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Original article

Association of TNF-α polymorphisms (−857, −863 and −1031), TNF-α serum level and lipid profile with acne vulgaris

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ABSTRACT

Background: Acne is an inflammatory condition principally affected by genetic and dietary factors. Investigation into functional polymorphisms of TNF-α gene and their association with acne vulgaris will be helpful in exploring genetic influence on skin immune mediated inflammatory events. In the present study, we analyzed association of TNF-α gene polymorphisms, its expression levels and lipid profiles in a large cohort of acne patients and controls.

Methods: We used PCR-RFLP to study association of TNF-α polymorphisms at −857C/T, −863C/A and −1031 T/C sites with acne vulgaris. Lipid profiles were measured using enzymatic end-point method. The serum levels of TNF-α and apolipoprotein a were measured using ELISA. NIH, LDlink was used to investigate patterns of linkage disequilibrium across south Asian reference genome (Punjabi from Lahore Pakistan).

Results: We found that TNF-α −863 polymorphism is strongly associated with acne in overall population as well as in gender and severity based groups of acne patients. Polymorphisms at −863 and −1031 position were in linkage disequilibrium. Importantly, TNF-α serum level was significantly increased in acne patients with severe disease symptoms. Furthermore, levels of total cholesterol (TC) and triglycerides (TG) were significantly increased, whereas high density lipoprotein cholesterol (HDL-C) level was significantly decreased in acne patients. The levels of apolipoprotein a varied widely in studied populations and no significant difference was found in the analyzed groups.

Conclusion: In conclusion, we found that TNF-α expression increases in acne patients affected by TNF-α polymorphisms, and that the lipid profile is specifically disrupted in acne patients.

1. Introduction

Acne vulgaris (AV) is a worldwide disorder of sebaceous glands and associated hair follicles, affected by several genetic and environmental factors (Harper, 2020). AV is a widespread skin disease in all cultures, ages and ethnic groups, and ranked 8th in global prevalence of the 50 most common diseases in 2010 (Vos et al., 2012). Ethnicity and gender determine the precise prevalence and duration of acne in different regions. Asian population is at maximum risk of acne development (Han et al., 2019). It is also consistently ranked in top 3 most frequent cutaneous disorders in the populations of UK, USA and France (Rea et al., 1976; Johnson and Roberts, 1978, Wolkenstein et al., 2003). Notably, in

Abbreviations: **AV**, Acne vulgaris; **OPD**, Out-patient department; **C. acnes**, Cutibacterium acnes; **TNF-α**, Tumor necrosis factor alpha; **IL-1**, Interleukin-1; **LGL**, Low glycemic load; **Apo-a**, Apolipoprotein a; **HDL**-C, High density lipoprotein cholesterol; **TC**, Total cholesterol.

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Pakistan, acne is responsible for 1/3 of the visits to dermatology OPD (out-patient department) (Asim et al., 2014). Moreover, it is also a leading cause of health care visits worldwide (Pawin et al., 2007). In the USA alone, more than 3 billion $ are spent on acne treatment yearly (Bickers et al., 2006). The worldwide prevalence and high treatment cost necessitates further investigations into acne development mechanism, which will lead to discovery of novel therapeutic strategies.

Inflammation seen in acne lesions is a consequence of interplay between Cutibacterium acnes (C. acnes) infection and innate immunity through production of cytokines, chemokines and antimicrobial peptides from epidermal- as well as immune-cells. The ultimate effect of C. acnes infection, hyperkeratinization and innate immunity activation; is a prolonged inflammatory event that leads to development of severe acne lesions. Cytokines, the potential inflammation causing agents, are found in non-infected sebocytes, however, during acne development cytokines expression further increases (Clarke et al., 2007).

The inflammatory mediators also affect hyperkeratinization, the first visible step of the microcomedones development. In a study, it was proposed that abnormal production of some beneficial lipids leads to the injury as well as interleukin-1 (IL-1) release from the epithelial cells. Consequently, IL-1, due to its effects on keratinocytes, initiates the cascade of events involved in the acne initiation and development. In addition, IL-1α induces the production of other inflammatory markers especially tumor necrosis factor alpha (TNF-α) from keratinocytes (Younis and Javed, 2015). These factors further activate keratinocytes (Paithankar et al., 2015). The role of primary and secondary inflammatory cytokines in the inflammatory events of acne pathogenesis is still controversial, as revealed from genetic association studies in different populations.

