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ORIGINAL ARTICLE

The association of Wnt – Factor TCF7L2 (TCF4) gene polymorphism and treated Alopecia Areata (Platelet-rich plasma Vs conventional)

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ABSTRACT

Background: Alopecia areata is an autoimmune disorder with a varied, typically relapsing or remitting nature. Numerous concomitant conditions, such as celiac, hypothyroidism, hyperlipidemia, type 1diabetes, and atherosclerosis, are linked to alopecia areata.

Methods: This study was performed on (86) subjects who were categorized into two groups; Group (1): included 43 healthy individuals. Group (2): included 43 patients diagnosed with alopecia areata. This group was divided into 2 groups; group A: 20 patients were treated by conventional therapy. Group B: 23 patients were treated by Platelet Rich Plasma (PRP) (local intradermal injection).Whole blood samples were used for genotyping of TCF7L2 polymorphisms (rs7903146).

Results: Regarding TCF7L2 genotyping: CT, TT, and CT+TT genotypes of TCF7L2 were significantly higher in cases compared to controls. There was no significant difference between different TCF7L2 genotypes among alopecia patients regarding to demographic data and Clinical findings except white hair before treatment. There was no significant variation between different TCF7L2 genotypes in PRP and steroid-treated alopecia patients regarding demographic data and clinical findings except exclamation mark in PRP treated group.

Conclusions: TCF7L2 gens polymorphismis associated with alopecia areata, however, there was no significant difference between PRP treatment and conventional therapy regarding the different genotypes.

Keywords: TCF7L2;Alopecia areata; PRP; Polymorphism

INTRODUCTION

Alopecia areata (AA) is an autoimmune disorder with a varied, often relapsing or remitting pattern that can be permanent, especially when hair loss is severe. The prevalence of AA is ~2% [1]. In Egypt, the prevalence of alopecia areata is ~1% of population and is ~0.6% among children [2]. The pathogenesis of alopecia areata is still unclear. The primary pathogenic mechanisms that cause this condition are thought to be the activation of immune systems and the impairment of the immune privilege of hair follicles [1].

The Wnt/-catenin/TCF4 signaling pathway has a significant role in hair cycling. It aids the telogen-to-anagen transition by determining the fate of hair follicles [3]. The alterations in its activity could result in hair follicle quiescence and an early transition into catagen. TCF7L2 gene is one of the most studied gene in this pathway. This gene produces the transcription factor (TCF-4) that activates many genes by binding to β-catenin [4].

The TCF7L2 gene is positioned on the tenth chromosome and rs7903146 is effective variant. This variant is suggested to increase incidence of diabetes type 2, latent autoimmune diabetes in adult (LADA) [5], many cancer types (stomach, breast, and colon) [6], and schizophrenia [7]. Hereditary varieties may cause unsteadiness in this pathway; as a result, hair follicles may be less susceptible to an immune response. They could also, clarify the disappointment within the therapeutic methodologies which targeted for immunologic responses [8].

Platelet rich plasma (PRP) is considered as a new approach for inducing hair regeneration that combines an autologous plasma preparation with concentrated platelets. PRP is made up of a variety of growth factors and cytokines that enhance hair follicle regeneration and repair [9]. These growth factors, which include epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF), and connective tissue growth factor enhance hair regrowth [10]. These growth factors bind to the corresponding receptors made by stem cells in the bulge of the hair follicle and surrounding tissues. The anagen follicular unit is formed as a result of this binding, which stimulates hair growth and promotes proliferative activity [11].

So, we aimed in this work to determine the genetic polymorphisms in TCF7L2 gene in alopecia areata. Also, to evaluate the role of PRP and compare it with the conventional therapy in treatment of alopecia areata.

METHODS

This study was done within the departments of Medical Biochemistry (clinical chemistry and stem cell lab) and Dermatology, Faculty of Medicine, Zagazig University. Approval for the study was obtained from the Institutional Review Board (IRB) Faculty of Medicine, Zagazig Univesity (reference number is 9056/7-11-2021). This study is a case-control study and randomized controlled clinical trial included (86) subjects who were categorized into two groups; Group (1): included 43 healthy individuals. Group (2): included 43 patients diagnosed as alopecia areata. This group was divided into 2 groups:

group A: 20 patients were treated by conventional therapy. Group B: 23 patients were subjected to PRP (local intradermal injection). Patients diagnosed alopecia areata and with age >20 were included in the study.

