Egypt. J. Chem. **Vol. 64,** No. 11 pp. 6195 - 6201 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Serum Angiopoietin-2: A Sensitive Inflammatory Marker in Psoriasis Patients

Noha M Hammad,[a,*] Tawhed M Ismail,[b] Mohamed A Teama,[b] Hagar Bessar[b]

[a] *Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

[b] *Dermatology, Venerology and Andrology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

Abstract

Psoriasis is an immune-mediated inflammatory skin disease associated with comorbidities. Angiogenesis plays a pivotal role in psoriasis pathogenesis which contributes to the initial events that trigger an autoimmune inflammatory response. Angiopoietins 1 and 2 are glycoproteins. Their binding to the tyrosine kinase receptor is essential to angiogenesis. Dysregulation of angiopoietins together with angiopoietin-2 overexpression induce vascular destabilization and regression. This study aimed to explore the relationship between systemic angiopoietin-2 and psoriasis severity. A total of 45 psoriasis vulgaris patients and 45 matched healthy controls were enrolled in the study. The severity of disease was evaluated by Psoriasis Area and Severity Index. Angiopoietin-2 serum level was measured using enzyme linked-immunosorbent assay. The relation between serum level of angiopoietin-2 and psoriasis severity was assessed. Angiopoietin-2 serum level was significantly more elevated in patients than controls ($p<0.001$). Angiopoietin-2 serum level was indicatively direct correlated with disease severity ($r = 0.7$, $p < 0.001$) with 100% sensitivity and 90.9% specificity in identifying mild and moderate to severe psoriasis patients. Serum angiopoietin-2 directly correlates with psoriasis severity. Angiopoietin-2 is nominated to be a potential sensitive biomarker for the ongoing inflammatory process to monitor the clinical and therapeutic outcomes in psoriasis patients.

Keywords: Angiogenesis; angiopoietin-2; inflammatory biomarker; psoriasis

1. Introduction

Psoriasis is a long-standing inflammatory, non-contagious, and disfiguring disease that involves the skin, nails, joints and is linked to several comorbidities [1]. Experts believe that psoriasis is an autoimmune disorder that has a strong genetic predisposition [2]. Psoriasis influences 1 to 3% of the population worldwide [3]. It is a lifelong disease that impairs the quality of life of the affected patients [4]. Understanding the pathogenesis of psoriasis has always increased the knowledge about the biology of the skin. Deciphering the inflammatory mediators of the immunologic pathways implicated in the pathogenesis of psoriasis has always been a target to identify how to interrupt the inflammatory psoriatic pathways to control the disease Despite the achievements in psoriasis treatment, there are still etiological aspects that have not been investigated yet [5]. Angiogenesis is one of the early incidents that happens in psoriasis even before plaque formation [6]. Angiogenesis is a strictly controlled process of new blood vessel sprouting from pre-existing blood vessels. In psoriasis, angiogenesis occurs early and precedes epidermal hyperplasia. These vascular changes are believed to be the initial trigger to the autoimmune inflammatory response in psoriasis [4].

The angiopoietin (Ang) family includes Ang-1, Ang-2, Ang-3, and Ang-4. Their activities are facilitated through the tyrosine kinase (Tie) receptors; Tie-1 and Tie-2. The Angiopoietin/Tie signaling pathway is one of the main pathways involved in angiogenesis. Ang-1 and Ang-2 angiopoietins are the best- characterized [7]. Ang-2, a glycoprotein, is produced and stored in Weibel-Palade bodies which are in endothelial cells and have many components that interleukin 8 (IL-8), eotaxin-3, endothelin-1, osteoprotegerin, and the P-selectin cofactor cluster of differentiation 63 (CD63) [8, 9].

The Ang-2 plays a fundamental role in diseases related to vascular leakage, abnormal vessel structure, and angiogenesis. It enhances the proinflammatory signals in endothelial cells that lead to inflammation [10]. It is evident that the Angiopoietin/Tie signaling

*Corresponding author e-mail: nmhammad@medicine.zu.edu.eg.; (Noha M Hammad).
Receive Date: 23 April 2021, Revise Date: 02 June 2021, Accept Date: 06 June 2021
DOI: 10.21608/ejchem.2021.73608.3636

pathway is stimulated in the papillary dermis in psoriasis [11]. Ang-2 promotes the adhesion of inflammatory cells to vascular endothelium, triggering the psoriatic inflammation and the aberrant angiogenesis. Moreover, the reduced expression of Ang-2, following adequate treatments, endorses the significance of this glycoprotein in the pathogenesis of psoriasis [6, 11].

