



ISSN: 0067-2904
GIF: 0.851

The role of Interleukin -18 / Interleukin -18 Binding Protein in Rheumatoid Arthritis patients

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritic disease associated with remission and exacerbation with different immunological features. Rheumatoid factor (RF) screening test was used quantitatively and qualitatively using an enzyme immunoassay (EIA), RF-isotypes (RF-IgG, RF-IgM, RF-IgA) , Interleukin-18 (IL-18) and Interleukin – 18 binding protein (IL-18BP) were detected quantitatively using enzyme linked immunosorbent assay (ELISA). Results indicated a 51% positivity in RF among RA patients and a prevalence of the RF-isotype (RF-IgM) with the highest level at $(41.6 \pm 16.3 \text{ U/ml})$ ($p < 0.05$) among other RF-isotypes at a percentage of 85.5% , results also indicated an elevated serum level of IL-18 and IL-18BP ($225 \pm 24.8 \text{ pg/ml}$), ($5.2 \pm 2.1 \text{ ng/ml}$) respectively compared to healthy control (HC) group ($65 \pm 8.4 \text{ pg/ml}$) ($p < 0.01$) , ($2.9 \pm 1.3 \text{ ng/ml}$) ($p < 0.05$) respectively, and ratio of IL-18/IL-18BP serum levels in RA patients were significantly higher than in HC group ($p < 0.05$) which increased the importance of IL-18BP in therapeutic strategy. In conclusion RF-IgM may be an important complimentary detection method for RA among the other isotypes , IL-18 with its IL-18BP may play an important role in RA which need to be further investigated.

Keywords: Rheumatoid arthritis, Interleukin-18, Interleukin – 18 binding protein.

دور الانترلوكين-18 والبروتين الرابط للانترلوكين-18 لدى مرضى التهاب المفاصل الرثوي

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الخلاصة

التهاب المفاصل الرثياني هو مرض التهابي مزمن يرتبط مع سكون وتفاقم للمرض بالتناوب مع ملامح مناعية مختلفة . فحص العامل الريماتويدي (RA) من خلال التحري عنه كميًا ونوعيًا باستخدام تقنية التحري المناعي الإنزيمي (EIA) ، أما العامل الريماتويدي الخاص بالأصناف (RF-isotypes, IgG,IgM,IgA) و الانترلوكين -18 (IL-18) والبروتين الرابط للانترلوكين-18 (IL-18BP) تم التحري عنها كميًا باستخدام تقنية التحري المناعي الممتز المرتبط بالأنزيم (ELISA). أظهرت النتائج أن 51% من المرضى كانت فحوصهم ايجابية لفحص (RA) مع غلبة العامل الريماتويدي الخاص (RA-IgM) عند أعلى مستوى له (41.6±16.3 U/ml) (P< 0.05) أمام غيره من الأنواع ونسبة مئوية مقدارها 85.5%. أظهرت النتائج أيضًا ارتفاع المستوى المصلي ل (IL-18) و (IL-18BP) (225 ± 24.8 pg/ml) (5.2 ± 2.1 ng/ml) على التوالي مقارنة بمجموعة السيطرة (65 ± 8.4 pg/ml) (p<0.01) (2.9 ± 1.3 ng/ml) (P<0.05) على التوالي. إن نسبة المستوى المصلي IL-18/IL-18BP لدى مرضى التهاب المفاصل (RA) كانت أعلى من تلك التي وجدت لدى مجموعة السيطرة (P<0.05) مما يزيد من أهمية هذا البروتين في احتمالية استخدامه في إستراتيجية العلاج لهذا المرض. وكاستنتاج للدراسة نجد أن RF-IgM من الممكن أن يكون فحص تكميلي للتحري عن المرض دونًا عن غيره من الأنواع وان IL-18 مع بروتيينه IL-18BP ممكن أن يلعبان دورًا مهمًا في مثل هذا المرض مما يتطلب تحريات أكثر في دوره بمرض التهاب المفاصل الرثياني.