Patients on hormonal therapy, presence of other disease that may interfere with the study parameters, patient refuses to give consent and lack of cooperation, and Alopecia areata due to infection or wound were excluded from this study.

Verbal and written informed consents were collected from all cases after an explanation of the procedure and medical research.

All subjects of the study will be subjected to the following: full history taking, complete general examination, dermoscopic examination, blood tests to determine autoimmune conditions, and scalp swab.

Sampling:3ml of patient's blood was collected in EDTA tubes under complete aseptic condition then samples were stored at (-20°) C until the DNA isolation process.

DNA extraction:DNA was extracted as directed by the manufacturer (Geneaid Biotech Ltd).DNA Quantification and purity was performed. This was achieved by calculating the A260/A280 ratio. This ratio was determined to be between 1.7 and 1.9 for pure double-stranded DNA.

Genotyping of *TCF7L2* (*rs7903146*) single nucleotide gene polymorphism by amplification refractory mutation system-PCR:ARMS-PCR was used to identify of TCF7L2 (rs7903146) by utilizing forward primer with sequence: 5′ – GGAGC CGTCA GATGG TAATG-3′ and 2 reverse primers: TCF4-C: 5′ – GGTG CCTCA TACGG CAATT AAATT ATATAG −3′ unique for C allele, and TCF4-T: 5′ – GGTG CCTCA TACGG CAATT AAATT ATATAA −3′ unique for T allele.

The PCR was performed in a final volume of 20 µl comprising 10 µl of 2x i-Taq™ PCR Master Mix (iNtRON Biotechnology, Seongnam-Si, Korea), 5 µl of template DNA, 1 µl of (respectively forward and reverse primer TCF4-C for allele C) and (1µl of respectively common forward and reverse primer TCF4-T for allele T (Biolegio, Nijmegen, Netherland)), and 3 µl DNase-free water.

DNA Thermal Cycler was used for the amplification. The PCR process involved 30 cycles of denaturation at 95 °C for 30 s, followed by annealing at 58 °C for 40 s, extension at 72 °C for 30 s, and the final extension step at 72 °C for 7 min. The two percent agarose gel electrophoresis of the PCR products (188 bp for TCF7L2) was followed by ethidium bromide staining and UV transilluminator visualization.

***Platelet rich plasma (PRP) preparation*:**PRP extraction using double-spin method as reported by El-Husseiny et al [12]. 10 mL of venous autologous whole blood was collected into tubes containing tri-sodium citrate as anticoagulant, then centrifuged at 112g units (1000 rpm) for 10 min at room temperature to separate red blood cells at the bottom of the tube, buffy coat (containing white blood cells) in the middle and plasma above (soft spin). The portion of plasma was transferred into other plain tubes (free of tri-sodium citrate) and centrifuged further at 448g units (2000 rpm) for 10 min (hard spin) to obtain a platelet pellet at the bottom of the tube and platelet-poor plasma (PPP) in the upper part**.**

Platelet rich plasma (PRP) injection:Using sterilized needles of 30 gauge and a 1 mL

syringe, the injection was administered intradermally. After administering local anesthetic, 0.1 mL of PRP was injected into the affected area. Vertex, parietal, and frontal regions of the scalp are the three anatomical injection sites. The target scalp surface was properly cleansed with alcohol pads before injection. For a total of five sessions, patients received one session every two weeks. Patients find remarkable relief in discomfort after the first or second sessions. Although PRP injections are not unpleasant, there may be a small amount of discomfort. After two treatments, initial hair regrowth was frequently noticeable [13].

Intralesional steroids for alopecia areata: An intradermal injection of triamicinolone acetinoide was administered using an insulin syringe and a 0.5-inch, 30-gauge needle. A series of 0.1 mL injections spaced 1 cm apart were administered. The dilution utilized was sterile saline. To reduce discomfort from the injections, an optional topical anesthetic was administered 30 to 60 minutes prior to treatment; the optimum dose for the scalp was 5 mg/mL (maximum volume of 3 mL per session). Patients were seen in five appointments, one of which was every two weeks. In the first four to eight weeks, new hair growth was frequently noticeable. After five sessions, if there was no improvement, the intralesional steroid was discontinued [13].