This study aimed to explore the association between the serum level of Ang-2 and the severity of psoriasis to improve the clinical and therapeutic outcomes.

2. Materials and methods

2.1 Ethical approval

The study was approved by Institutional Review Board, Faculty of Medicine, Zagazig University, Zagazig, Egypt (Approval no:5207). Written informed consent was obtained from each study participant. The study protocol conforms to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2 Subjects

A total of 45 patients affected by psoriasis vulgaris and 45 healthy controls matched for age and sex participated in the study. Patients were recruited from attendants to Dermatology and Andrology outpatient clinic of Zagazig University Hospitals, Zagazig, Egypt. Written informed consent was obtained from each of the study participants.

Patients were either recently diagnosed with psoriasis vulgaris or had not received topical therapy (for at least 2 weeks) or systemic therapy (for at least one month) and had no pertinent comorbidities before enrollment in the study. Besides, patients did not take drugs that could interfere with the activity of the disease. Full personal history was taken from all patients followed by a comprehensive general and dermatological examination to describe the lesions criteria. The severity of psoriasis was evaluated by Psoriasis Area and Severity Index (PASI) according to the method described by Robinson in 2012 [12]. Depending on psoriasis severity, patients were assigned into three categories (mild <10, moderate 10-20, severe >20).

2.3 Blood sampling

Three mL venous blood was withdrawn from each study participant by venipuncture. At once, blood samples, after incubated in water bath for 10 min at 37°C, were centrifuged at 1000 × g for 10 min. Serum was separated and stored at -20 °C for quantitation of Ang-2 serum level.

2.4 Ang-2 serum level Measurement

Measurement of Ang-2 serum level was performed at Immunology Research Laboratory, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. A quantitative sandwich ELISA technique was implemented according to the manufactures' protocol (Human Ang-2, Sunred, China). The results were measured via using an ELISA reader (Stat Fax® 303 Plus) set to 450 nm and corrected to wavelength set to 630 nm. According to standards' concentrations and their corresponding absorbance, the standard curve linear regression equation was calculated with subsequent calculation of the samples' concentrations.

2.5 Statistical analysis

Normality distribution was check using Kolmogorov-Smirnov test. Numerical data were expressed as mean ± SD, median, and range. Mann–Whitney *U* and Kruskal-Wallis tests were used to compare between abnormally distributed independent variables. Fisher exact test was used to evaluate the association between qualitative variables. The correlation coefficient r was generated using Spearman's correlation. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of serum Ang-2 of maximum sensitivity and specificity to judge psoriasis severity [13]. All analyses were 2-tailed and performed by IBM SPSS software package version 24. P values more than 0.05 were deemed to be non-significant.

3. Results

The present study involved 90 participants divided into equal two groups: 45 patients with psoriasis and 45 healthy subjects in the control group. The mean age of the study participants was 41.1 ± 8.1 years in the psoriasis group and 38.5 ± 8.2 years in the control group. Males were the plurality in both groups representing 33 (73.3 %) and 27 (60.0%) patients and controls, respectively. The baseline demographic characteristics are demonstrated in Table 1.

The average disease duration in the psoriasis group was 18.5 ± 19.3 months. Among the psoriasis group, thirty patients (66.7%) had previously received conventional therapy. The mean value of PASI among the patients was 10.9 ± 6.7. According to the analysis of psoriasis severity, thirty patients (66.7%) had mild, nine patients (20%) had moderate, and six patients (13.3%) had severe disease. The PASI was remarkably higher in male patients (p= 0.02) and severe psoriasis was more frequent in male patients (p= 0.017) (Table 2 and Figure 1A).