Introduction :

Rheumatoid arthritis (RA) is a systemic inflammatory disease that usually affects the synovium , with local symptoms of joint swelling, morning stiffness and pain as well as inflammation and subsequent cartilage damage with destruction [1]. It is also considered as an immune mediated disease that could possibly be triggered by an environmental factor in genetically susceptible individuals [2]. RA result from chronic inflammatory process mediated through a complex of cytokine network which participate in all phase of immune response [3] . The release of specific cytokines into the systemic circulation has been observed in a variety of inflammatory disorders including RA , there concentration levels usually reflect disease severity and prognosis. The cytokine expression in rheumatoid joints began to appear in the pathogenesis and treatment of disease, blocking the action of these cytokines has been used in RA treatment [4]. Interleukin-18 is a pleiotropic cytokine involved in both innate and adaptive immune response [5], and is widely expressed in monocytes , macrophages, keratinocytes and other cells , usually identified as interferon inducing factor[6]. IL-18 is characterized by both structural and functional homology to the IL-1 β family cytokine[7]. IL-18 appears to be a pivotal mediator of T-helper type-1 cell responses such as induction of T-helper 1 cytokines including (IFN- γ) synergistically with IL-12 [8], it was also shown to facilitate the development of Th2 response in the absence of IL-12 [9]. IL-18 exerts its function by binding to the IL-18 receptor complex (the ligand binding chain IL-18R α and the signaling chain IL-18R β) , these both chains are required for IL-18 signaling [10].

Interleukin -18 is unique among other cytokines in that it has a specific binding protein named interleukin -18 binding protein which is expressed in the spleen, colon and small intestine and found in plasma , it belongs to a growing family of soluble binding proteins [11]. Its major splice variant IL-18BP α is a unique , constitutively secreted protein , which binds IL-18, thus blocking its biological activity . Although the binding of IL-18 to IL-18BP α is highly specific, it is structurally unrelated to the two components of the cell bound IL-18 receptor complex (IL-18R α and IL-18R β)[12].IL-18 activates IL-18 receptor through two step binding mechanism , according to this model , the inhibitory mechanism of IL-18BP and the Fab antibody fragment are different , Figure-1.

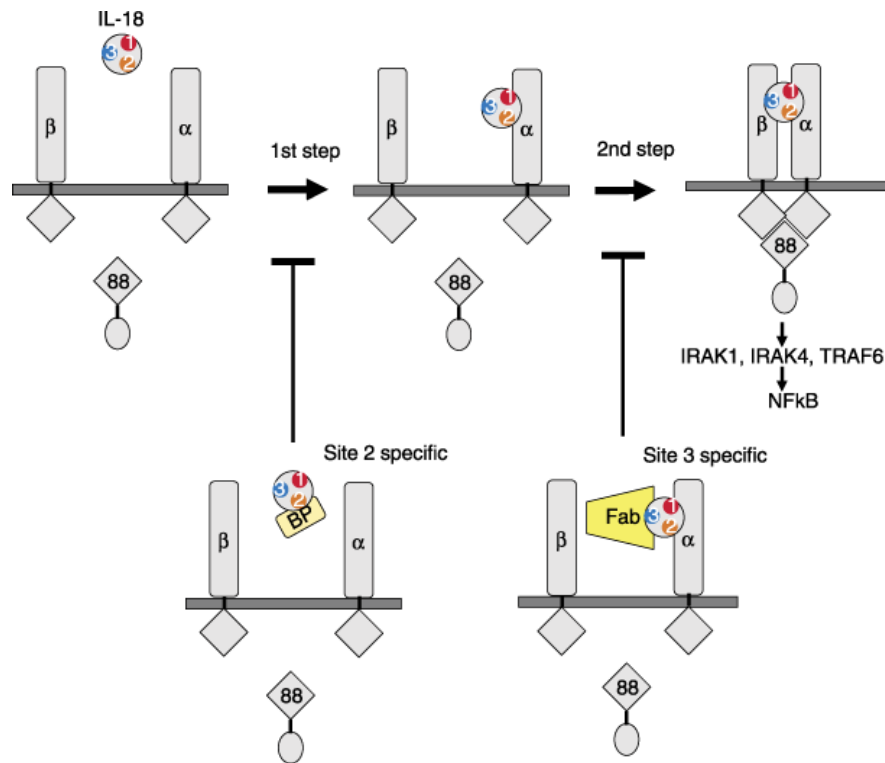


Figure 1-A schematic model of IL-18 receptor activation and inhibition. A two-step complex formation model of IL-18 signaling is shown at the top. The inhibitory mode of IL-18BP or Fab fragment is shown at the bottom. [13].