Two blind dermatologists analyzed the images to determine the clinical response after comparing the pre- and post-treatment photographs taken with a digital camera. Determine the number of dystrophic hairs and their indicators, such as yellow, black spots, exclamation marks, short broken, short villous and white hair, using dermoscopic photomicrographs.

STATISTICAL ANALYSIS

Data were collected and analyzed using SPSS software (IBM, Version 20.0). For characterization of quantitative data (IQR), mean, and standard deviation were used. Fisher's exact test was utilized. All tests were two-sided. P-value < 0.05 was considered statistically significant, p-value ≥ 0.05 was considered statistically insignificant.

RESULTS

The present study revealed that 43 cases with AA were subjected to this study with mean age of 32.2 years, 79% were females while 21% were males. While the control group included 43 cases with mean age of 34 years, 72% were females, and 28% were males. There was no significant variation between alopecia patients and healthy subjects regarding to age and gender.

Concerning the clinical data of AA patients, there was no remarkable variation between PRP and steroid treated alopecia patients regarding to clinical findings of alopecia (Table 1).

Regarding TCF7L2 genotyping; the CC genotype was detected in 13 (30%) patients; CT and TT were in 25 (58%) and 5 (12%), respectively and CC+TT was in 30 (70%). In the healthy group, CC was present in 25 (58%) individuals, CT and TT were in 17 (40%) and 1 (2%) control. CT, TT and CT+TT genotypes of TCF7L2 were significantly higher in cases compared to controls. Concerning TCF7L2 allele distribution, C allele was detected in 51 (59%) cases and T allele in 35 (41%) cases, while in the control group, C allele was found in 67 (77%) controls and T allele in 19 (23%)

controls. There was significant difference between alopecia patients and healthy subjects regarding to T allele of TCF7L2 (Table 2) ,(figure 1).

There was no significant difference between different TCF7L2 genotypes among alopecia patients regarding to demographic data and Clinical findings except white hair before treatment (Table 3) (figure 2).

There was no significant difference between different TCF7L2 genotypes in PRP and steroid treated alopecia patients regarding to demographic data and clinical findings except exclamation mark in PRP treated group (Table 4).

(Table 5) shows that there was no significant difference between CC genotype of PRP treated group and steroid treated group regarding different parameters. There was significant difference between yellow dots before and after treatment, black dots before and after treatment in CC genotype of PRP treated group. There was significant difference between yellow dots before and after treatment in CC genotype of steroid treated group.

This table showed that there was no significant difference between CT genotype of PRP treated group and steroid treated group regarding different parameters except broken hair. There was significant difference between yellow dots before and after treatment, black dots before and after treatment, white hair before and after treatment, short villous before and after treatment and broken hair before and after treatment in CT genotype of PRP treated group. There was significant difference between yellow dots before and after treatment, black dots before and after treatment and white hair before and after treatment in CT genotype of steroid treated group.

This table showed that there was no significant difference between TT genotype of PRP treated group and steroid treated group regarding different parameters. Also, there was no significant difference between different parameters before and after treatment in TT genotype of PRP treated group and TT genotype of steroid treated group except black dots in PRP treated group.

Table (1): Clinical findings of the alopecia patients

	PRP Treated (N=23)	**Steroid Treated** (N=20)	t/ X^2	P
Age	34.3 ± 10.1	31.3 ± 9.1	T= 0.99	0.3
Gender **Female** **Male**	 3 (13%) 20 (87%)	 6 (30%) 14 (70%)	X^2= 1.8	0.17
Duration	11.5 ± 8.1	16.6 ± 11.3	T=1.7	0.09
Onset **Acute** **Gradual**	 20 (87%) 3 (13%)	 18 (90%) 2 (10%)	X^2= 0.09	0.75
Course **Progressive** **Stationery**	 5 (22%) 18 (78%)	 5 (25%) 15 (75%)	X^2=0.06	0.8
Family History	2 (9%)	1 (5%)	X^2=0.22	0.6
Yellow dots before treatment	23 (100%)	20 (100%)		