3.1 Ang-2 serum level

The Ang-2 serum level was significantly higher (p <0.001) in psoriasis patients (mean: 3.2 ± 6.8, median: 0.3, and range: 0.1 – 23.6 ng/mL) compared to controls (mean: 0.14 ± 0.02, median: 0.15, and range: 0.1 – 0.2 ng/mL) (Figure 1B). Ang-2 serum level was significantly higher (p <0.001) in male patients (mean: 4.2 ± 7.7, median: 0.4, and range: 0.1 – 23.6 ng/mL) in comparison to female patients (mean: 0.2 ± 0.08, median: 0.2, and range: 0.2 – 0.4 ng/mL). Ang-2 serum level was neither correlated with age and disease duration (r = 0.1, p = 0.3 and r = -0.07, p = 0.7, respectively) nor affected by previous treatment, and different therapeutic agents (p = 0.3, and p = 0.6, respectively). The serum level of Ang-2 substantially correlated with PASI (r=0.7 and p<0.001) and was significantly diverse (p<0.001) among the psoriasis severity groups (mild, moderate, and severe groups) (Table 3 and Figure 1A).

According to ROC curve analysis, Ang-2 serum level could distinguish between patients with mild psoriasis vulgaris and those with moderate or severe disease at a cut-off value ≥ 0.7 ng/mL (the optimal sensitivity and specificity were 100% and 90.9%, respectively) (Table 4 and Figure 2A), and between those with moderate and severe disease at a cut-off value ≥ 10.3 ng/mL (the optimal sensitivity and specificity were 100% and 100%, respectively) (Table 4 and Figure 2B).

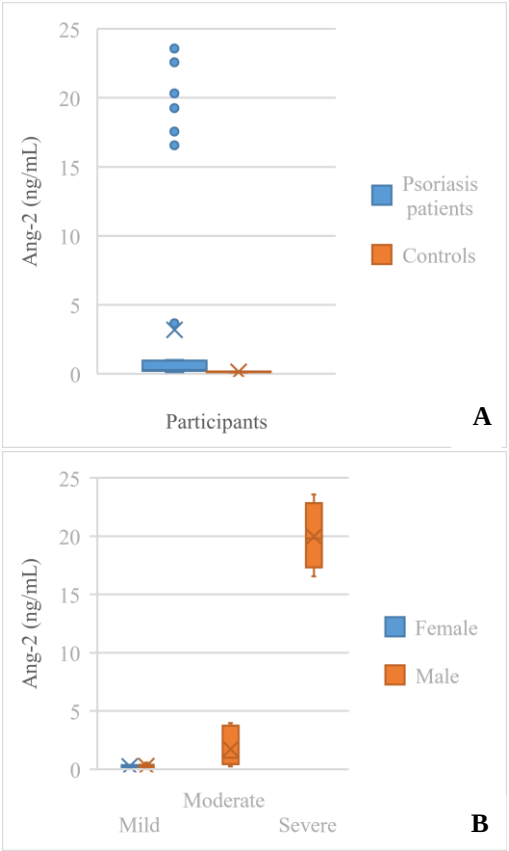

Fig. 1. Boxplots showing the analysis of Ang-2 serum level (ng/mL). A) The median of Ang-2 serum level was significantly higher in psoriasis patients (n = 45) than in controls (n = 45) (p <0.001). B) The median Ang-2 serum level was significantly different among psoriasis severity groups (p <0.001); mild (n = 30), moderate (n = 9), and severe (n =6) groups and the highest median value was seen in the severe group. The median of Ang-2 serum level was significantly higher in male patients compared to female patients (p <0.001). All female patients had mild psoriasis (p = 0.017).

Table 1
Demographic characteristics of the studied groups (N=90)

Variable	Psoriasis group (n=45)	Control group (n=45)
Age (Years)		
Mean ± SD	41.1 ± 8.1	38.5 ± 8.2
Range	25-52	22-48
Gender (%)		
Female	12 (26.7)	18 (40.0)
Male	33 (73.3)	27 (60.0)

Table 2
Clinical characteristics of psoriasis patients and treatments

Clinical Variable	Psoriasis patients (n=45)
Disease duration (Months)	
Mean ± SD	18.5 ± 19.3
Median	10.0
Range	1.0 - 72.0
Previous treatment (%)	
Yes	30 (66.7)
No	15 (33.3)
Therapeutic agents (%)	
Vitamin D analogue	18 (40.0)
Corticosteroids	6 (13.3)
Vit analogue + methotrexate	6 (13.4)
N/A	15 (33.3)
PASI	
Mean ± SD	10.9 ± 6.7
Median	8.6
Range	4.2 -28.9
Severity	No (%)
Mild	30 (66.7)
Moderate	9 (20.0)
Severe	6 (13.3)

