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Evaluation of Serum Substance P Level in Chronic Urticaria and Correlation with Disease Severity

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ABSTRACT

Substance P (SP) is a neurotransmitter emitted from neurons that plays a role in the pathogenesis of itching conditions including chronic urticarial (CU). The present research aims to investigate the serum level of S.P among CU patients and compare them with healthy subjects and explore how it correlates with the severity of urticaria.

The present research was conducted on 87 CU patients who visited the allergy clinic of Ghaem Hospital, Mashhad, Iran from October 2017 to June 2018. Besides, 86 healthy subjects were recruited as the control group. Background information of patient was collected including age, sex, duration of the disease and the co-occurrence of angioedema. S.P serum level was measured in two groups by ELISA method. In the patients group, the autologous serum skin test (ASST) was performed along with the urticaria evaluation questionnaire include Urticaria Activity Score 7 (UAS7), Urticaria Control Test (UCT) and Chronic Urticaria Quality of Life (CU-Q2OL).

Among the patients, the SP serum level showed to be about two times higher than the healthy subjects (p<0.001). SP showed to be increased as patients' age grew (p=0.010). In patients with a positive ASST, SP level was higher (p=0.012). No correlation was found between SP and the presence of angioedema among patients. There was no correlation between the SP serum level and the scores obtained from urticaria evaluation questionnaires.

SP among CU patients was higher than healthy subjects. SP was also higher among female, older and positive ASST patients. The SP value was not correlated with the severity of urticaria, angioedema. In conclusion, Using SP antagonist drugs could be a potential treatment for chronic urticaria.

Keywords: Chronic urticaria; Substance P; Urticaria severity

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INTRODUCTION

Urticariais characterized by the appearance of pruritic, erythematous papules or plaques, with superficial swelling of the dermis. More than twenty percent of ordinary people in society experience hives at least once in their lives.[1]If urticaria remains on the skin most of the weekdays and for a length of at least six weeks, it is called chronic urticaria (CU).[2]CU usually afflicts more than one percent of the population and in eighty percent, the cause is not known. Though CU is not a fatal disease, it tremendously affects the quality of life. Controlling the clinical symptoms of CU is also costly.[2] Drugs such as antihistamines and corticosteroids often reduce the symptoms temporarily. Yet, in the long run, they can have certain adverse effects. Moreover, they do not work on all patients.[3] Investigation of CU patients has shown that the presence of angioedema, more severe urticaria, positive autologous serum skin test (ASST) are correlated with a longer duration of the illness.[4] The above-mentioned issues attest to the significance of the disease and recognition of the mechanisms involved so as to develop new and more efficient medical therapies.

As for the pathogenesis of CU, there have been numerous theories. Yet, none have been proven on an absolute basis. Though the majority of the related literature has looked into the autoimmunity theory, there are several investigations of mastocyte abnormalities of skin cells and basophils.[5,6] A variety of reasons can lie behind the degranulation of mastocytes leading to the emergence of urticaria and itching.[7,8] In an investigation, such neurotransmitters as SP showed to be involved in the degranulation of mastocytes and production of chemokines.[9] In some other research, SP was found to be the most prevalent neurotransmitter released after a local trauma to the skin leading to the emergence of wheal and flare at the traumatic site.[10] Substance P (SP) is a neuropeptide (neurotransmitter) with 11 amino acids which belongs to a family of peptides known as tachykinins. SP is released from certain neurons and belongs to the non-adrenergic non-cholinergic category (NANC). Once released, it is capable of activating some other inflammatory factors. It poses its effect through an axon reflex.[11] SP is widely spread in the peripheral nervous system. It is not only present in neural tissues but also in such other tissues as skin.[12] It from neurons or inflammatory cells such as macrophage, eosinophil, lymphocytes, and dendritic cells. It acts through binding to Neurokinin-1 receptor (NK1R) which is the main receptor.[12-14]

A body of research on patients afflicted with chronic itchy skin diseases such as psoriasis showed that the rate of free plasma SP and its tissue as well as NK1R in patients suffering from itching is significantly higher than those without this problem. Moreover, an increase in the free plasma SP is closely related to the higher severity of such clinical symptoms as itchy skin. Thus, these researchers suggested that probably SP and NK1R play a role in the pathophysiology of chronic itchy skin diseases such as Psoriasis.[15]In a body of research on chronic urticaria, first, no statistically significant difference was found between the SP level of those afflicted with CU and the control group.[16] Newer investigations, however, reported the SP level many times as high.[17-19] In some research, the level of SP showed to the with the severity of the disease.[17]In another investigation, overregulation the in the NK1 receptor in eosinophil of CU patients. Besides, the rate of SP was higher than the normal control group.[20]

Aprepitant drug, which is an antagonist of the NK1 receptor (SP receptor), is applied in the treatment of chemotherapy-resistant vomiting and its efficiency have been investigated.[21] It has shown to be effective in treating chronic itching and CU resistant to therapy.[22-27] If the correlation of SP and CU is proven and SP is targeted, its main receptor i.e. NK1 can be a new treatment for skin diseases that involve chronic itching including CU.