	PRP Treated (N=23)	Steroid Treated (N=20)	t/ X^2	P
Black dots before treatment	20 (87%)	17 (85%)	X^2= 0.03	0.85
White Hair before treatment	13 (57%)	11 (55%)	X^2=0.01	0.9
Exclamation mark before	5 (22%)	4 (20%)	X^2=0.02	0.8
Short villous before	14 (61%)	10 (50%)	X^2= 0.5	0.4
Broken hair before	13 (57%)	12 (60%)	X^2=0.05	0.8
Hair regrowth	14 (61%)	15 (75%)	X^2=	
Yellow dots after treatment	3 (13%)	2 (10%)	X^2= 0.09	0.7
Black dots after treatment	3 (13%)	2 (10%)	X^2= 0.09	0.7
White Hair after treatment	3 (13%)	2 (10%)	X^2= 0.09	0.7
Exclamation mark after	2 (9%)	2 (10%)	X^2=0.02	0.8
Short villous after	3 (13%)	3 (15%)	X^2= 0.03	0.8
Broken hair	3 (13%)	2 (10%)	X^2= 0.09	0.7

Data are represented as mean ± SD or number (%). Data are analyzed using Chi square (X^2) or independent student t test.

Table (2): Genotype and allele frequency distribution of TCF7L2 in alopecia patients and control subjects

TCF7L2	**Alopecia patients (N=43)**	**Healthy Subjects (N=43)**	**OR (95%CI)**	**P- value**
CC	13 (30%)	25 (58%)	**Ref (1)**	
CT	25 (58%)	17 (40%)	**2.8 (1.13-7.02)**	**0.02***
TT	5 (12%)	1 (2%)	**9.6 (1.01-91.1)**	**0.04***
CT+TT	30 (70%)	18 (42%)	**3.2 (1.3-7.7)**	**0.01***
Dominant CT+ TT Versus CC	-	-	**3.2 (1.3-7.7)**	**0.01***
Recessive TT versus CC + CT	-	-	**5.5 (0.6-49.4)**	**0.12**
C allele	**51 (59%)**	**67 (77 %)**	**Ref (1)**	
T allele	**35 (41%)**	**19 (23 %)**	**2.4 (1.2-4.7)**	**0.009***

Data are represented as number (%). Data are analyzed using odd ratio.

Table (3): Demographic data and Clinical findings of different TCF7L2 genotypes among Alopecia patients.

	CC (N=13)	CT (N=25)	TT (N=5)	F/ X^2	P
Age	35.1 ± 10.6	30.6 ± 8.8	39 ± 10.5	F=2	0.14
Gender **Female** **Male**	 1 (8%) 12 (92%)	 8 (32%) 17 (68%)	 0 5 (100%)	**Fischer exact 0.12**	
Duration	15.6 ± 11.9	13.1 ± 9.6	12.8 ± 6.6	F=0.29	0.7
Onset **Acute** **Gradual**	 12 (92%) 1 (8%)	 22 (88%) 3 (12%)	 4 (80%) 1 (20%)	X^2=0.54	0.76

	CC (N=13)	CT (N=25)	TT (N=5)	F/ X^2	P
Course **Progressive** **Stationery**	 2 (15%) 11 (85%)	 7 (28%) 18 (72%)	 1 (20%) 4 (80%)	X^2= 0.79	0.67
Family History	0	3 (12%)	0	**Fischer exact 0.68**	
Yellow dots before treatment	13 (100%)	25 (100%)	5 (100%)	-	
Black dots before treatment	11 (85%)	22 (88%)	4 (80%)	X^2=0.25	0.88
White Hair before treatment	8 (62%)	16 (64%)	0	**Fischer exact 0.03***	
Exclamation mark before	5 (38%)	4 (16%)	0	**Fischer exact 0.17**	
Short villous before	4 (31%)	16 (64%)	4 (80%)	X^2= 5.1	0.07
Broken hair before	7 (54%)	15 (60%)	3 (60%)	X^2= 0.14	0.93
Hair regrowth	7 (54%)	11 (44%)	2 (40%)	X^2= 0.4	0.8
Yellow dots after treatment	2 (15%)	2 (8%)	1 (20%)	X^2=0.84	0.65
Black dots after treatment	2 (15%)	3 (12%)	0	**Fischer exact 1**	
White Hair after treatment	3 (23%)	2 (8%)	0	**Fischer exact 0.3**	
Exclamation mark after	3 (23%)	1 (4%)	0	**Fischer exact 0.13**	
Short villous after	2 (15%)	4 (16%)	0	**Fischer exact 1**	
Broken hair after	2 (15%)	2 (8%)	1 (20%)	X^2=0.8	0.6