The TNF-α superfamily and associated receptors are implicated in variety of cell functions. Moreover, their role in different pathogenic conditions, especially in skin disorders, has been extensively investigated. Study of the TNF-α gene promoter in different populations revealed numerous SNPs in the TNF-α promoter region (Li et al, 2015). The genetic factors that modulate TNF-α expression may thereby affect the development of acne lesions. TNF-α promoter polymorphisms association study in Central European population showed that TNF-α −857C/T polymorphism has a protective role in acne development whereas TNF-α −863C/A and −1031 T/C polymorphisms are not associated with acne development (Szabó et al., 2011). We have recently published the data for TNF-α −238 and −308 association with acne in a preliminary study on the Pakistani population (Aisha et al, 2016). The limited number of studies on TNF-α promoter polymorphisms association with acne requires further investigations in Asian acne patients, where the disease is highly prevalent.

Dietary factors and lipid levels are also important in acne pathogenesis. Various research groups investigated association of consumed carbohydrates, dairy products and chocolate with AV (Karadağ et al., 2017). A cross-sectional questionnaire-based world-wide-web study including data from 2528 subjects reported that low glycemic load (LGL) diet consumption improves acne symptoms and reduces medication requirement (Rouhani et al., 2009). The studies of diet and acne prevalence have limitations, for example data collection questionnaire based personal assessment or smaller number of study subjects. Hence, the relationship of diet and acne development is still disputed (Bowe et al., 2010). The importance of sebaceous glands in acne pathogenesis and evidence that its activity is modulated by dietary lipids provoked many researchers to study association of serum lipids in acne patients (Sobhan et al, 2020). Recently, elevated expression of apolipoprotein a (apo-a), a core protein component of high density lipoprotein cholesterol (HDL-C) has been identified in a proteome analysis of the follicle infundibulum in acne patients (Bek-Thomsen et al., 2014) Increased levels of total cholesterol (TC), but decreased levels of HDL-C and apo-a were consistently observed in acne patients from different populations (Jiang et al., 2015; Sobhan et al., 2020). In addition, El-Akawi and colleagues reported that serum HDL-C levels decrease with increase in acne severity (El-Akawi et al., 2007).

The role of TNF-α promoter polymorphisms in the etiology of Asian acne patients still requires further investigation. Lipid profile is important in acne pathophysiology, as already observed from review of the available reports. However, limited number of study subjects and conflicting results from different populations demands further investigation. Therefore, in the current study we explored the association of TNF-α polymorphisms at −857, −863 and −1031, TNF-α level in serum and lipid profile with AV in large cohort of acne patients from Pakistan.

2. Methods:

2.1. Sample collection

The sex, age and ethnicity matched acne patients and healthy controls were recruited from Benazir Bhutto Hospital and Quaid-e-Azam University Islamabad, respectively. Informed written consent was obtained from each participant of the study in accordance with the Helsinki Declaration of 1975 revised in 1997 and a questionnaire was filled through personal interviews of the participants. Inclusion criteria required participants to have acne on face or back for at least six months and absence of acne cosmetica or polycystic ovary syndrome. Acne severity was rated according to Combined Acne Severity Classification (Lehmann et al., 2002). Inclusion criteria for healthy controls included subjects who met the following conditions**:** never had any episode of acne, any other skin or systemic disorder in past, had a few pimples, which never persisted for more than a week and had not acne marks or ice pick scars on face or back.

2.2. Genetic testing

For genetic study, DNA was extracted from patients and controls blood samples according to the manufacturer's protocol enclosed with commercially available kit (Jena Bioscience, Germany). The TNF-α functional polymorphisms (-857C/T, −863C/A and −1031 T/C) were studied using PCR-RFLP technique. The primers were adopted from previously published articles. PCR conditions and information about forward and reverse primer sequences are given in Supplement 1.

2.3. Lipid profile determination

Lipid profile (TC, TG and HDL-C) was determined in acne patients and controls serum samples using commercially available kits (AMP diagnostics) according to manufacturer's protocol.

2.4. Enzyme linked immunosorbant assay for determination of TNF-α and apo-a levels in serum

TNF-α and apo-a levels in sera of age- and sex-matched mild to moderate and severe groups of acne patients were measured using ELISA technique. Commercially available enzyme immunoassay kit (Human tumor necrosis factor-alpha, ELISA, Kit, Immunotech, France) for determination of TNF-α level in patients groups. The level of apo-a in samples was determined using commercially available enzyme immunoassay kit [Mercodia apo (a) ELISA].