Due to the different result of studies, we design this project to evaluate the role of SP in CU.

PATIENTS AND METHODS

Data Collection

In the present research, two cross-sectional studies were conducted on 87 CU patients visiting the allergy clinic of Ghaem Hospital in Mashhad, Iran from October 2017 to June 2018. Full consent was obtained from the participant. Those included were patients diagnosed with CU by an allergist. Those with physical urticaria, those with systemic diseases and those with depression and anxiety were excluded.

So as to compare SP between patients and healthy population, a control group was used with 86 healthy subjects matched with the patient group in terms of age and sex, willing to take part in the study. The healthy have exclusion criteria like patients.

Questionnaire Completion

87 CU patients filled out a checklist to record demographic information such as age, gender, and duration of the disease.

In order to evaluate the effect of urticaria on their quality of life, the Persian version of CU-Q2oL (Chronic Urticaria Quality of Life) was used in this research.[28] Completion of this questionnaire was followed by the Urticaria Activity Score7(UAS-7) questionnaire.[29] The same physician completed the UAS-7 questionnaire for all patients.

The Urticaria Control Test (UCT) questionnaire was used to rate the extent to which urticaria was controlled in patients.[30]

Sampling Procedure and Conducting ASST

Once the participants consented to undergo ASST, 5 cc blood sample was taken under sterilized conditions. The samples were then stored at room temperature for 30 minutes and when fully coagulated, they were segregated in a centrifuge device.A (31-gauge) syringe was used to inject .05 cc of patient's serum, histamine (10 µg/mL) and normal saline (0.9%) as a positive and negative control to the front side of the forearm in a distance of 3-5 centimeters intradermal. Within 30 minutes of the test, the injection site was read. The negative control was supposed to be asymptomatic while the positive control was expected to be swollen with a red rash in the surroundings. ASST result would be taken as positive only when the injection site was 1.5 mm more swollen than the negative control site.[31] The remaining blood serum was segregated and stored at -80 degrees centigrade until the test time. Then the S.P level was measured through Elisa kit (Made in Zell Bio GmbH, Germany, Cat No: ZB-11528C-H9648).

Ethical Considerations

Informed consent to take part in the research was obtained from the subjects. This research was approved by the Ethics Committee of Mashhad University of Medical Sciences (N. IR.MUMS.fm.REC.1396.610).

Statistical Analysis

The normality of data was checked through the Kolmogorov-Smirnov test. To compare normally-distributed variables in the two research groups, T-test was used and for compare abnormally distributed data, Mann-Whitney U-test was used for both groups. To compare quantitative variables across different age groups in terms of the type of distribution, ANOVA or Kruskal-Wallis were used.

Pearson or Spearman Correlation test was used to check whether there was a correlation between the variables. *p*-value was set at<05 to check the significance of findings. IBM SPSS, version 23 (IBM Corp., Armonk, N.Y., USA) was used to do all the analyses.

RESULTS

Participants' Demographic Information

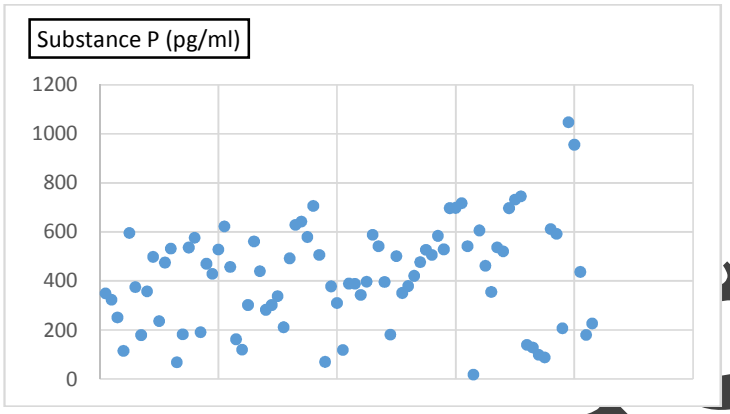
From 87 patients, 57 were female and 30 were male. There were 86 healthy subject participants, 56 of them were female and 30 were male. The age difference between the two groups was checked with Mann-Whitney U-test and the results showed that it is insignificant (p=0.926). The gender difference between the two groups was checked through the Chi-squared test and the results showed that it was not significant (p=0.956) (Table 1).