PASI, Psoriasis Area Severity Index

Table 3
Relations between Ang-2 serum level, and demographic and clinical characteristics in psoriasis patients (n=45)

	Ang-2 Serum level (ng/mL)		
	Test of significance	r_s	p
Age (years)		0.1	0.3
Disease duration (months)	Spearman correlation	-0.07	0.7
PASI		0.7	<0.001[a]
	Test of significance		p
Previous treatment	Kruskal-Wallis		0.3
Therapeutic agents			0.6

Ang-2, Angiopoietin-2; r_s, Spearman coefficient; PASI, Psoriasis Area Severity Index

[a]Statistically significant at $P \leq .05$

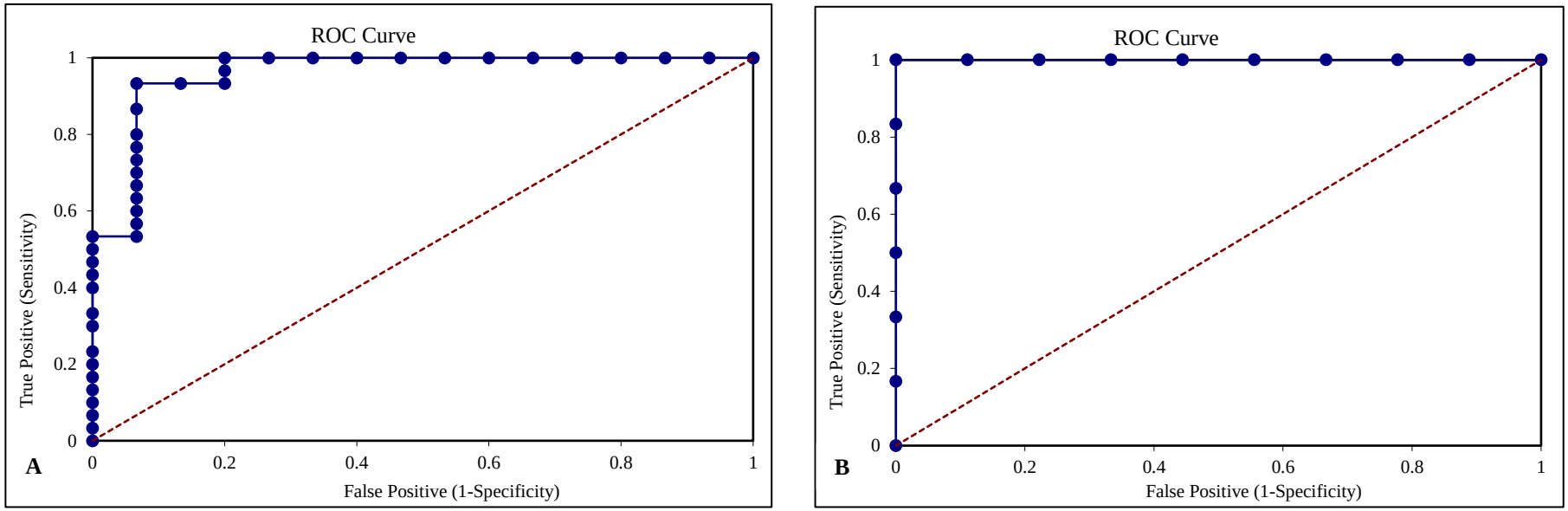

Fig 2. ROC (Receiver operating characteristics) curve analysis demonstrating the validity of Ang-2 serum level (ng/mL) in judgement of psoriasis severity. A) The sensitivity and specificity at cut-off value ≥ 0.7 ng/mL were 100% and 90.9%, respectively. Patients were dichotomized into mild versus moderate to severe psoriasis ($p < 0.001$). B) The sensitivity and specificity at cut-off value ≥ 10.3 ng/mL were 100% and 100%, respectively. Patients were dichotomized into moderate versus severe psoriasis ($p = 0.001$).