Data are represented as mean ± SD or number (%). Data are analyzed using Chi square (X^2) or Fischer exact or One way ANOVA

Table (4): Demographic data and clinical findings of different TCF7L2 genotypes among alopecia patients

	PRP Treated				**Steroid Treated**			
	CC (N=6)	**CT (N=13)**	**TT (N=4)**		**CC (N=7)**	**CT (N=12)**	**TT (N=1)**	**P**
Gender Female Male	 **1 (17%)** **5 (83%)**	 2 (15%) 11 (85%)	 0 4 (100%)	1	 0 7 (100%)	 6 (50%) 6 (50%)	 0 1(100%)	0.06
Onset Acute Gradual	 **5 (83%)** **1 (17%)**	 11 (85%) 2 (15%)	 4 (100%) 0	**1**	 **7** (100%) **0**	 **11 (92%)** **1 (8%)**	 **0** 1(100%)	**1**
Course Progressive Stationery	 **1 (17%)** **5 (83%)**	 3 (23%) 10 (77%)	 1 (25%) 3 (75%)	1	 1 (14%) 6 (86%)	 4 (33%) 8 (67%)	 0 1(100%)	0.7
Family History	**0**	2 (15%)	0	**1**	**0**	**1 (8%)**	**0**	**1**
Before treatment								
Yellow dots	6 (100%)	13 (100%)	4 (100%)		7 (100%)	12 (100%)	1(100%)	-
Black dots	6 (100%)	11 (85%)	4 (100%)	0.7	5 (71%)	11 (92%)	1(100%)	0.5
White Hair	4 (67%)	9 (69%)	0 (0%)	0.06	4 (57%)	7 (58%)	0 (0%)	0.8
Exclamation mark	4 (67%)	1 (8%)	0 (0%)	0.01*	1 (14%)	3 (25%)	0 (0%)	1

	PRP Treated				Steroid Treated			
	CC (N=6)	CT (N=13)	TT (N=4)		CC (N=7)	CT (N=12)	TT (N=1)	P
Short villous	1 (17%)	10 (77%)	3 (75%)	0.05	3 (43%)	6 (50%)	1 (100%)	1
Broken hair	2 (33%)	9 (69%)	2 (50%)	0.3	1 (14%)	1 (8%)	0 (0%)	1
After treatment								
Hair regrowth	3 (50%)	9 (69%)	2 (50%)	0.6	5 (71%)	9 (75%)	1(100%)	1
Yellow dots	1 (17%)	1 (8%)	1 (25%)	0.7	1 (14%)	1 (8%)	0 (0%)	**1**
Black dots	1 (17%)	2 (15%)	0 (0%)	**1**	1 (14%)	1 (8%)	0 (0%)	**1**
White Hair	4 (67%)	1 (8%)	0 (0%)	0.2	1 (14%)	1 (8%)	0 (0%)	**1**
Exclamation	4 (67%)	0 (0%)	0 (0%)	0.08	1 (14%)	1 (8%)	0 (0%)	**1**
Short villous	1 (17%)	2 (15%)	0 (0%)	1	1 (14%)	2(17%)	0 (0%)	1
Broken hair	0 (0%)	2 (15%)	1 (25%)	0.7	2 (29%)	0 (0%)	0 (0%)	0.2

Data are represented as number (%). Data are analyzed using Fisher exact test

Table (5): Demographic data and clinical findings of different TCF7L2 genotypes among alopecia patients**.**

