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تقييم دور تعدد الأشكال الوراثية والمناعية لبعض الحركيات الخلوية في مرضى داء الثعلبة

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Evaluation the role of genetic polymorphisms and immunological of some cytokinesis in patients with alopecia areata

A Thesis
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1- 1 المقدمة Introduction

يعد داء الثعلبة Alopecia Areata(AA) من أمراض المناعة الذاتية Autoimmune diseases يحدث نتيجة مهاجمة الخلايا المناعية لبصيلات الشعر، ويتمثل بتساقط الشعر على هيئة بقع دائرية صغيرة الحجم، وعادة ما تبقى هذه البقع متمركزة في مواضع معينة دون الإنتشار إلى أماكن أخرى في الجسم، ويعد ظهور هذه البقع عاملاً مميزاً لداء الثعلبة، إذ تظهر في جميع مناطق الجسم وبالأخص في الوجه والرأس(Rajabi وآخرون،2018) وتجدر الإشارة إلى ان داء الثعلبة يمكن ان يصيب الأشخاص من مختلف الفئات العمرية سواء كان من الذكور أم الاناث وفي أي مرحلة عمرية، إلا أنَّه عادة ما تظهر الإصابة به في مرحلة الطفولة، ولا يؤثر في قدرة المصاب على ممارسة حياته الطبيعية. ولكن من الممكن ان ينتج عنه توتر واضطرابات نفسية بظهور بقع ملساء ورطبة دون شعر ولا يمكن التنبؤ بفترة الاصابة بداء الثعلبة إلى حد كبير، يمكن ان تحدث في أي عمر منذ الولادة وحتى العقود الأخيرة من العمر، ليس له عمر أو جنس أو ميول عرقية محددة (Strazzulla وآخرون، 2018).

تؤدي بروتينات نظام المتمم دورًا في حث الاصابة وتنشيط التفاعلات الألتهابية لدى المرضى المصابين بداء الثعلبة وتحفز الخلايا التائية على إنتاج الحركيات الخلوية التي تحفز بدورها الخلايا البائية التي تتمايز إلى خلايا بلازمية لإنتاج الاجسام المضادة التي تهاجم مكونات متعددة من بصيلات الشعر منها الطور التنامي وأماكن تكون الميلانين بأعتبارها مستضد مستهدفة ولم يكتشف لحدالآن مستضد ذاتي محدد للأجسام المضادة خاص ببصيلات الشعر لدى الافراد الاصحاء (Zeberkiewicz وآخرون، 2020). وبالإضافة إلى ذلك ، تؤدي الحركيات الخلوية الألتهابيةCytokines دوراً مهماً في التسبب بداء الثعلبة، إذ ان إرتفاع مستويات IFN-γ أعلى بكثير

في مرضى الثعلبة الكلية أو الشاملة مقارنة بمجموعة السيطرة وذات أشكال أكثر اتساعًا (Gregoriou وآخرون،2010). تشير الدراسات الحديثة إلى إرتفاع اعداد خلايا CD_8^+ T cell آحادية النسيلة لدى مرضى الثعلبة التي ترافق معقد التوافق النسيجي الكبير من الصنف الأول MHC−1 وبدورها تؤثر على الخلايا التائية T lymphocytes التي تعبر بشكل أساسي عن IFN−γ و IL−17 في وقت مبكر من الأصابة، مع زيادات كبيرة في انتاج الحركيات الخلوية الآخرى IL−4 و IL−10 مع تطور المرض إلى داء الثعلبة الشامل، مما قد يشير إلى التكرار في استجابات الحركيات الخلوية (Alli وآخرون، 2012؛ Guo وآخرون، 2015).

يرتبط داء الثعلبة البقعي بخلل في التنظيم الجهازي للخلايا التائية Th1 و Th2 و Th17 ويدل على ذلك إرتفاع تركيز السايتوكينات الخلوية كل من IL−2 وIL−12 و TNF و IL−17 و IL−17 E وبالإضافة إلى زيادة مستوى IL−13 و IL−14 في مصل المصابين بداء الثعلبة (Waśkiel−Burnat،2021) بحيث يرتفع مستوى IL−17A و IL−17F و IL−23 و IL−31 و IL−33 في مصل المرضى المصابين بداء الثعلبة ويعد الاكتئاب كعامل نفسي مرتبط بشدة الاصابة (Bain وآخرون،2020). أنَّ تعدد الإشكال لجين IL−16 له علاقة بالتاريخ العائلي للمصابين بداء الثعلبة(Lew وآخرون2014). يعد IL−36 و IL−31 من الانترلوكينات المنشئة للألتهاب وكل من IL−37 و IL−38 مضادة للألتهاب وتعود جميعها إلى عائلة IL−1 ويعدُّ إرتفاعها في مصل المرضى المصابين بداء الثعلبة كمؤشر بايلوجي في التشخيص وكهدف علاجي لداء الثعلبة (Boutet وآخرون ، 2019).