SP Serum Level in Patients vs. Healthy Participants

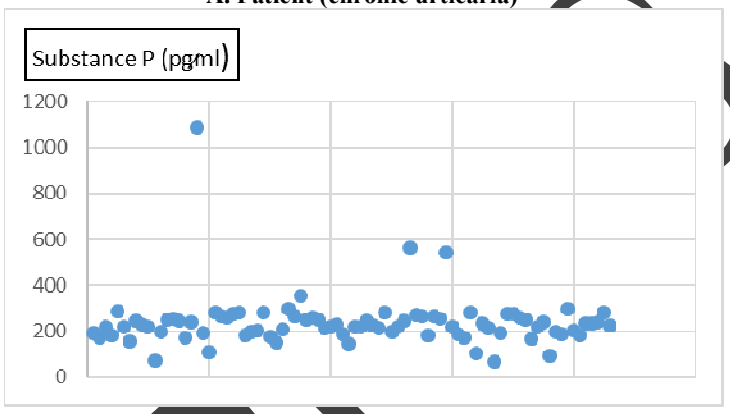
The mean SP serum level of the patient group was 434.37±227.917pg./mLwhich was about twice as high as that of the healthy (238.21±116.980pg./mL). Mann-Whitney U-test showed a statistically significant difference between the two research groups with this respect (p<0.001). Figure 1 indicates the SP serum level of all participants of both research groups.

Table 1.Demographics of chronic urticaria patients and normal control subjects in an of evaluation of serum Substance Plevels Chronic urticaria patients Normal

n	87	86
Age (years)		
(mean±SD)	35.72±12.00	35.81±12.30
Sex		
Male N (Percent)	30(34.5)	30(34.9)
Female N (Percent)	57(65.5)	56(65.1)



A. Patient (chronic urticaria)



B: controls

Figure 1. Scatter plot of Substance P serum level of participants comparing Group A (urticaria patients) and Group B (normal control)

SP Serum Level in Patients vs. Healthy Participants

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SP Serum Level Compared in terms of Patients' Gender

In Group A (patients), the mean SP level of male participants was 400.13±184.954 and the mean of female participants was 452.39±247.188. In group A, the difference between male and female was not statistically significant (p=0.312). In group B (control), the SP level of the male was estimated at 268.23±172.98 and that of the female group was 222.13±67.94 (Figure 2). In the control group, the two sexes showed no statistically significant divergence (p=0.136).

SP Serum Level Compared in Terms of Age

Pearson correlation test was run for all participants (n=173) which showed a statistically significant correlation between SP value and increase of age (p=0.010, r=0.197). In Group A (patients), the Spearman test was run which similarly showed a statistically significant correlation between increasing age and SP value (p=0.004, r=0.307) (Table 2).

SP Serum Level Compared in Terms of Patients' ASST

Among 87 CU patients, 53 (61%) and 34 (39%) had respectively positive and negative test result. Among the former, the mean SP was significantly higher than

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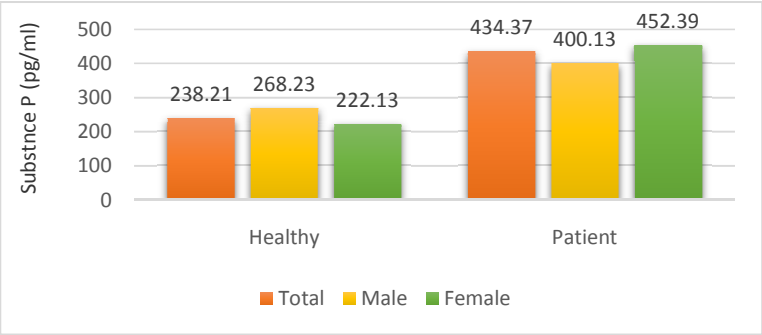

Figure 2. Substance P serum level inchronic urticariapatientsgroup vs. healthycontrol group in terms of gender

the latter (p=.0012) (Table 2). In terms of ASST size, among 53 CU patients with a positive ASST result, the mean diameter was 7.21±2.332 mm (min=3, max=13 mm).