Interleukin-18BP inhibits the receptor activation by competing for site 2 on IL-18 , which is also a binding site for IL-18 α . On the other hand , the Fab antibody fragment bind to the site 3 on IL-18 to block IL-18 from binding to IL-18BP [13]

Evaluation of IL-18 and IL-18BP in RA patients was an important goal of this study , to stand upon their role and to shed light on such unique protein as a normal inhibitor of IL-18.

Materials and Methods :

Study population:

This study was carried out on 70 (RA) patients . Certain exclusion criteria were followed to avoid interference with our results ; patients who take immunosuppressant agents or other autoimmune disorders and pregnant women. The patients were diagnosed and treated in Baghdad Teaching hospital / Medical city . Clinical examinations were performed in the Department of Rheumatology under supervision of a specialist physician . Healthy control subjects (HC) with total number of 30 were included in this study , they had no history of clinical evidence of RA or any other chronic disease .

Measurement of RF, RF- isotypes, IL -18 and IL-18 BP :

Blood samples (10)ml were collected from both patients and controls by vein puncture using disposable syringes . The aspirated blood was immediately transferred into plane test tubes and was allowed to clot at room temperature for 30 min. and then was centrifuged for 5 min. at 2000 r.p.m, then sera was separated and kept at deep freeze (-20C°) to be used later for further investigations including:

1. Quantitative and Qualitative detection of rheumatoid factor (RF) screening test (RF check enzyme immunoassay kit, Aesku, Diagnostic GMBH, Mickroforum).
2. Quantitative measurement of RF isotypes (ELISA kit of RF-IgG and IgM , Euroimmun, Germany).
3. Enzyme immunoassay for serum IL-18 and IL-18BP estimation (ELISA kit Biosource, Belgium ; ELISA kit BioRad, USA) respectively .The minimal detection level for IL-18 was 12.5 pg/ml and for IL-18BP was 0.062 ng/ml , levels below the detection limits is considered as zero.

Statistical analysis:

The results were expressed either as frequencies with their percentage , or presented as Mean \pm SEM . A paired t-test was used to compare the parameters between patients with RA and healthy control subjects. Tests also included Chi-Square test(X^2). Statistical analysis was assumed for P value less than 0.05, 0.01.

Results and discussion :-

1. Distribution of study groups:

Patient group subject ages was ranged between (20-65) years , the mean age of RA was (45.4 \pm 9.4) and (36.0 \pm 3.5) for HC group , Table 1.

Table 1-Distribution of the studied groups according to age.

Study group		Age range (years)			Total	Mean age \pm SEM	Male:Female
		20-40	41-60	>65			
RA	No	20	34	16	70	45.4 \pm 9.4	18:52**
	%	28.6	48.6	22.8	100		
HC	No	15	10	5	30	36.0 \pm 3.5	12:18
	%	50	33.3	16.6	100		

RA: Rheumatoid arthritis , **HC:** Healthy control , **: highly significant as compared to HC group at ($p < 0.01$).

The majority of RA patients were of the age range at (41-60) years , according to gender distribution the majority of RA patients were of females (74.3%)with a M:F ratio of 18:52 , while frequency of females among the HC group was 60% with M:F ratio 12:18 , Figure-2. female prevalence among RA patients was significantly higher compared to HC group ($p<0.01$).