1-2 الهدف من الدراسة Amis of Study

تم أختيار محافظة ديالى لأجراء الدراسة الحالية للوقوف على واقع الحال ودراسة المرض، إذ استهدفت الدراسة الحالية ما يأتي:

1- التحري عن شدة الاصابة بداء الثعلبة وعلاقتها بالبيانات الوصفية .

2- تقييم مستويات الاضداد المناعية IgG و IgM وبروتينات المتمم C3 و C4 في مصل دم المرضى المصابين بداء الثعلبة واعتمادها كعوامل لتشخيص المرض .

3- تقييم مستويات الحركيات الخلوية IL-16 و IL-37 في مصل دم المرضى المصابين بداء الثعلبة .

4- التحري عن تعدد الإشكال الوراثية Polymorephism للانترلوكينات IL-16 و IL-37 بإستعمال تقنية(Polymerase chain reaction Sequence-specific primer (PCR-SSP ومدى علاقتهما بمرض داء الثعلبة.

5- التحري عن وجود علاقة تربط الطرز الوراثية لتعدد الاشكال النيوكلوتيدة المفردة ل IL-16 و IL-37 مع مستوى تركيزهما في مصل المرضى المصابين بداء الثعلبة ومقارنة بمجموعة السيطرة .

Summary

Alopecia areata is one of the autoimmune disorders that causes hair loss in the form of patches in all parts of the body and is concentrated in the areas of the scalp, eyebrows, beard and eyelashes. There are many factors, including the genetic factor, family history, infection with other immune diseases, viral and bacterial causes, in addition to psychological stress and environmental factors. It was treated with natural and pharmaceutical methods. Recently, (topical) immunosuppressants have been adopted as a therapeutic method, and in view of the role of genetic factors in the incidence of alopecia areata, the current study suggested to investigate some immunological changes associated with alopecia areata and to know the extent of the genetic association of single nucleotide polymorphisms (SNPs) for some genes and their relationship with alopecia areata.

The current study was conducted on patients with alopecia areata who were attending Baquba General Hospital, Muqdadiya General Hospital, Baladruz General Hospital, and Mansouriya Hospital in Diyala Governorate from December 2021 to June 2022.The number of patients with alopecia areata was (51) in different ages, in addition to them (50) person as a control group (uninfected). The proportion of infected males with alopecia was higher (62.7%) than in females, (37.3%), the age group (31-40) years was more group that being infected with rate (33.33%) while the age group (51- 60) years was the less categories being infected in percentage (1.96%) at mean was (27.90 ± 1.66)). The infected percentage with alopecia areata among employees increased (68.6%) compared to non-employees (31.4%), with a significant difference at the level of probability (P<0.05).The presence of hair loss areas distributed over body of the patient or the entire body, as it constituted the high percentage infection rate in the scalp was high (74.5%) than from the head and beard areas (7.8%), while the incidence of chin and beard was (3.9%). According to its clinical forms, alopecia areata, which constituted (92.16%) in a significant difference at the level of at a probability less than (0.05). Individuals with single spots without hair (one spot and only two) were significantly higher (60.78%) than multiple spots (three and more) (35.29%) at (3.84 ± 0.68) compared to control group . The mean duration of onset of infection in weeks at (78.98 ± 38.46), in statistically significant difference at the level less than 0.05. The patient's family history is a major factor in the incidence of alopecia areata, as the number of patients with alopecia areata who had a family history of infection increased at (98.00%) among other chronic diseases, this increase formed a significant difference at (P<0.05).

The results of the immunological study showed after some immunological changes were investigated, there was a highly significant, statistically significant increase at the level the values of some immunological factors that were associated with the infection by measuring the levels of IgG , IgM , Complement proteins

(C3 and C4) and IL-16 and IL-37. The level IgG was elevated in serum of alopecia areata patients and arched to (2439.75± 69.58) mg/dl compared to control group(389.09 ± 17.85) mg/dl. As well as the case of IgM which their concentration in serum of alopecia areata patients was reached to (509.26±9.85) mg/dl while,the mean IgM in control group was(73.48 ± 2.69) mg/dl. An increase in the level of complement protein C3 and C4 were observed in the group of alopecia patients (221.96 ± 5.07)mg/dl and (87.12 ± 1.85) mg/dl, respectively, but in control group the concentrations were (31.89 ± 1.89) mg/dl and (17.75 ± 1.15) mg/dl, respectively. As well as the concentration of IL-16 and IL-37were high alopecia areata patients group that were reached (214.56 ± 22.48) pg/ml and (184.18 ± 69.45) pg/ml, respectively compared to its concentration in the control group (167.07 ± 23.59) pg/ml and (153.28 ± 48.17) pg/ml, respectively. This increase formed a non-significant difference at aprpbability($P > 0.05$),