2.5. Statistical analysis

The clinical and biochemical features of study population were presented as mean ± SD. SPSS and Stata was used for statistical analyses. For, comparison of continuous variables (for example lipid profile) in controls *vs.* patients analyses unpaired *t* test was used. The frequencies in 2 × 2 and 2 × 3 and 3 × 3 contingency tables (gender, genotype, allele, acne severity, acne type, skin type, family history) were compared using Fisher's exact test or Chi-square test according to sample size. The levels of TNF-α, apo-a and PF4 among wild type and variant type genotypes of the inflammatory cytokines and adipokine were analyzed using unpaired *t* test. The continuous variables in skin type and acne type groups were calculated using one way non-parametric analysis of variance (ANOVA). LDlink (https://ldlink.nci.nih.gov) was used to investigate haplotype frequencies, and patterns of linkage disequilibrium. The p-Values<0.05 were considered statistically significant. The Benjamini-Hochberg adjusted p-Value were calculated for false discovery rate correction in multiple comparison analysis. LDlink (https://ldlink.nci.nih.gov) as used to investigate patterns of linkage disequilibrium across south Asian reference genome (Punjabi from Lahore Pakistan).

3. Results

3.1. Association of TNF-α polymorphisms with AV

TNF-α is a primary inflammatory cytokine and molecular analysis has confirmed presence of functional polymorphisms in TNF-α gene promoter, with increased levels of this cytokine activating secondary inflammatory cytokines and leading to inflammation (Szabó et al., 2011). In the present study, three polymorphisms in the promoter region of the TNF-α gene at −857C/T, −863C/A and −1031 T/C were genotyped to investigate their importance in inflammatory events of acne progression (Table 1). TNF-α −857C/T was genotyped in 297 controls and 468 patients. The genotype frequency was not different in control and patients group statistically, as observed in general model of association penetrance (p = 0.072). The major (C/C), and heterozygous (C/T) genotype frequencies were increased in patients whereas minor genotype (T/T) frequency was same in both groups as observed in general genotype model. Furthermore, the major allele frequency was increased in patients group relative to the controls, as shown in additive model (p = 0.140). TNF-α −863C/A polymorphism was studied in 303 controls and 441 patients. The genotyping of C/A polymorphism at −863 position indicated that genotype distribution was significantly different in the control and patient groups (p = 0.009). Interestingly, frequencies of major (C/C) and minor (A/A) genotypes were increased in the control group whereas the heterozygous genotype (C/A) was more frequent in the patients group. The comparison of allele frequency in additive model presented that minor allele (A) was more frequent in patients than controls; however, the p-Value was non-significant. The comparison of TNF-α −1031 T/C polymorphism in 310 controls and 505 patients showed that this polymorphism is not associated with AV (Table 1a). The study of TNF-α promoter polymorphisms suggested that TNF-α −863C/A polymorphism is associated with AV. The TNF-α −857 T allele may has protective role in acne disease and TNF-α polymorphism at −1031 T/C is not associated with acne pathogenesis in Pakistani population.

3.2. Association of TNF-α gene polymorphisms with AV in gender and acne severity based groups

Because of the association of acne with sex hormones, the promoter polymorphisms of TNF-α gene were also analyzed separately in the two genders based groups. The polymorphism at −857C/T site was genotyped in 297 controls (94 females and 203 males) and 468 patients (302 females and 166 males). The polymorphism at −1031C/A site was analyzed in 310 controls (103 females and 207 males) and 505 patients (311 females and 194 males). The genotype and allele frequencies were not statistically different in female and male patients from their respective controls for −857C/T and 1031C/A. The polymorphism at −863C/A site was analyzed in 303 controls (111 females and 192 males) and 441 patients (287 females and 154 males). The analysis of −863C/A genotypes and allele frequency distribution showed that this polymorphism was associated with acne in females according to general model and in males according to the dominant model of association penetrance. These results suggested that both female and male patients with heterozygous genotype at TNF-α −863C/A site are at risk of acne development (Table 1b). The comparison of TNF-α in patients groups with varying degrees of acne severity and acne types has suggested that studied TNF-α polymorphisms are not associated with acne severity and acne type (Table 1c and d).

3.3. Comparison of TNF-α and apo-a levels in acne patient versus controls, acne severity and acne type groups

We expected that the TNF-α gene polymorphisms, especially at −863 effected its expression. Therefore, the TNF-α and apo-a protein levels were measured in acne patients using ELISA. A total of 89 patients were grouped according to the severity of acne disease (Controls: n = 45, mild to moderate acne symptoms and Patients: n = 44, severe acne symptoms). The comparative values of TNF-α level in population and genders based groups (54 female and 33 male subjects) are presented in Table 2. The TNF-α protein levels were significantly higher in patients than in control subjects (p = 0.014). The level was also significantly different in female patients versus female controls (p = 0.015). Although, the p-Value for TNF-α levels in the comparison of male controls versus male patients did not reach statistical significance, still in male patients higher levels of TNF-α protein were observed than in respective controls (p = 0.377). This study revealed that increase in expression of TNF-α is positively associated with acne severity independent of the gender. The p-Value was non-significant for the apo-a levels comparison among acne patients (Supplement 2).