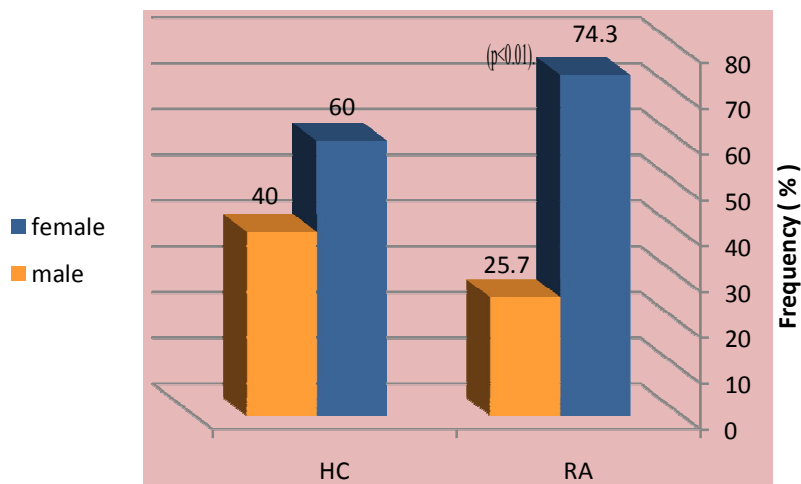


Figure 2-Percentage distribution of study groups according to gender.

Detection of RF and RF isotypes :

Rheumatoid factors has been used as markers for RA and were included into RA classification criteria [14]. These included quantitative and qualitative screening test , the quantitative determination for RF isotypes has been applied for positive samples .

In this study , there was a significant difference ($p<0.05$) between RA patients and HC group in the frequency , showing a 85.5% for RF-IgM as a predominant isotype, as shown in Figure-3. The positivity percentage of RF for RA patients was (51%), whereas , among RF-isotypes , the RF-IgM showed the highest level of 41.6 ± 16.3 U/ml Table 2, which was significantly different when compared with other RF-isotypes in the RA group ($p<0.05$).

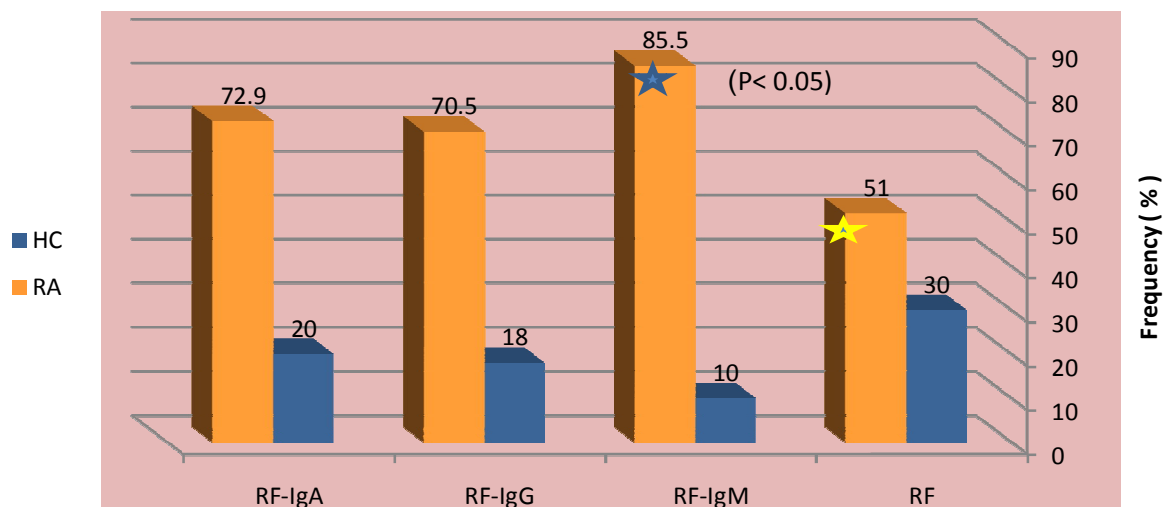


Figure 3- Frequency percentage of RF and its isotypes among RA patients and HC group .

Table 2- Mean distribution of RF screening and RF isotypes among RA and HC group .