The present study recorded a strong direct and statistically significant relationship at a significant level of (0.01) between the immunological variables IgG, IgM, C3, and C4 with a Pearson correlation coefficient of (0.910, 0.951 and 0.893, respectively), with a significance value of (0.000). The Pearson coefficient between IgM and protein complements C3 and C4, with a cumulative rate of 0.935 and 0.934, respectively, with a significance value of (0.000), and the correlation coefficient determined for C3 with C4 only with a value of (0.909) is smaller than (0.01). It has a very strong correlation, ($r>0.80$). While there is no relationship between variables C3, IL-16 and IL-37 with other variables within the studied groups and it is statistically significant (Sig.(2-tailed)>0.01).

As for the results of the molecular study, the analysis of the genetic sequence of SNPs IL-16 and SNPsIL-37 was found the presence of two genetic variants of the IL-16 SNPs were G>T rs11325 and T>C rs1131445 in the group of alopecia areata patients and control group, as two *G* and *T* alleles were recorded for the SNPs rs11325 IL-16 corresponding to three genotypes (GG, GT and TT), as the dominant genotype, GG and allele *G* had the highest percentage among affected patients (64.6%, OR:1.4; 81.0% OR:1.31, respectively), Therefore, the dominant type, GG, and its allele *G* was considered an etiological agent for the occurrence and progression of alopecia areata. While hybrid genotype GT, the recessive genotype TT and the allele *T* was decreased with a frequency of (33.3%, 2.1% and 19.0% with OR:0.69, 0.89, and 0.76, respectively),therefore GTand TT and *T* were considered as a preventive agent for infection . While rs1131445 IL-16 SNPs had two alleles, *T* and *C*, corresponding to three genotypes (TT, TC, and CC), the dominant genotype TT and allele *T* recorded a significant decrease in frequency in the infected group was (37.5%) while, the control group was (60.0%) , with OR:0.83 and 0. 86, respectively), therefore TT and *T* were considered a preventive agent for alopecia areata. While a significant increase observed of the hetero genotype TC, and the recessive genotype CC and allele *C* for patients with alopecia

areata (45.8%, 16.7%,40.0%, with OR: 1.07%, 1.23 and 1.16, respectively) also,TC , CC and *C* were considerd an etiological agent for alopecia areata. Through statistical analysis, this increase did not constitute a significant difference at p>0.05.

In addition, the results of the present study indicated by conducting a genetic sequence analysis of the IL-37 gene that there are also three important genetic variants of IL-37 gene known as (T>Crs3811045, G>Trs3811046 and A>Grs3811047), IL-37SNP T>Crs3811045 was recorded two alleles *T* and *C* compatible with three genotypes (TT, TC and CC), and the frequency of the dominant TT allele and its *T* allele in the group of affected patients was non-significantly decreased by (6.25% and 34.0%; OR:0.29, 0.56, respectively), which it's considered as a preventive factor for alopecia areata. While the hetero genotypes TC and recessive genotypes CC and *C* allele considered an etiological factor with OR:1.02, 1.75 and 1.78, respectively). Whereas, the dominant genotype GG, allele *G*, and the hetero genotype GT of IL-37SNPG>Trs3811046 recorded a significant decrease (8.33%, 52.08%, and 34.0%, respectively), and each of them was considered a protective factor for the disease OR:0.34, 0.95 and 0.57, respectively). As well as the recessive genotype TT and *T* allele were considered as an etiological factor with a frequency (39.58% and 66.0%; OR:1.91 and 1.74, respectively). This increase did not constitute a significant difference at the probability level P>0.05. The dominant genotype AA and A allele of IL-37SNPs gene A>Grs3811047 showed a significant decrease in patients with alopecia areata with a frequency (12.50% and 41.0% ;OR:0.47 and 0.75, respectively), and that were considered a protective factor for the disease. While the hetero genotype AG, the recessive GG and allele *G*, recorded a significant increase in affected patients compared to the healthy group (56.25%, 31.25%, and 59.0%; OR:1.35, 1.17, and 1.33, respectively). More over, the AG and GG genotypes and *G* allele considered etiological factors and risk factors for alopecia areata. Through statistical analysis, a non-significant correlation between the genotypes of each of the IL-16 gene and the IL-37 gene for different genetic variants, despite the significant increase in the level of their concentrations in the serum Patients with alopecia areata compared to control group.which are involved in the genetic predisposition and susceptibility to alopecia areata, because they are all considered among the genetic factors causing the disease and the risk of developing alopecia areata . Because heterogeneity occurs in the nitrogenous bases, which results in point mutations of the type of substitution within the sequence of nucleotides and thus leads to a change in the code of the type of amino acid. This is the first study of its kind in Iraq.