3.4. Linkage disequilibrium statistics for studied polymorphisms across south Asian population

LDlink (https://ldlink.nci.nih.gov) was used to investigate patterns of linkage disequilibrium across south Asian reference genome (Punjabi from Lahore Pakistan). Population-specific haplotype frequencies were calculated. LD Matrix was used to create interactive heatmap matrix of pairwise linkage disequilibrium statistics and LDpair was used to investigate correlated alleles for a pair of variants in high LD. We found that −857 was in linkage equilibrium with −863 and −1031 mutation sites. However, −863 and −1031 were in linkage disequilibrium. Furthermore, −1031 (C) allele is correlated with −863 (A) allele and −1031 (T) allele is correlated with −863 (C) allele (Supplement 3–4).

3.5. Comparison of serum lipid profile in acne patients versus controls

The lipid profile measurements are presented in Table 3. The levels of TC and TG were significantly higher in acne patients than in controls (p < 0.001). Interestingly, acne patients had significantly lower levels of HDL-C than controls (p < 0.001). Similar results were observed for TC, TG and HDL-C levels comparison in female group. The TC and TG levels were higher in male acne patients sig-

Table 1
Genotype and allele frequencies of TNF-α polymorphisms including −857, −863 and −1031 in overall population, gender, acne severity and acne type based groups.

Characteristics		TNF-α −857					TNF-α −863					TNF-α −1031				
		C/C	C/T	T/T	C	T	C/C	C/A	A/A	C	A	T/T	T/C	C/C	T	C
a.																
Population	**Controls**	96 (32)	192 (65)	9 (3)	384 (65)	210 (35)	187 (62)	93 (31)	23 (8)	467 (77)	139 (23)	159 (51)	140 (45)	11 (4)	458 (74)	162 (26)
	Patients	192 (41)	261 (56)	15 (3)	645 (69)	291 (31)	228 (52)	189 (43)	24 (5)	645 (73)	237 (27)	251 (50)	240 (48)	14 (3)	742 (73)	268 (27)
	OR	0.6866			0.825		0.7662			1.234		1.066			1.021	
	CI (95%)	0.5060–0.9315			0.6635–1.026		0.5856–1.003			0.9702–1.571		0.8030–1.414			0.8136–1.282	
	p-Value*	0.072			0.140		**0.009**			0.268		0.073			0.862	
b.																
Female	**Controls**	26 (28)	66 (70)	2 (2)	118 (63)	70 (37)	65 (59)	34 (31)	12 (11)	164 (74)	58 (26)	47 (46)	49 (48)	7 (7)	143 (69)	63 (31)
	Patients	123 (41)	172 (57)	7 (2)	418 (69)	186 (31)	148 (52)	123 (43)	16 (6)	419 (73)	155 (27)	159 (51)	145 (47)	7 (2)	463 (74)	159 (26)
	OR	0.5564			0.7501		1.327			1.046		0.8023			0.7795	
	CI (95%)	0.3351–0.9239			0.5325–1.057		0.8521–2.067			0.7359–1.487		0.5131–1.255			0.551–1.103	
	p-Value*	0.276			0.216		0.384			0.858		0.222			0.297	
Male	**Controls**	70 (34)	126 (62)	7 (3)	266 (66)	140 (34)	122 (64)	59 (31)	11 (6)	303 (79)	81 (21)	112 (54)	91 (44)	4 (2)	315 (76)	99 (24)
	Patients	69 (21)	89 (37)	8 (2)	227 (68)	105 (32)	80 (52)	66 (43)	8(5)	226 (73)	82 (27)	92 (47)	95 (49)	7 (4)	279 (72)	109 (28)
	OR	0.7399			0.8789		1.612			1.357		1.307			1.243	
	CI (95%)	0.4846–1.130			0.6453–1.197		1.047–2.483			0.9544–1.930		0.8826–1.936			0.906–1.706	
	p-Value*	0.336			0.472		0.384			0.251		0.353			0.296	
c.																
Mild n (%)		67 (40)	96 (57)	4 (2)	230 (69)	104 (31)	76 (50)	68 (44)	9 (6)	220 (72)	86 (28)	94 (54)	78 (45)	1 (1)	266 (77)	80 (23)
Moderate n (%)		70 (38)	111 (60)	5 (3)	251 (67)	121 (33)	90 (53)	71 (42)	9 (5)	251 (74)	89 (26)	98 (48)	99 (49)	7 (3)	295 (74)	113 (26)
Severe n (%)		55 (48)	54 (47)	6 (5)	164 (71)	66 (29)	62 (53)	50 (42)	6 (5)	174 (74)	62 (26)	59 (46)	63 (49)	6 (5)	181 (71)	75 (29)
p-Value*		0.478			1.053		0.981			1.112		0.648			0.561	
d.																
Comedonica n (%)		19 (50)	18 (47)	1 (3)	56 (74)	20 (26)	17 (53)	15 (47)	0 (0)	49 (77)	15 (23)	20 (50)	20 (50)	0 (0)	60 (75)	20 (25)
Papulopustular n (%)		91 (37)	149 (60)	9 (4)	331 (66)	167 (34)	122 (53)	93 (40)	15 (17)	337 (73)	123 (27)	134 (49)	130 (48)	8 (3)	398 (73)	146 (27)
Nodulocystic n (%)		61 (51)	56 (47)	3 (3)	178 (74)	62 (26)	56 (47)	58 (49)	4 (3)	170 (72)	66 (38)	68 (52)	60 (45)	4 (3)	196 (74)	68 (26)
p-Value*		0.570			0.871		0.532			1.150		1.002			0.992	

