inflammation and stimulates macrophages to produce various inflammatory cytokines, such as IL-1β and TNF (Jovanovic*et al*., 1998).

CONCLUSION

Serum IL-1β and IL-17 are significantly higher in RA patients than in healthy controls confirming their important role in the pathogenesis of RA and possible target for future therapy.

REFERENCES

1. Alison Finnegan and Paul D. Doodes Pathways for IL-1 driven arthritis. Arthritis Rheum, 2008; 58(11): 3283–3285.
2. Al-Saadany, Hanan M. Hussein, Manal S. Gaber, Rasha A. Zaytoun, Hossam A. Th-17 cells and serum IL-17 in rheumatoid arthritis patients: Correlation with disease activity and severity, The Egyptian Rheumatologist. issn, 2015; 1110-1164.
3. Altomonte, L. Zoli, A. Mirone, L. Scolieri, P. Magaro, M. Serum levels of nterleukin-1b, tumour necrosis factor-a and interleukin-2 in rheumatoid arthritis. Correlation with disease activity. Clinical rheumatology, vol11, 1992; 202-205.
4. Cabhaud M., J. M. Durand N. Buchs F. Fossiez G. Page, L. Frappart, and P. Miossec. Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. Arthritis Rheum, 1999; 42: 963–970.
5. Cantagrel A., Navaux F., Loubet-Lescoulie P., *et al.* Interleukin-1β eta, interleukin-1 receptor antagon- ist, interleukin-4, and interleukin-10 gene polymorphisms: Relationship to occurrence and severity of rheumatoid arthritis. *Arthritis & Rheumatism*, 1999; 42: 1093-1100.

6. Chabaud M, Fossiez F, Taupin JL, Miossec P. Enhancing effect of IL-17 on IL-1induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritissynoviocytes and its regulation by Th2 cytokines. J Immunol, 1998; 161: 409–414.
7. Ebringer A, Rashid T, Wilson C. Rheumatoid arthritis, proteus, anti-CCP anibodies and Karl Popper. Autoimmunity Reviews, 2010; 9(4): 216-23.
8. Filipovic I, Walker D, Forster F, Curry AS. Quantifying the economic burden of productivity loss in rheumatoid arthritis. Rheumatology (Oxford), 2011; 50(6): 1083-90.
9. Flynn, J. L. and Chan, J. Immunology of tuberculosis. Annu. Rev. mmunol., 2001; 19: 93-129.
10. Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, Pin JJ, Garrone P, Garcia E, Saeland S, *et al*. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J Exp Med*, 1996; 183: 2593-2603.
11. Goronzy, J. and Weyand, E. Developments in the scientific understanding of rheumatoid arthritis. Arthritis Res. Ther., 2009; 11: 249.
12. Hambright D, Henderson RA, Cook C, Worrel T, Moorman CT, Bolognesi MP. A comparison of perioperative outcomes in patients with and without rheumatoid arthritis after receiving a total shoulder replacement arthroplasty. Journal of Shoulder and Elbow Surgery, 2011; 20(1): 77-85.
13. Janeway CA, Bottomly K. Signals and signs for lymphocyte responses. Cell, 1994; 76: 275-285. *134*.
14. Jin, WeiDong, Chen. IL-17 cytokines in immunity and inflammation. Emerging Microbes & Infections, 2013; 2: 9.
15. Jovanovic DV, Di Battista JA, Martel-Pelletier J, et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J Immunol*, 1998; 160(7): 3513–3521.
16. Kay, Jonathan Calabrese, Linda. The role of interleukin-1in the pathogenesis of rheumatoid arthritis. Rheumatology-Oxford, 2004; 43(3): 1462-0324.
17. Lahiri, M.; Morgan, C.; Symmons, D. P. M. and Bruce, I. N. Modifiable risk factors for RA: prevention, better than the cure? Rheumatology., 2012; 51: 499-512.
18. Martínez EO, Ramírez DFH, Núñez-Álvarez CA, Cabiedes J Proteínas citrulinadas en artritis reumatoide. Reumatología. Clínica, 2011; 7(1): 68-71.
19. McInnes, I.B.; Schett, G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol, 2007; 7: 429–42.

20. Metawi SA, Abbas D, Kamal MM, Ibrahim MK. Serum and synovial fluid levels of interleukin-17 in correlation with disease activity in patients with RA. Clin heumatol, 2011; 30: 1201–7.
21. Miossec P. Interleukin-17 in rheumatoid arthritis: if T cells were to contribute to inflammation and destruction through synergy. *Arthritis Rheum*, 2003; 48: 594-601.
22. Moran EM, Mullan R, McCormick J, Connolly M, Sullivan O, Fitzgerald O, et al. Human rheumatoid arthritis tissue production of IL-17A drives matrix and cartilage degradation: synergy with tumor necrosis factor-alpha, Oncostatin M and response to biologic therapies, Arthritis Res. Ther, 2009; 11: R113.
23. Muhammed Noora*, Hassan J Hasony*, Mubder A Mohamed Saeed The Clinical Significance of Interleukin-15 and Interleukin -17 in Patients with Rheumatoid Arthritis.The Iraq postgraduate medicaljournal, 2014; 13: 4.
24. Paramalingam, Sivalingam Suppiah Thumboo, Julian Sheila Thio, Szu Tien Tse, Connie Fong, Kok-Yong. In vivo Pro- and Anti-inflammatory Cytokines in Normal and Patients with Rheumatoid Arthritis. ANNALS-ACADEMY OF MEDICINE SINGAPORE, 2007; 36: 2-96.
25. Schiff MH. Role of interleukin 1 and interleukin 1 receptor antagonist in the mediation of rheumatoid arthritis. *Ann Rheum Dis*, 2000; 59(I): i103-i108.
26. Tobon, G. J.; Youinou, P. and Saraux, A. The environment, geo-epidemiology and autoimmune disease: rheumatoid arthritis. Autoimmunity Review., 2010; 9: A288-292.
27. Weyand, C. M. and Goronzy, J. J. The molecular basis ofrheumatoid arthritis. J. Mol. Med., 1997; 75: 772-785.
28. Wilder, R. L. Adrenal and gonadal steroid hormone deficiencyin the pathogenesis of rheumatoid arthritis. J. Rheumatol., 1996; S44: 10-12.
29. Ziolkowska M, Koc A, Luszczykiewicz G, et al. High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. *J Immunol*, 2000; 164(5): 2832–2838.