	CC Genotype			CT Genotype			TT Genotype		
	PRP treated (N=6)	Steroid treated (N=7)	P	PRP treated (N=13)	Steroid treated (N=12)	P	PRP treated (N=4)	Steroid treated (N=1)	P
Gender Female Male	1 (17%) 5 (83%)	0 (0%) 7 (100%)	0.46	2 (15%) 11 (85%)	6 (50%) 6 (50%)	0.09	0 (0%) 4 (100%)	0 (0%) 1(100%)	-
Onset Acute Gradual	5 (83%) 1 (17%)	7 (100%) 0 (0%)	0.46	11 (85%) 2 (15%)	11(92%) 1 (8%)	0.9	4 (100%) 0 (0%)	0 (0%) 1(100%)	**0.9**
Course Progressive Stationery	1 (17%) 5 (83%)	1 (14%) 6 (86%)	0.9	3 (23%) 10 (77%)	4 (33%) 8 (67%)	0.67	1 (25%) 3 (75%)	0 (0%) 1(100%)	0.9
Family History	0 (0%)	0 (0%)	-	2 (15%)	1 (8%)	0.9	0 (0%)	1 (33%)	**0.2**
Yellow dots (Before)	6 (100%)	7 (100%)	-	13 (100%)	12 (100%)	-	4 (100%)	1(100%)	-
Yellow dots (After)	1 (17%)	1 (14%)	0.9	1 (8%)	1 (8%)	0.9	1 (25%)	0 (0%)	**0.9**
P value	0.01*	0.004*		<0.0001 *	<0.0001*		0.14	0.9	
Black dots (Before)	6 (100%)	5 (71%)	0.9	11 (85%)	11 (92%)	0.9	4 (100%)	1(100%)	-
Black dots (After)	1 (17%)	1 (14%)	0.9	2 (15%)	1 (8%)	0.9	0 (0%)	0 (0%)	-

	CC Genotype			CT Genotype			TT Genotype		
P value	0.01*	0.1		0.001*	0.001*		0.02*	0.9	
White Hair (Before)	4 (67%)	4 (57%)	0.9	9 (69%)	7 (58%)	0.68	0 (0%)	0 (0%)	-
White Hair (After)	4 (67%)	1 (14%)	0.1	1 (8%)	1 (8%)	0.9	0 (0%)	0 (0%)	-
P value	0.9	0.26		0.001*	0.02*		-	-	
Exclamation mark (Before)	4 (67%)	1 (14%)	0.1	1 (8%)	3 (25%)	0.32	0 (0%)	0 (0%)	-
Exclamation mark (After)	4 (67%)	1 (14%)	0.1	0 (0%)	1 (8%)	0.48	0 (0%)	0 (0%)	-
P value	0.9	0.9		0.9	0.59		-	-	
Short villous (Before)	1 (17%)	3 (43%)	0.55	10 (77%)	6 (50%)	0.22	3 (75%)	1 (100%)	0.9
Short villous (After)	1 (17%)	1 (14%)	0.9	2 (15%)	2(17%)	0.9	0 (0%)	0 (0%)	-
P value	0.9	0.55		0.004*	0.19		0.14	0.9	
Broken hair (Before)	2 (33%)	1 (14%)	0.55	9 (69%)	1 (8%)	0.003*	2 (50%)	0 (0%)	0.9
Broken hair (After)	0 (0%)	2 (29%)	0.46	2 (15%)	0 (0%)	0.48	1 (25%)	0 (0%)	0.9
P value	0.45	0.9		0.01*	0.9		0.9	-	
Hair regrowth	3 (50%)	5 (71%)	0.59	9 (69%)	9 (75%)	0.9	2 (50%)	1(100%)	0.9