Table 2
Comparison of TNF-α serum level between mild to moderate *vs* severe acne patients in overall population and gender based groups.

Characteristics	Controls	Patients	p Value	
Population	n (%)	45 (51)	44 (49)	
	Age (years)	23.04 ± 7.27	22.39 ± 6.00	0.637
	TNF-α (pg/mL)	73.11 ± 90.21	138.00 ± 149.00	**0.014***
	Med (Min-Max)	36 (0.00–279.00)	64 (10.00–407.00)	
Female	n (%)	**28 (50)**	**28 (50)**	
	Age (years)	24.39 ± 8.30	23.03 ± 6.38	0.496
	TNF-α (pg/mL)	73.11 ± 90.21	138.00 ± 149.00	**0.015***
	Med (Min-Max)	26 (0.00–279.00)	65 (10.00–406.00)	
Male	n (%)	**17 (51)**	**16 (49)**	
	Age (years)	20.82 ± 4.56	21.25 ± 5.26	0.805
	TNF-α (pg/mL)	92.47 ± 103.6	134.30 ± 159.60	0.377
	Med (Min-Max)	40 (0.00–279.00)	57 (10.00–407.00)	
	n (%)	**56 (63)**	**33 (37)**	
Female *vs.* Male	Age (years)	23.71 ± 7.37	21.03 ± 4.84	0.065
	TNF-α (pg/mL)	100.80 ± 123.30	112.70 ± 133.30	0.670
	Med (Min-Max)	39 (0.00–406.00)	47 (0.00–407.00)	

Controls group represented acne patients with mild to moderate disease; Patients group represented acne patients with severe disease; Continuous data is represented as mean ± standard deviation; p Values were calculated using two tailed unpaired *t* test; p Values<0.05 were considered statistically significant; n: Number of subjects; Med, Median; Min, Minimum value; Max, Maximum value.

nificantly as well (TC, $p < 0.001$; TG, $p < 0.001$). However, the HDL-C levels were higher in male patients than in male controls ($p = 0.014$). In addition, the observed p-Value for HDL-C comparison in male patients was weaker than the p-Values for overall and female acne patient's comparisons. This data represent that dyslipidemia is important in acne pathogenesis. The p-Values for comparison of apo-a levels between analyzed groups were not statistically significant and in acne patients apo-a levels were widely scattered, ranging from 39.62 mg/L to 779.60 mg/L (Table 3, Supplement 2 and 5).

4. Discussion

This study indicates that TNF-α promoter polymorphism at −863 position, TNF-α serum levels as well as serum lipids are associated with AV in Pakistani population. Similar results were observed in a study conducted on Turkish population. TNF-α levels were significantly elevated in severe acne patients (Akoglu et al, 2019). We found that polymorphism at −1031 is not associated with acne and high frequency of −857 T allele in controls shows that it may has protective role in acne development. Similarly,

Table 3
Comparison of serum levels of TC, TG and HDL-C in acne patients *vs.* controls.