SP Serum Level Compared in Terms of patients' Angioedema

In group A (patients), 41.4% had angioedema while 58.6% had none. There was no statistically significant difference between those with angioedema and those without (p=0.872) (Table 2).

SP Serum Level Compared in Terms of the Duration of Disease

The mean duration of CU was 36.85±56.981 months. No statistically significant correlation was found between the SP level and duration of the disease (p=.57, r=-0.062).

SP Serum Level Compared in Terms of CU Severity Measured in Questionnaires

Spearman correlation test was then run and found no statistically significant correlation between higher SP level and higher CU-Q2oL score (p=0.631),higher UAS7 score(p=0.898) and lower UCT score (p=0.092) (Table 3).

ASST Result Compared with Urticaria Questionnaire Results

To compare urticaria evaluation questionnaires as subjective measures and ASST result as an objective instrument of testing urticaria severity, positive and negative ASST subjects were compared that all results are showed in Table 4.

ASST Size (mm) Compared with Questionnaire Results

In the positive ASST group, the test size in millimeter was compared to the questionnaire results. Statistically significant correlations were found between UAS7 and Pruritus (p=0.042, r=.280) in one hand and CU-Q2oL and Pruritus on the other (p=0.032, r=295).

Table 2. Substance P serum level inchronic urticaria patients across sex, age group, ASST result and angioedema

Factor		N (%)	S.P (pg/mL) (Mean±SD)	*p*-value
Gender	Male	30 (34.5)	400.13±184.95	0.312*
	Female	57 (65.5)	452.39±247.19	
Age(years)	≤20	7 (8)	412.86±307.78	0.054**
	21-40	52 (59.8)	388.12±227.99	
	41-60	24 (27.6)	540.46±189.59	
	60-80	4 (4.6)	110.3±436.758	
ASST	Positive	53 (61)	483.23±234.65	**0.012***
	Negative	34 (39)	358.21±196.98	
Angioedema	Presence	36 (41.4)	429.64±216.54	0.872*
	Absence	51 (58.6)	437.70±237.69	

ASST: Autologous Serum Skin Test, * T-test, **Kruskal-Wallis

Table 3. Spearmancorrelation of SubstanceP serum level and urticaria evaluation questionnairesdata in chronic urticaria patients

Test		UAS7			UCT	Cu-Q2oL						
Domain		Total	urticaria	Pruritus	Total	Total	Pruritus	Swelling	Activities	Sleep	Limit	Look
S.P	*p*-value*	0.898	0.599	0.957	0.092	0.631	0.260	0.318	0.980	0.971	0.499	0.795
(pg./ml)	r	0.014	0.057	-0.006	0.182	-0.052	-0.122	-0.108	-0.003	0.004	0.073	-0.028

ASST: Autologous Serum Skin Test, SP: substance P, * Spearman correlation

UAS7: Urticaria Activity Score 7, UCT: Urticaria Control Test, Cu-Q2Ol: Chronic Urticaria Quality of Life

Table 4. Correlation of urticaria evaluation questionnaires data and Autologous Serum Skin Test result in chronic urticaria patients

Test			UAS7			UCT	Cu-Q2oL						
Domain			Total	urticaria	Pruritus	Total	Total	Pruritus	Swelling	Activities	Sleep	Limit	Look
A	Positive	Mean	26.45	12.60	13.85	5.50	62.77	7.60	4.24	16.02	14.50	7.79	12.60
S		S.D	10.63	5.49	5.75	2.68	17.05	1.84	2.39	5.71	4.87	2.71	4.71
S	Negative	Mean	23.47	12.03	11.44	4.82	62.61	7.76	3.55	15.56	14.47	7.94	13.32
T		S.D	12.23	6.65	6.29	2.87	17.13	1.89	2.19	5.54	4.80	2.67	5.02
		p-value	0.232*	0.626**	**0.043****	0.261*	0.967*	0.611**	0.166**	0.712*	0.971*	0.749**	0.500*
		r	0.248	-0.487	-2.020	0.269	0.967	-0.511	-1.385	0.710	0.971	-0.320	0.5-7

ASST: Autologous Serum Skin Test, *T-test, ** Mann Whitney test, UAS7: Urticaria Activity Score 7

UCT: Urticaria Control Test, Cu-Q2Ol: Chronic Urticaria QualityofLife

DISCUSSION

According to our knowledge, central histamine mediator often causes itching in CU patients. It is primarily released from the degranulation of mastocytes and basophils.[7] Neurotransmitters such an SP, through degranulation of mastocytes and basophils, can cause itching and hives in people.[9] The present research aimed to explore the role of SP in CU patients and the results indicated a higher level (twofold) of SP in CU patients in comparison with the healthy group.