Table 4
Validity of Ang-2 serum level in judgement of psoriasis severity

Diagnosis	Cut-off	AUC	P- value	Specificity	Sensitivity	+ve PV	-ve PV	Accuracy
Mild/Moderate or Severe (n=45)	≥ 0.7 ng/mL	0.96	<0.001[a]	80%	100%	90.9%	100%	93.3%
Moderate/ Severe (n=15)	≥ 10.3 ng/mL	1.00	0.001a	100%	100%	100%	100%	100%

Ang-2, Angiopoietin-2; AUC, Area Under Curve; +ve PV, Positive Predictive Value; -ve PV, Negative Predictive Value

[a]Statistically significant at $P \leq .05$

4. Discussion

Psoriasis is a lifelong immune-mediated cutaneous disorder recognized by remissions and exacerbations. It remarkably affects the patients physically and psychologically. The expense of long-term therapy coupled with the social burden of psoriasis has a major influence not only on the patient but also on healthcare sector [14]. Moreover, the severity and chronicity of the disease is highly connected to associated disorders and shorter life span due to cardiovascular mortality [3]. The identification of new surrogate biomarkers for psoriasis evaluation and monitoring of the therapeutic options should be a helpful tool to promote the clinical and the therapeutic management of the disease.

In the present work, psoriasis patients had a significantly higher Ang-2 serum level than controls. Moreover, the study could identify significant distinctions regarding Ang-2 serum level among the three psoriasis severity groups. Also, a strong correlation between Ang-2 serum level and PASI was found. In other words, the larger the disease extent, the higher the Ang-2 serum level. That supported the preliminary findings of Takahashi et al., 2017 [15]. On the other hand, the molecular analysis of the Ang-2 gene identified a polymorphism that might affect vascular development and angiogenesis in psoriasis patients [16]. The histopathologic studies also revealed that Ang-2 is expressed in the papillary dermis of psoriatic skin; however, its signals are exclusive to vascular endothelium. The upregulation of Ang-2 in endothelial cells could be the initial trigger of vascular expansion in psoriasis [11]. Recent reports have identified the association between serum Ang-2 and the severity of atopic dermatitis and lichen planus [17, 18]. Furthermore, the high Ang-2 serum level in psoriasis patients might contribute to the increased cardiovascular risk factors associated with psoriasis suggesting the systemic role of Ang-2 in psoriasis associated comorbidities [19].

The observation that all severe cases included in the study were males and all-female patients had mild psoriasis supports the assumptions of sex-specific difference in patients with psoriasis [20, 21]. Furthermore, male patients had higher Ang-2 serum levels than females, which could be attributed, in this study, to the predominance of severity in male patients; however, the high frequency of males in the study might be responsible for this inclination.

In the present work, two-thirds of psoriasis patients had received previous conventional treatment, albeit this had no impact on Ang-2 serum level when compared to the other third. However, it has been reported that anti-TNF therapy significantly suppressed Ang-2 serum level, which proposed that TNF induces the release of Ang-2 and provokes the adhesion of inflammatory cells to endothelial cells and then initiating the psoriatic inflammation and angiogenesis [15].

The implications of the mentioned findings are advocated by ROC curve analysis. ROC curve displayed that Ang-2, at a cut-off point $\geq$ 0.7 ng/mL had 100% sensitivity and 90.9% specificity when dichotomizing the patients into mild and moderate/severe psoriasis. Albeit at a cut-off point $\geq$ 10.3 ng/mL, it had 100% sensitivity and 100% specificity when dichotomizing the patients into moderate and severe psoriasis. All these findings could propose serum Ang-2 as a potential prognostic biomarker in patients with psoriasis vulgaris.

5. Conclusion

The Ang-2 has a pivotal role in psoriasis pathogenesis. It is upregulated not only locally but also systemically. It can be a promising inflammatory marker in psoriasis vulgaris. Ang-2 is valuable in prognosis and judgment of severity and might be an optimistic therapeutic target for psoriasis in the future.

6. Conflicts of interest

There are no conflicts to declare.

7. References

[1] E. Christophers, Psoriasis− epidemiology and clinical spectrum, Clinical and experimental dermatology 26(4) (2001) 314-320.

[2] J.C. Prinz, Autoimmune aspects of psoriasis: heritability and autoantigens, Autoimmunity reviews 16(9) (2017) 970-979.

[3] A. Rendon, K. Schäkel, Psoriasis pathogenesis and treatment, International journal of molecular sciences 20(6) (2019) 1475.

[4] A. Mohd Affandi, I. Khan, N. Ngah Saaya, Epidemiology and clinical features of adult patients with psoriasis in Malaysia: 10-year review from the Malaysian Psoriasis Registry (2007–2016), Dermatology research and practice 2018 (2018).