Characteristics	Controls (n = 550)	Patients (n = 530)	p Value
Age (years)	23.51 ± 6.17	23.07 ± 7.04	0.278
Gender (Male/Female)	332/218	329/201	0.574
TC (mg/dL)	177.96 ± 22.36	217.80 ± 25.96	**<0.001***
TG (mg/dL)	196.49 ± 29.72	231.04 ± 30.02	**<0.001***
HDL-C (mg/dL)	42.62 ± 7.64	33.03 ± 7.84	**<0.001***

Continuous data is represented as mean ± standard deviation; p Values were calculated using two tailed unpaired *t* test; p Values<0.05 were considered statistically significant; n: Number of subjects.

Szabo and coworkers found lack of −1031 association and protective role of −857 T allele in Central European population (Szabó et al., 2011). LD Matrix analysis showed that −863 A and −1031C alleles; and −863C and −1031 T alleles are correlated. However, R square value is<0.8 explaining that these alleles are less likely to be inherited together.

We have also found that TNF-α polymorphisms and serum levels are not associated with acne type (Data not shown). However, there are few differences concerning the association of TNF-α polymorphisms with acne and acne severity between Pakistani and Central European population. In contrast to our results, Szabo and coworkers did not find association of −863 with acne in gender or severity based groups (Szabó et al., 2011). In addition, −857 frequency distribution trend in acne type groups was different in the two populations. This difference in association of the −863 polymorphism can be attributed to dissimilarity in sample size and ethnic groups. Additionally, for the −857 polymorphism, difference in acne grading system could be a reason for different findings in two populations. Positive association of TNF-α polymorphisms and serum levels with acne provide another evidence of significance of genetic factors in acne development. Because C. *acnes* infection and IL-1α also regulate TNF-α expression in keratinocytes and immune cells (Tilg and Moschen, 2006) Therefore, the TNF-α polymorphisms are not independent determinant of TNF-α levels in acne patients. Genetic and environmental factors including diet, C. *acnes* infection collectively effect the TNF-α levels and thus its contribution in acne pathogenesis.

We have also showed that lipid profile is unbalanced in Pakistani population with acne. Importantly, this is first study comparing lipid levels in a large cohort of healthy population versus acne patients, including representatives from both genders. Similar to our study, increased levels of TG and decreased levels of HDL, were observed in non-obese female acne patients from Saudi population (Abulnaja, 2009). Moreover, Arora and coworkers reported increased levels of TC and decreased levels of HDL-C in Indian female patients with severe acne symptoms (Arora et al., 2010). Recently, it was reported that levels of HDL-C and TG were increased in female and male patients treated with isotretinoin in a Brazilian population. This research group also reported that female patients have increased risk of TG derangements (Schmitt et al., 2011).

We did not find significant difference in apo-a levels between healthy control and patient groups, whereas studies conducted by Abulnaja and Arora reported significant p-Values for comparison of apo-a levels in female subjects with and without acne. Moreover, Abulnaja observed increased levels of apo-a in non-obese acne patients while Arora reported high levels of apo-a in healthy controls (Abulnaja, 2009; Arora et al., 2010). The possible reason for the different results for apo-a levels in the three populations could be a difference in study designs in three reports; specifically, Abulnaja focused on female obese and non-obese subjects with or without acne while Arora focused on the female acne patients with severe symptoms and sampling was done during luteal phase of menstrual cycle (Abulnaja, 2009; Arora et al., 2010). In Pakistani populations the levels of lipid measurements especially for apo-a, were very widely dispersed. Evidences that serum lipids levels are affected by multiple factors, for example genetics, diet, hormones and glucose levels, could further explain this variation (Bouche et al., 2002; Drew et al., 2012; Gil-Campos et al., 2004).

We observed significant difference in TC, TG and HDL-C levels between acne patients and controls. Therefore, we suggest that glycemic index should be considered in acne pathogenesis and treatment. Further investigation of the mTORC1 and FOXO, which are sensor of nutrients and control skin homeostasis and regulate protein production, lipid formation, proliferation as well as differentiation of the skin cells these factors in relation to acne development will be helpful to elucidate importance of dietary factors in acne development (Melnik, 2010). In summary, we found that TNF-α promoter polymorphism at −863 is significantly associated with AV and that TNF-α levels are increased in acne patients with severe symptoms. We have also proved that lipid profile is important in pathogenesis of acne in both genders.

5. Conclusion

Acne vulgaris is affected by genetics and environmental factors. Among genetics the inflammatory genes play a vital role in the acne development. TNF-α, a primary cytokine induces inflammatory responses *via* multiple signaling pathways. The mutations in TNF-α affect its expression and therefore, contribute to the altered inflammatory response. Current study proved that the subjects with high frequency of heterozygous variant at TNF-α −863 position are at high risk of acne development. TNF-α serum level was also significantly increased in the severe acne patients as compared to patients with mild to moderate. Furthermore, this study showed that the levels of TC and TG were significantly increased in acne patients whereas HDL-C levels were significantly decreased. Nutrition plays an important role in acne development, though exact mechanism is still unknown. On the basis of the current information and our study, dermatologists may recommend low glycemic diet to acne patients.