In previous researches, only 4 other investigations explored the SP serum level in CU patients. Basak et al. had 57 CU patients along with 47 healthy subjects matched in terms of age and gender. They found a twofold increase in the CU patient group which was similar to the present finding.[18] In another study, Metz et al. studied 118 CU patients compared with 30 healthy subjects. Similar to the present study, the SP level was higher in the CU group than the healthy (4 times as higher).[17] In another research, Zheng et al reported a higher SP level in their CU group as compared to the control (3 times as higher).[19] The only research which observed no difference between the SP level of the CU group and that of the healthy was conducted by Tedeschi et al with 117 patient subjects and 24 healthy individuals. They found no significant difference between these two groups.[16] What was common between our research finding and that the research by Basak, Metz, and Zheng was the higher SP level in CU patients in comparison to healthy people. Contrary to the present research and others, the study by Tedeschi et al reported no statistically significant difference between the two groups. However, it is noteworthy that the experimental kits used in the newer researches including our study have been more sensitive.

In case the serum is segregated from blood after an hour or the cold chain is not stored in a freezer at the temperature of -80 degrees, the SP level is decreased. One advantage of the present research over others is the large health sample size (n=86) as compared to that of Tedeschi (n=24), Zheng (n=15) and Basak (n=46). This provided a more precise comparison of the healthy and patient groups.[16-19] Moreover, in the present research, those with psychological disorders, anxiety or major depression were excluded from both groups; those with a physical disease other than CU which might have induced an increase in SP level. Two research groups were matched in terms of age and gender. In the patient

group, the SP showed to be higher among female participants and SP increase with the increase of age. In other investigations, SP level was not compared in terms of age or gender. As SP has been recognized as a factor involved in the severity of CU[4,9] and positive ASST result is also considered a factor underlying the severity of CU and duration of disease,[32] SP levels were compared in the two ASST result groups (positive and negative). The former group showed a significant higher SP level than the latter one. In Tedeschi's investigation, 3 patients were positive ASST and marked as high SP level.[16] In Basak's research which looked into the correlation of ASST result and SP level, two groups showed no statistically significant divergence.[18] Mind that the number of patient participants in the present research was higher than that Bask research.

Due to the fact that angioedema is also a factor involved in the severity of CU,[4] the presence of angioedema was also examined along with SP level. The result showed no statistically significant difference between those with and without angioedema in terms of SP level. This finding was similar to that of Metz.[17] These groups were not compared in the related literature. There is a need for further research into the correlation of SP in CU patients and the prognostic factors of disease duration and CU severity (e.g. ASST, angioedema, duration of disease, etc.). SP level was taken as objective data of CU severity and its correlation was tested with the scores obtained from questionnaires as subjective data to test the severity of urticaria. No statistically significant correlation was found between UAS7, UCT, and CU-Q2oL scores and SP level. In the related literature, only Metz tested the correlation of SP level and UAS7 score and found a statistically significant correlation.[17] None of the previous studies explored the correlation of S.P serum level and duration of disease. In the present research, however, the correlation between the two was tested and showed to be insignificant. Thus, according to the present findings, SP is not a prognostic factor of CU severity and duration.

Another interesting point raised in the present research but absent in the related literature was the positive correlation between the questionnaire scores and positive ASST result. Statistically,a significant correlation was found between the pruritus domain of UAS7 and positive ASST result. So it is possible there is a correlation between positivity of ASST and severity of pruritus in chronic urticaria.

Similarly, statistically significant correlations were found between the induced diameter of ASST and the pruritus domain of UAS7 (p=0.042) and CU-Q2oL (p=0.032). This would show that for those with more itching (a higher pruritus score from UAS7 and CU-Q2oL), ASST would turn positive at a larger diameter.

The limitations of our study were the lack of evaluation of NK1 as a receptor of substance Pand the small sample size. We suggest these performed in the future studies.

In light of the present findings, the serum level of SP in CU patients was twice as high as the healthy individuals. SP level showed to increase along with an increase in age. The serum level of SP was higher in the 40-60-year-old age group and among the female gender. In patients with positive ASST result, SP level was higher than the negative ASST group. SP value showed no statistically significant correlation between the severity and duration of urticaria, angioedema and the evaluation scores of the disease.

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