Study groups	No.(%)	Mean \pm SEM (U/ml)			
		RF	RF-IgM	RF-IgG	RF-IgA
RA	36 (51)	38.2 \pm 21.1	41.6 \pm 16.3*	28.7 \pm 15.8	32.3 \pm 20.5
HC	10(33)	10.0 \pm 4.9	6.71 \pm 4.1	7.05 \pm 3.1	12.5 \pm 6.3

*(p<0.05)

IL-18 and IL-18 BP levels among sera of RA and HC groups:

Serum levels of IL-18 had been elevated significantly in RA group (225 \pm 24.8 pg/ml) , compared to HC group (65 \pm 8.4 pg/ml) at (p<0.01). serum levels of IL-18BP were also elevated significantly in RA patients (5.2 \pm 2.1 ng/ml) compared to HC group (2.9 \pm 1.3 ng/ml) at (p<0.05), Figure-4.

Serum IL-18/IL-18BP ratio :

The ratio of serum levels of IL-18 and IL-18BP in patients with RA was significantly higher than the HC group (p<0.05) , Figure-5.

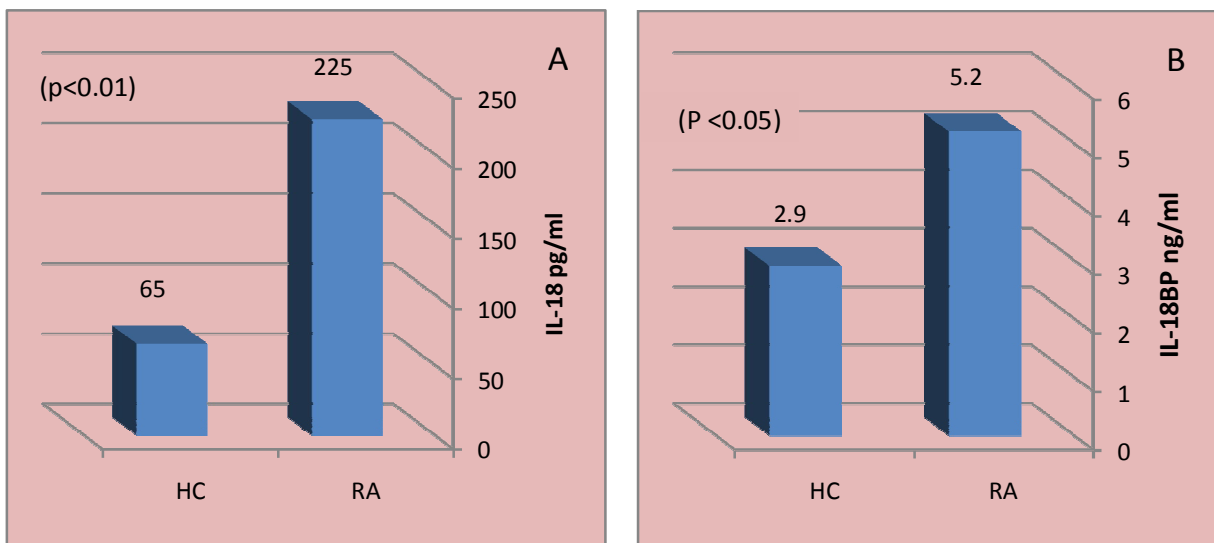


Figure 4-A: represent the concentration of IL-18 pg/ml, **B:** represent the concentration of IL-18BP ng/ml.