6. Ethical approval and consent to participate

The Quaid-i-Azam University ethical committee approved all project material including consent to participate in Performa. All participants gave written consent to participate.

7. Consent for publication

All the authors consented for the paper publication.

8. Availability of data and material

The data used and analyzed during the current study available from the corresponding authors declared between the authors.

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Authors contribution

Sidra Y. designed the study, designed the questionnaire, collected data from participants, applied statistics and drafted this

manuscript. Qamar J. contributed to study design and helped in finalizing the manuscript. Sana S. and Kanwal N. participated in sample processing and molecular characterization. Sara M. and Sabba M. helped in manuscript preparation. Farah D and Miroslav B. helped in manuscript revision. All authors critically reviewed the manuscript and approved the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.07.042.

References

Abulnaja, K.O., 2009. Changes in the hormone and lipid profile of obese adolescent Saudi females with acne vulgaris. Braz. J. Med. Biol. Res. 42 (6), 501–505.
Aisha, N.M., Haroon, J., Hussain, S., Tahir, C.M., Ikramullah, M., Rahim, H., Kishwar, N., Younis, S., Hassan, M.J., Javed, Q., 2016. Association between tumour necrosis-α gene polymorphisms and acne vulgaris in a Pakistani population. Clin. Exp. Dermatol. 41 (3), 297–301.
Akoglu, G., Tan, C., Ayvaz, D.C., Tezcan, I., 2019. Tumor necrosis factor α-308 G/A and interleukin 1 β-511 C/T gene polymorphisms in patients with scarring acne. J. Cosmetic Dermatol. 18 (1), 395–400.
Kataria Arora, M., Seth, S., Dayal, S., 2010. The relationship of lipid profile and menstrual cycle with acne vulgaris. Clin. Biochem. 43 (18), 1415–1420.
Asim, S.A., Ali, A., Nisa, Q.U., Salam, J.U., 2014. Dermatological manifestations in obese patients presenting in outpatient department. Rawal Med. J. 39 (2), 141–144.
Bek-Thomsen, M., Lomholt, H. B., Scavenius, C., Enghild, J. J., & Brüggemann, H. (2014). Proteome Analysis of Human Sebaceous Follicle Infundibula Extracted from Healthy and Acne-Affected Skin. PLoS ONE, 9(9).
Bickers, D.R., Lim, H.W., Margolis, D., Weinstock, M.A., Goodman, C., Faulkner, E., Gould, C., Gemmen, E., Dall, T., 2006. The burden of skin diseases: 2004: A joint project of the American Academy of Dermatology Association and the Society for Investigative Dermatology. J. Am. Acad. Dermatol. 55 (3), 490–500.
Bouche, C., Rizkalla, S.W., Luo, J., Vidal, H., Veronese, A., Pacher, N., Fouquet, C., Lang, V., Slama, G., 2002. Five-week, low–glycemic index diet decreases total fat mass and improves plasma lipid profile in moderately overweight nondiabetic men. Diabetes Care 25 (5), 822–828.
Bowe, W.P., Joshi, S.S., Shalita, A.R., 2010. Diet and acne. J. Am. Acad. Dermatol. 63 (1), 124–141.
Clarke, S.B., Nelson, A.M., George, R.E., Thiboutot, D.M., 2007. Pharmacologic modulation of sebaceous gland activity: mechanisms and clinical applications. Dermatol. Clin. 25 (2), 137–146.
Drew, B.G., Rye, K.-A., Duffy, S.J., Barter, P., Kingwell, B.A., 2012. The emerging role of HDL in glucose metabolism. Nature Rev. Endocrinol. 8 (4), 237–245.
El-Akawi, Z., Abdel-Latif, N., Abdul-Razzak, K., Al-Aboosi, M., 2007. The relationship between blood lipids profile and acne. J. Health Sci. 53 (5), 596–599.
Gil-Campos, M., Cañete, R., Gil, A., 2004. Hormones regulating lipid metabolism and plasma lipids in childhood obesity. Int. J. Obes. 28 (S3), S75–S80.
Han, G., Armstrong, A., Desai, S., Guenin, E., 2019. Novel Tretinoin 0.05% Lotion for the Once-Daily Treatment of Moderate-to-Severe Acne Vulgaris in an Asian Population. Journal of Drugs. Dermatology 18 (9), 910–916.