Data are represented as number (%). Data are analyzed using Fisher exact test

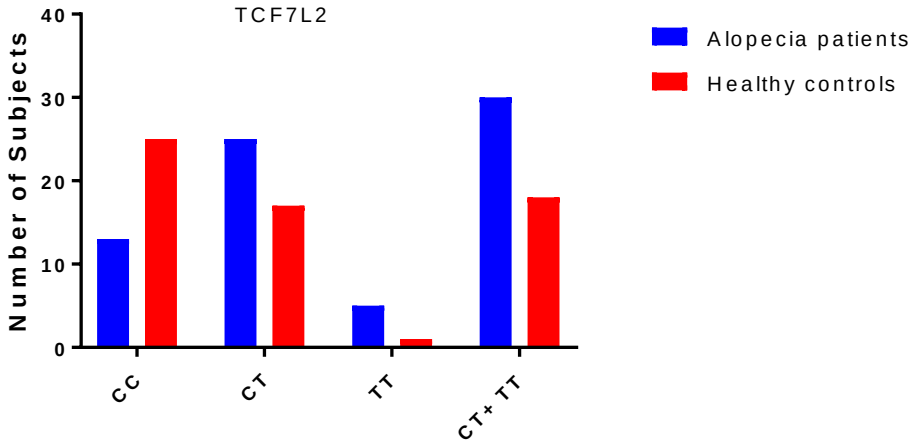

Figure 1: Genotypes among different groups

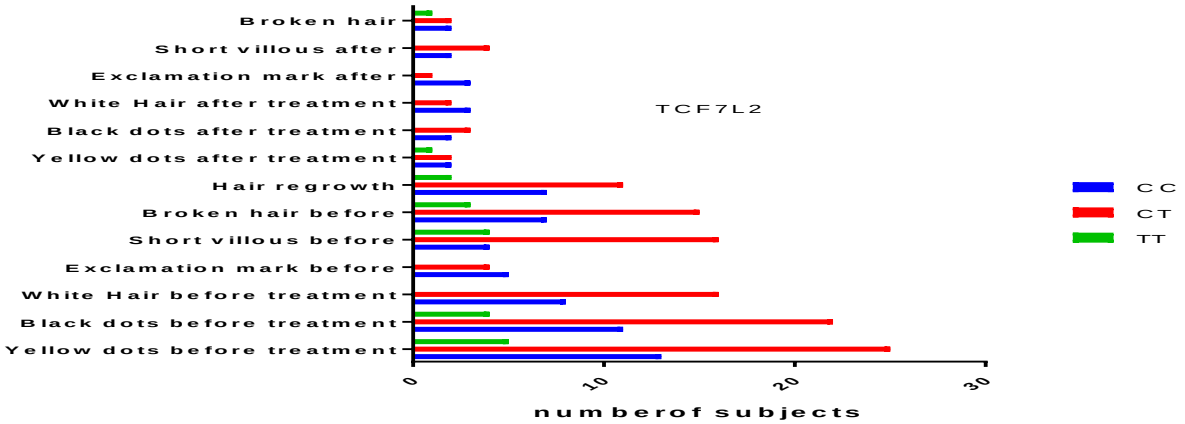

Figure 2: Clinical findings among different genotypes

DISCUSSION

Alopecia areata considered a multifactorial condition in which genetic factors, environmental, immunological and neuro-endocrinological are playing apart [14]. Other autoimmune disorders like hypothyroidism, vitiligo, pernicious anemia, diabetes, and atopic dermatitis are usually associated with it [15].We aimed in the present study, to determine genetic polymorphisms TCF7L2 gene, in alopecia areata and to demonstrate the role of PRP in treatment. Our results demonstrated that there was no remarkable variation between PRP and steroid treated alopecia patients regarding to clinical findings of alopecia.

In accordance with our study, Shumez et al. reported that difference was statistically insignificant between patients treated with PRP and those treated with steroids regarding clinical data [16].In disagreement with our study, Albalat and Ebrahim**,** reported that in the PRP group, a greater increase in the percentage of pigmented hair (75% vs. 70% in the ILCs group) was found. Yellow spots are the most precise dermoscopic sign of AA in terms of dystrophic hair markings. Black spots, which are the remains of broken hairs and were found in all of the patients, have been recommended as a marker for the activity and severity of the condition. This difference may be due to different techniques of treatments [13].

In the current study, the analysis of dominant and recessive genetic models showed significant difference in dominant CT + TT versus CC while showed an insignificant difference in recessive TT versus CC + CT between alopecia and healthy groups for TCF7L2 polymorphism. There was significant difference between alopecia patients and healthy subjects regarding to T allele of TCF7L2. An analysis of the TCF7L2 rs7903146 (C > T) polymorphisms in AA patients using a case-control design revealed that patients express the T-allele more frequently than controls do [17].