[5] T. Yamamoto, Angiogenic and inflammatory properties of psoriatic arthritis, International Scholarly Research Notices 2013 (2013).

[6] L. Sankar, D. Arumugam, P.P. Sudha Boj, Expression of angiogenic factors in psoriasis vulgaris, Journal of clinical and diagnostic research: JCDR 11(3) (2017) EC23.

[7] R.G. Akwii, M.S. Sajib, F.T. Zahra, C.M. Mikelis, Role of angiopoietin-2 in vascular physiology and pathophysiology, Cells 8(5) (2019) 471.

[8] M. Jeansson, A. Gawlik, G. Anderson, C. Li, D. Kerjaschki, M. Henkelman, S.E. Quaggin, Angiopoietin-1 is essential in mouse vasculature during development and in response to injury, The Journal of clinical investigation 121(6) (2011) 2278-2289.
[9] E.L. Doyle, V. Ridger, F. Ferraro, M. Turmaine, P. Saftig, D.F. Cutler, CD63 is an essential cofactor to leukocyte recruitment by endothelial P-selectin, Blood 118(15) (2011) 4265-4273.
[10] H. Menden, S. Welak, S. Cossette, R. Ramchandran, V. Sampath, Lipopolysaccharide (LPS)-mediated angiopoietin-2-dependent autocrine angiogenesis is regulated by NADPH oxidase 2 (Nox2) in human pulmonary microvascular endothelial cells, Journal of Biological Chemistry 290(9) (2015) 5449-5461.
[11] K. Kuroda, A. Sapadin, T. Shoji, R. Fleischmajer, M. Lebwohl, Altered expression of angiopoietins and Tie2 endothelium receptor in psoriasis, Journal of Investigative Dermatology 116(5) (2001) 713-720.
[12] A. Robinson, M. Kardos, A.B. Kimball, Physician Global Assessment (PGA) and Psoriasis Area and Severity Index (PASI): why do both? A systematic analysis of randomized controlled trials of biologic agents for moderate to severe plaque psoriasis, Journal of the American Academy of Dermatology 66(3) (2012) 369-375.
[13] I. Elgharib, N. Khalifa, O. Elasser, M. ElGhareeb, D. Hosny, Serum Endocan Levels in Patients with Psoriasis Vulgaris as a Marker of Disease Severity, Zagazig University Medical Journal 26(3) (2020) 517-523.
[14] S. Mehrmal, P. Uppal, N. Nedley, R.L. Giesey, G.R. Delost, The global, regional, and national burden of psoriasis in 195 countries and territories, 1990 to 2017: a systematic analysis from the Global Burden of Disease Study 2017, Journal of the American Academy of Dermatology 84(1) (2021) 46-52.
[15] T. Takahashi, Y. Asano, S. Shibata, Y. Tada, S. Sato, Serum angiopoietin-2 level as a potential biomarker in psoriasis vulgaris, The Journal of dermatology 44(2) (2017) 205-206.
[16] L. He, L. Dang, J. Zhou, J. Bai, Y.Z. Li, Association of angiopoietin-1, angiopoietin-2 and caspase-5 polymorphisms with psoriasis vulgaris, Clinical and experimental dermatology 40(5) (2015) 556-563.
[17] F.M. Khattab, N.M. Said, Serum angiopoietin-2 level as a novel biomarker in atopic dermatitis, International journal of dermatology 59(1) (2020) 3-7.
[18] H. Bessar, N.M. Hammad, T. Ismail, M. Teama, A. Youssef, Serum angiopoietin-2 level as a novel potential biomarker in oral lichen planus, Journal of Cosmetic Dermatology (2021).
[19] S.C.-S. Hu, C.-C.E. Lan, Psoriasis and cardiovascular comorbidities: focusing on severe vascular events, cardiovascular risk factors and implications for treatment, International journal of molecular sciences 18(10) (2017) 2211.
[20] D. Hägg, A. Sundström, M. Eriksson, M. Schmitt-Egenolf, Severity of psoriasis differs between men and women: a study of the clinical outcome measure psoriasis area and severity index (PASI) in 5438 Swedish register patients, American journal of clinical dermatology 18(4) (2017) 583-590.
[21] D. Colombo, N. Cassano, G. Bellia, G.A. Vena, Gender medicine and psoriasis, World Journal of Dermatology 3(3) (2014) 36-44.