Harper, J.C., 2020. Acne vulgaris: What's new in our 40th year. J. Am. Acad. Dermatol. 82 (2), 526–527.
Jiang, H., Li, C.Y., Zhou, L.u., Lu, B., Lin, Y., Huang, X., Wei, B., Wang, Q., Wang, L., Lu, J., 2015. Acne patients frequently associated with abnormal plasma lipid profile. J. Dermatol. 42 (3), 296–299.
Johnson, M. T., & Roberts, J., 1978. Skin conditions and related need for medical care among persons 1-74 years. United States, 1971-1974. Vital Health Statistic series 11, 212, i-v,1-72.
Karadağ, A.S., Balta, I., Saricaoğlu, H., Kilic, S., Kelekci, K.H., Yildirim, M., Bilgili, S.G., 2017. The effect of personal, familial, and environmental characteristics on acne vulgaris: a prospective, multicenter, case controlled study. Giornale italiano di dermatologia e venereologia: organo ufficiale, Societa italiana di dermatologia e sifilografia 154 (2), 177–185.
Lehmann, H.P., Robinson, K.A., Andrews, J.S., Holloway, V., Goodman, S.N., 2002. Acne therapy: a methodologic review. J. Am. Acad. Dermatol. 47 (2), 231–240.
Li, L., Wu, Y., Li, L., Cai, Y.F., Geng, L., Gao, X.H., Chen, H.D., 2015. The tumour necrosis factor-α 308G> A genetic polymorphism may contribute to the pathogenesis of acne: a meta-analysis. Clin. Exp. Dermatol. 40 (6), 682–687.
Melnik, B.C., 2010. FoxO1–the key for the pathogenesis and therapy of acne? JDDG J. der Deutschen Dermatologischen Gesellschaft 8 (2), 105–114.
Paithankar, D.Y., Sakamoto, F.H., Farinelli, W.A., Kositratna, G., Blomgren, R.D., Meyer, T.J., Faupel, L.J., Kauvar, A.N.B., Lloyd, J.R., Cheung, W.L., Owczarek, W.D., Suwalska, A.M., Kochanska, K.B., Nawrocka, A.K., Paluchowska, E.B., Podolec, K. M., Pirowska, M.M., Wojas-Pelc, A.B., Anderson, R.R., 2015. Acne treatment based on selective photothermolysis of sebaceous follicles with topically delivered light-absorbing gold microparticles. J, Invest. Dermatol. 135 (7), 1727–1734.
Pawin, H., Chivot, M., Beylot, C., Faure, M., Poli, F., Revuz, J., Dréno, B., 2007. Living with Acne. Dermatology 215 (4), 308–314.
Rea, J.N., Newhouse, M.L., Halil, T., 1976. Skin disease in Lambeth. A community study of prevalence and use of medical care. J. Epidemiol. Community Health 30 (2), 107–114.
Rouhani, P., Berman, B., & Rouhani, G., 2009, March. Acne improves with a popular, low glycemic diet from South Beach. In Journal of the American Academy of Dermatology (Vol. 60, No. 3, pp. AB14-AB14). 360 PARK AVENUE SOUTH, NEW YORK, NY 10010-1710 USA: MOSBY-ELSEVIER.
Schmitt, J.V., Tavares, M., Cerci, F.B., 2011. Adult women with acne have a higher risk of elevated triglyceride levels with the use of oral isotretinoin. An Bras. Dermatol. 86 (4), 807–810.
Sobhan, M., Rabiei, M.A.S., Amerifar, M., 2020. Correlation Between Lipid Profile and Acne Vulgaris. Clin. Cosmetic Invest. Dermatol. 13, 67.
Szabó, K., Tax, G., Teodorescu-Brinzeu, D., Koreck, A., Kemény, L., 2011. TNFα gene polymorphisms in the pathogenesis of acne vulgaris. Arch. Dermatol. Res. 303 (1), 19–27.
Tilg, H., Moschen, A.R., 2006. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat. Rev. Immunol. 6 (10), 772–783.
Vos, T., Flaxman, A.D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., Abraham, J., 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380 (9859), 2163–2196.
Wolkenstein, P., Grob, J.J., Bastuji-Garin, S., Ruszczynski, S., Roujeau, J.C., Revuz, J., 2003. French people and skin diseases: results of a survey using a representative sample. Arch. Dermatol. 139 (12), 1614–1619.
Younis, S., Javed, Q., 2015. The interleukin-6 and interleukin-1A gene promoter polymorphism is associated with the pathogenesis of acne vulgaris. Arch. Dermatol. Res. 307 (4), 365–370.