Additionally, Jabbari et al. demonstrated a substantial distinction in TCF2L2 expression between people with AA totalis/universalis and healthy control patients [18].

This came in agreement with Rajabi et al. who demonstrated that in both groups; the genotype frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$). Those with AA had significantly greater frequencies of the T allele and the combined TT and CT genotypes [17].Also, Coda et al. reported a Significantly more Wnt/-catenin/TCF signaling genes were up-regulated in AA patients and their unaffected siblings than in healthy controls (2.7 folds increase). The

diseased cases had TCF7L2 levels with 5.74 times higher than those of their healthy siblings [19].

In different ethnicities, the rs7903146 T-allele had the highest association with type 2 diabetes. In addition to predicting type-2 diabetes, the CT/TT genotype is linked to a higher risk of complications from diabetes in the future [20]. Despite this particular polymorphism is intron-positioned, it presumably has no effect on how the protein functions, but it could alter how the gene is expressed. The T allele has been linked to both gene over- and under-expression in the development of diabetes [21].

The Wnt/-catenin/TCF4 signaling pathway is the most significant in cycling of hair. It aids the telogen-to-anagen transition by determining the fate of hair follicles [3], and alterations in its activity could result in hair follicle quiescence and an early transition into catagen [4]. It's possible that a genetic variant could change the way this pathway functions, lowering the threshold for hair follicles to enter and stay in quiescence.

TCF7L2's function in controlling dendritic cells may also provide an explanation for its involvement in the pathophysiology of AA. Dendritic cells are in charge of the skin's strong IFN signature in AA lesions [22],and They most likely have a role in moderating the positive effects of contact sensitizers when treating AA [23]. Through promoting the transcription of a number of target genes, it is hypothesized that the β-catenin signaling pathway has a crucial role in the phenotypic alterations of dendritic cells [24].

Therefore, variations in the TCF7L2's function and structure, maybe brought on by genetic polymorphisms, may have a significant impact on processes of cell that significantly affect the immune system. It makes sense that the elements of the -catenin/TCF pathway would be involved in mediating dendritic cells' involvement because they are also implicated in the pathogenesis of AA due to the numerous research that have been done on remarkable therapeutic points in the Wnt/-catenin/TCF pathway [25].

In the present study, we found that the functional variation of TCF7L2 increases the risk of AA. The minor allele T frequency in the general population is around 23%. Also, in our study it was like wise 23% in the control subjects and 41% in the alopecia group, this result was in agreement with Zerbino et al. [26]. This reveals that AA is more likely to occur in TCF7L2 rs7903146 T-allele carriers.

It makes sense to study how these pathways are related to AA and see whether these targeted treatments might help with AA. Numerous immune-mediated illnesses, including rheumatoid arthritis, lupus erythematosus, psoriasis, and Sjogren's syndrome, include abnormal dendritic cells and dysregulated Wnt/-catenin pathways as part of their pathogenesis [27].

We demonstrated that there was no significant difference between different TCF7L2 genotypes in PRP and steroid treated alopecia patients regarding to demographic data and clinical findings except exclamation mark in PRP treated group (before starting therapy) that refer to increase disease activity and explain the insignificant variation between the studied therapeutic modalities asexclamation marks hairs were discovered to be associated with disease activity[28].

We also found that PRP treatment is less effective than conventional therapy in treatment of alopecia areata associated with TCF7L2 gene polymorphism as the result

showed that hair regrowth was (50% in CC genotype, 69% in CT genotype and 50% in TT genotype) in PRP treated patient, while in steroid treated patient it was (71%in CC genotype, 75% in CT genotype and 100% in TT genotype).

LIMITATION OF THE STUDY

The relatively small sample size was the most important limitation of this study.

CONCLUSIONS

TCF7L2 gens polymorphism (rs7903146)is associated with alopecia areata, however, there was no significant difference between PRP treatment and conventional therapy regarding the different genotypes.

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AUTHOR CONTRIBUTIONS:SHA constructed the study design. ANselected the patients and the control group. All laboratory tests were carried out by AT and ADE, who also analyzed patient data pertaining to each group under study. All authors wrote, read, and approved the final manuscript.

Data Availability

All data generated or analyzed during this study are included in this published article

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