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العلاقة بين التباين الوراثي في جينات بين الأبيضاضي IL1-α IL33,IL36, والأصابة بألتهاب المفاصل الرثوي وبعض المؤشرات المناعية في عينة لمرضى التيفوئيد

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Relation between IL-1α , IL-33 and IL36 gene polymorphism and the susceptibility to Rheumatoid Erthritis in a sample of typhoid patients

A thesis
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1- المقدمة (Introduction)

يعد ألتهاب المفاصل الروماتويدي (RA) من أمراض المناعة الذاتية المزمنة وأكثرها إنتشاراً، يصيب المفاصل في المقام الأول ويمكن أن يظهر هذا المرض الإصابات الخطيرة في العديد من المفاصل (القدم ، الركبة ،الورك ، العمود الفقري، الكتفان) وأصابات خارج المفصل (العقد الرثوية، ضعف العضلات ، اصابات العين ، أمراض القلب والتهاب الأوعية ، التهاب الرئة) له تأثير كبير على معدلات الاعتلال والوفيات لدى المرضى (Mitsuhiro وآخرون، 2022). لصعوبة تشخيصه لكونه يتضمن العديد من الخصائص السريرية والمظهرية وفترات من الأستقرار والتفاقم فقد وضعت الجمعية الأمريكية لأمراض الروماتيزم معايير لتصنيفه (Chang وآخرون، 2016). يهاجم مرض التهاب المفاصل الروماتويدي العديد من مفاصل الجسم ومختلف أنواع الخلايا مما يحفز الجهاز المناعي على تفعيل الاستجابة المناعية الذاتية فضلاً عن أحداث الالتهابات النسيجية المتفاقمة (Rada و Bungau ، 2021). يتميز إلتهاب المفاصل الروماتويدي بإلتهاب الغشاء المفصلي المستمر والألتهاب الجهازي وظهور الأجسام المضادة الذاتية (خاصةً العامل الروماتويدي والببتيد السيترولي). يُعزى خطر الإصابة بألتهاب المفاصل الروماتويدي إلى عوامل وراثية وعوامل بيئية والعديد من العوامل التي تشكل خطورة كالتدخين الذي يعد الخطر البيئي الرئيس وكذلك الأصابات الميكروبية والعدوى البكتيرية كبكتريا السلمونيلا وكذلك تأثير العمر والجنس والسمنة على المرض (Littlejohn و Monrad، 2018). يعد التهاب المفاصل الروماتويدي الأكثر شيوعًا عند النساء وكبار السن إذ يتسبب التهاب المفاصل الروماتويدي النشط غير المنضبط تلف المفاصل ، والعجز ، تشوهات بمفاصل الجسم تعيق ممارسة الحياة الطبيعية والأمراض القلبية الوعائية والأمراض المصاحبة الأخرى (Sefat وآخرون ،2019). يتعرض نظام المناعة الفطري (Innate Immune System) الى الاضطراب قد يكون هذا جزئيًا بسبب إنتاج الأجسام المضادة الذاتية مثل عامل الروماتويد (RF) والأجسام المضادة للبروتينات المضادة للسيترولين (ACPAs) التي قد يكون لها تأثيرات مهمة على الجهاز المناعي مما يؤدي إلى فقدان الجهاز المناعي قدرته على التمييز بين الأجسام الغريبة

وأنسجة الجسم هذا يقود الى مهاجمة تلك الاضداد مختلف خلايا وأنسجة الجسم (Narendra وآخرون،2019).

يصاحب الإصابة بألتهاب المفاصل الروماتويدي انتهاك التنظيم المناعي في الغشاء الزليلي للمفصل مما يؤدي إلى تلف بالغ وتدمير للغضاريف والعظام. لا يتسبب هذا المرض فقط في العديد من الأعراض الجهازية ، مثل الحمى ، وفقر الدم ، وهشاشة العظام ، أو ضعف العضلات ، ولكنه يزيد أيضًا من الإصابة بأمراض القلب والأوعية الدموية والتهاب الرئة وخطر الإصابة ببعض أنواع السرطان في موقع الإلتهاب الموضعي، أي المفصل، تؤدي الأضطرابات في مسارات الحركيات الخلوية والجاذبات الكيميائية إلى ترشيح مناعي يساهم في زيادة تكاثر الخلايا الليفية المفصلية، المسماة بالخلايا الزليليّة ومن المظاهر الأخرى للمرض هي المظاهر الدموية التي يرافقها حدوث الاضطرابات الدموية من نقص أو زيادة في أعداد خلايا الدم البيضاء والحمراء والصفيحات الدموية وفقر الدم(Azizoddin وآخرون، 2017) .