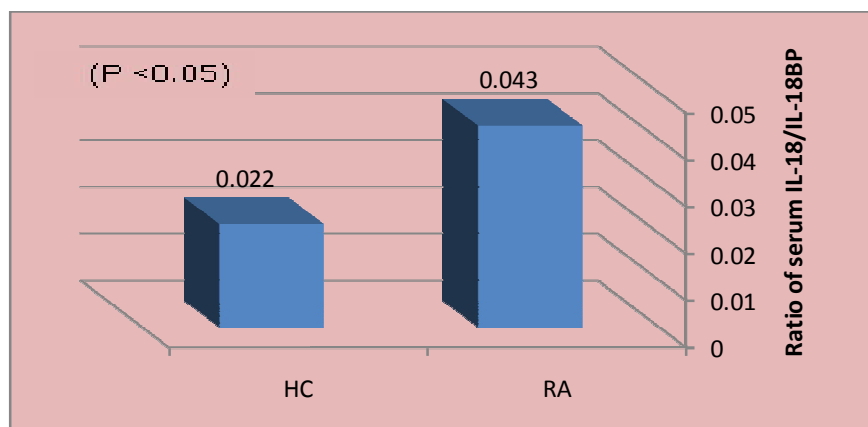


Figure 5- Ratio of IL-18 to IL-18BP in RA and HC at ($p < 0.05$).

Rheumatoid arthritis is considered as an immune-mediated disease that could be possibly triggered by environmental factors in a genetically susceptible individual [15], it is also a disease that occurs at any age and may increase with it [16]. The results of this study revealed that the age range for the majority of RA patients was at 41-60 years, which indicated RA within the fourth or fifth decade.

Among RF-isotypes, RF-IgM was most predominant and gave the highest level when detected by the means of quantitative manner at a level of 41.6 ± 16.3 U/ml, other studies showed that IgG isotype may be the predominant isotype [17]. These differences may be related to the stage of disease, in which the level of RF-IgG elevated during the chronic stage rather than the acute stage in which the RF-IgM level is elevated or during a specific therapy treatment [18], or the differences may be related to the severity of the case where the high level of RF-IgA is shown in more severe disease outcomes during the time of collecting blood samples, this may lead to a conclusion, that detection of RF-IgM in serum may be a strong indicator for RA [19].

Measurement of circulating cytokines in health and disease provides information about the process and progression of disease in terms of severity , effects of therapy , as well as, indicators of mortality and survival . Several studies reported circulating levels of cytokine – interleukine-18 in health and various disease [20]. However , in any study on the role of particular cytokine associated with disease concurrent measurement of naturally occurring specific inhibitor of that cytokine is desirable . In the case of assessing the role of IL-18 in disease , such naturally occurring inhibitor is the IL-18BP[21].Results of this study showed a significant increase in IL-18 level for RA patients , as well as a significant increase in the inhibitory factor IL-18BP for the RA patients, these findings suggest that IL-18 and its inhibitory factor IL-18BP may play an important role in the pathogenesis of RA , IL-18 therefore represent an exiting novel inflammatory mediator that is up regulated in numerous clinical situations including autoimmune rheumatic disease [22] .Many studies shed light on the role of IL-18BP and that administration of IL-18BP abolishes the induction of IFN- γ , IL-8 and activation of nuclear kappa- β [23]. Thus the IL-18BP acts as an inhibitor in the early Th1 response . Several studies mentions that several viruses produce proteins homologous to the IL-18BP which possess the ability to bind and neutralize human IL-18 in a fashion similar to that of IL18-BP[24], suggesting that viral products may interfere with cytokine T-cell response [25].

Elevated serum levels of IL-18BP have been reported in idiopathic thrombocytopenic purpura [26], systemic lupus erythematosus [27] , allergy [28] , inflammatory bowel disease [29] , and other diseases [30]. Experiments have shown that IL-18BP could prevent or attenuate the development of some autoimmune diseases [31].The results demonstrated that , despite the compensatory , raised levels of IL-18BP in serum of patients with RA , the ratio IL-18/IL-18BP in these patients was higher than that in HC group . this indicated that IL-18BP may not be present in amounts sufficient for blocking the proinflammatory activities of IL-18 in RA . This imbalance have been reported in other studies such as secondary hemophagocytic syndrome [32] and Henoch-Schonlein purpura [33].

In conclusion , the present study showed that RF-IgM was an important indicator of RA and that IL-18 and IL-18BP were constitutively raised, and resulted in an increase in the IL-18/IL-18BP ratio. Therefore , regulating the balance between IL-18 and IL-18BP might be a potential therapeutic strategy in the treatment of RA , and further studies are needed.

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