يتضمن التسبب في التهاب المفاصل الروماتويدي شبكة معقدة من الحركيات الخلوية والخلايا المختلفة التي تؤدي إلى تكاثر الخلايا الزليليّة وتسبب تلفًا لكل من الغضاريف والعظام وأهمها عائلة الحركيات الخلوية من البين الأبيضاضي 1 الذي يتكون من سبعة روابط ذات نشاط مؤيد للألتهابات (IL-1α و IL-1β و IL-18 و IL-33 و IL-36α و IL-36β و IL-36γ). من المعروف أن هذه الحركيات الخلوية تؤدي دورًا رئيساً في تعديل كل من استجابة المناعة الفطرية والتكيفية، مع خلل في التنظيم مرتبط بمجموعة متنوعة من أمراض المناعة الذاتية والألتهابات و التسبب في التهاب المفاصل الروماتويدي ، وبينت الدراسات الحديثة أن الحركيات الخلوية تؤدي دور في تطوير المرض (Kevin واخرون، 2019) .

يؤدي الجانب الوراثي دوركبير في تطور إلتهاب المفاصل الروماتويدي؛ إذ بينت الدراسات الى وجود علاقة بين الخصائص الجزيئية لبعض الجينات والإصابة بالمرض عن طريق ظهور حالات جديدة في الأسر التي يوجد مصاب بالمرض بين أفرادها فقد لوحظ أن التباين الوراثي في بعض الجينات مثل جينات الحركيات الخلوية قد يكون مؤشر جزئي مصاحب للإصابة بالمرض (Yapو Lai ، 2013). ومن هذه الحركيات الخلوية البين الأبيضاضي 1 الفا، البين ألابيضاضي 33

Abstract

The current study was conducted on a sample of Iraqi patients with rheumatoid arthritis and Salmonella typhi who attended the consulting clinics for joint diseases in the Baghdad Teaching Hospital of the Medical City in Baghdad Governorate and all governorates of Iraq who attended the consulting clinic for the period between December 2020 and February 2021. Blood samples were collected Of 60 patients with rheumatoid arthritis and infected with Salmonella typhi bacteria in a hospital with ages ranging from 21-70 years, the number of females was more than the number of males with a significant difference, as the number of females was 55, (91.7%) , while the number of males was 5, (8.3%). For the purpose of comparison, 28 other blood samples were collected from healthy people (healthy control), ranging from 21-60 years, and the number of males was 5, (17.9%) , and the number of females was 23(82.1%). The results indicated that the highest infection rate was in the age group 41-60 years, with a percentage of 51.7%, and the lowest was in the age group (≤20) years (3.3%). The results indicated that there was a statistically significant difference ($P<0.01$) between the infection rate. The disease occurred in married couples and was the largest compared to the infection rate among unmarried couples (80.0%) and (20.0%), respectively.

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for the same number of patients whose periods exceeded They were (20) years old. The results indicated that there were highly significant differences between the education levels of the patients, as the highest infection rate was among those with primary education at a percentage of (45%), while the lowest percentage was among patients with secondary and university education at a percentage of 11.7%. The disease was studied in patients infected with Covid 19 virus and those who were not infected with it, as the results showed that the number of people infected with the virus was (13), with a percentage of 21.7%, while the number of those without it was 47 with a percentage 78.3%, with a highly significant difference, $p<0.001$.

The results indicate that there is no statistically significant difference in the numbers of white and red blood cells and hemoglobin concentration between (patients and healthy groups), as the average number of white blood cells 8.14 (m/mm^3) was compared to the mean of healthy subjects, which amounted to8.22 (m/mm^3). While the average number of red blood cells (m/mm3) was 4.28 for patients compared to healthy subjects, which amounted to 4.44 (m/mm3), while the concentration of hemoglobin of patients was11.94 (g/dl) compared with healthy subjects, which amounted to 12.70 (g/dl). On the other hand, the results indicated that there was a significant difference, $P<0.05$, between the platelet counts of the patients, at a rate of 304.75, compared to the average of the healthy subjects, which was 247.75. The results also indicated that there was a significant increase ($P<0.01$) in the erythrocyte sedimentation rate in patients with the healthy control. The sedimentation rate was 46.10 mm/hour, while it was 30.68 mm/hour in the healthy control. The results indicated that there was a significant increase ($P<0.001$) in the concentration of cyclic citrullinated peptides antibody protein in blood samples of patients compared to healthy subjects, as the average protein in the blood of patients was 406.62 mg/L compared to 66.07 mg/L in healthy subjects. And that there was a significant increase ($P<0.001$) in the level of interleukin- 1 alpha in patients 2.87 units / ml, while in healthy subjects 1.36 units / ml. The results also indicated that there was a highly significant increase ($P < 0.001$) in the interleukin- 33 for the patient sample 19.09 mg / dL, while the healthy ones were 8.18 mg / dL. The results also indicated that there was a significant increase ($P < 0.001$) in the interleukin 36 In patients, interleukin 36 was

284.65 ng/l compared to healthy subjects, which was 238.59 ng/l. The results indicated that there was a highly significant increase (P<0.001) in the rate of immunoglobulin IgG in patients, which amounted to 3.39 (ug/ml) compared to healthy controls, which amounted to 0.72 (ug/ml). The results indicated that there was a highly significant increase (P<0.001) in the rate of immunoglobulin IgM in patients, which amounted to 8.75 (ug/ml) compared to healthy subjects, which amounted to 5.32 (ug/ml). The results indicated that the concentration of the extracted DNA for all samples ranged between 90-120 ng/microliter and a high purity ranged between 1.7 to 2. The results also indicated that sharp DNA bands were obtained after electrophoresis on an agarose gel (1.5%). Then, the exact locus rs1304037 of the amplification segment of 337 bps length that covered part of the 3′-UTR of the interlukin 1 alpha gene within chromosome 2 was amplified using primers specialized for this locus and the nucleic acid sequence of this gene was analyzed. One DNA substitution was detected in the amplified PCR outputs, namely 176T>C or T176C. This heterozygote was detected in three multiple genotypes. In patient samples, the heterozygous T/C genotype was observed more prevalently than the other examined samples in 26 out of 60 samples, which is followed, respectively, by the homozygous T/T genotype in 24 out of 60 samples and then the C genotype. / C homozygous and by 10 samples out of 60 samples. As for the control samples, it was noted that the homozygous T / T genotype is the most prevalent compared with the other samples that were examined, by 15 out of 28 samples, which is followed, respectively, by the heterozygous T / C genotype, by 10 out of 28 samples and from Then the C / C type is homozygous, with a rate of 3 out of 28 samples. Regarding genotype rs1304037 (T/G),The results indicated that the patient cohort exerted a nonsignificant association of the distribution of TT, TC and CC genotypes based on rs1304037 with RA. Individuals with the TC genotype showed no significant risk of developing RA among the compared groups. The results included that individuals with the C allele did not have a significant risk of developing rheumatoid arthritis. The interlukin -33 gene was amplified at the exact locus rs1375 of the amplification segment of length 421 base pairs that partially covers the regions of the second intron-2 of the interlukin-33 gene within chromosome No. 9 by using specialized primers for this site and analyzing the DNA sequence of all studied samples. One DNA substitution

was detected in the amplified PCR prohncts, that is, 165G>T or G165T. This heterozygote was detected in three polymorphic haplotypes, G165T. In patients' samples, the results indicated that the homozygous T/T genotype was found more frequently compared with other samples examined in patients' samples by 28 out of 60 samples in patients and 16 out of 28 samples in control, which was followed by genotype respectively. T / G heterozygous, 27 out of 60 in patient samples and 11 out of 28 in control samples, and homozygous G / G genotype, 5 out of 60 in patients and 11 out of 28 in control samples. The results also indicated that the homozygous T / T genotype is the most prevalent form compared with the other samples examined in (28 out of 60 samples in patients and 16 out of 28 samples in control). And followed, respectively, by the heterozygous T/G genotype (27 out of 60 samples in patients and 11 out of 28 in control), and the homozygous G/G genotype (5 out of 60 samples in patients and 1 out of 28). in control samples). The results indicated that the patient group showed a non-significant association regarding the distribution of TT, TG and GG genotypes based on rs1375 with RA. Individuals with the TG genotype showed no significant risk of disease among the comparison groups. The results also indicated that there were no statistically significant differences between individuals carrying the CC genotype in both patient and control samples. and that individuals carrying the G allele had no significant risk of developing rheumatoid arthritis compared with individuals carrying the reference T allele. The results indicated that the interlukin gene 36 alpha was performed with exact locus amplification. The variant rs2305152 and variant rs895497, respectively, were analyzed for the leukoplakia gene 36 alpha according to the Sanger method for the amplification segment of 226 pairs in length, partially covering the regions of the pre-translational segment - the first exon - the first intron - the exon. The second (3-UTR-exon 1- intron-1-exon-2) of this gene using specialized primers for this site and analyzing the nucleic acid sequences of all the studied samples. The rs2305152-target variant in which an adenine-cytosine DNA substitution was detected at position 77 of the PCR-amplified segment, namely 77A>C or A77C. This heterotroph has been detected in three polymorphic patterns. In the patient group, both the homozygous A/A genotype and the heterozygous A/C genotype were observed equally in prevalence compared to the other examined samples by 29 out of 60 samples

each, followed by the homozygous C/C genotype by 2. from a total of 60 samples. In the control group, the dominant form was found in the homozygous A / A genotype in 14 of the total 28 samples. After this pattern, the heterozygous A / C genotype comes (9 out of 28 samples), then the homozygous C / C genotype (5 out of 28 samples), respectively. The results also indicated that in the second variant that was discovered , in which the DNA was replaced by an adenine to a cosine at position 189 with a high frequency, i.e. 189A>G or A189G. This heterotroph has been detected in three multiple phenotypes. In the patient group, homozygous type A/A was observed at a very low frequency in both patients (2 out of 60) and control (1 out of 28) samples. The results also showed that the homozygous G/G genotype was found in the highest percentage in both groups (41 out of 60 and 20 out of 28 in patients and control, respectively). While the A/G genotype was found to be heterozygous in an intermediate proportion compared to the other two types (17 out of 60 and 7 out of 28, respectively). The detected A77C and A189G heterozygotes were found to be identified as rs2305152 and rs895497, respectively. The results also showed that the targeted variant rs2305152 was located in the intron sequences, while the non-targeting variant rs895497 was found in the coding region of the 36 alpha gene. The rs895497 variant causes a change in the amino acid sequence of albicans protein 36 alpha (a missense mutation) due to the substitution of the amino acid isoleucine with an alanine at position 36 of the full length of the protein (p.36Ile>Ala). With regard to the rs2305152 heterozygous, it was observed that the patient cohort showed a nonsignificant association of the rs2305152-based AC genotype distribution with RA. Individuals with AC genotype showed no significant risk of developing RA among the compared groups. The results indicated that there was a significant difference between individuals with CC genotype between patients and control samples. The results indicated that individuals with the CC genotype showed a significant protective ability against the development of rheumatoid arthritis compared to individuals with other genotypes. With regard to the rs895497 heterozygous, it was observed that the patient cohort showed a nonsignificant association of the rs2305152-based AG genotype distribution with RA disease. Individuals with the AG genotype showed no significant risk of developing RA among the compared groups. The results did not indicate any significant difference

between individuals carrying the CC genotype in patients and control, and the results indicated that the G allele did not show statistically significant differences in the distribution between patients and control. The results of the current study indicated that there were significant differences in the genotype distribution of rs2305152:CC between control and patient samples. The rs2305152:CC genotype showed a significant decrease in its ratio from control samples (17.9%) to patient samples (3.4%). The statistical calculations showed that there is a significant correlation between this genotype and rheumatoid arthritis for samples infected with Salmonella typhi (P = 0.0348). Statistical analyzes of this study clearly showed that individuals carrying the rs2305152:CC genotype showed a protective effect against the development of RA as their OR indicated significantly lower values in terms of the development of this autoimmune disease (P = 0.1586). This type of association was not reflected in the C allele itself in terms of its association with rheumatoid arthritis (P = 0.3847). The results indicated the recessive character of the C allele. Therefore, This allele cannot show any phenotypic effect on its own and requires another homozygous allele to exert such a protective effect. Interesting results of the current study between the CC genotype of this heterozygote and rheumatoid arthritis indicated a significant relationship despite the low number of study samples examined. This means that adding a larger number of study samples can produce more positive data in this regard. The results showed that no previous studies have been published in the PubMed database describing any association between this variant and the development of any significant cellular abnormalities. In addition, This variant was not detected in the ClinVar database, since there was no previous study in this regard, the result of the current study is the first to report a significant association between the rs2305152 polymorphism and rheumatoid arthritis. And the first study that studied patients with rheumatoid arthritis and those infected with Salmonella typhi.