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ABSTRACT

Background:

Thiol-disulphide balance plays a major role in health and diseases. This balance may be disrupted by various diseases. We aimed to determine status of the effect of thiol -disulphide balance in urticaria

Objectives

We aimed to investigate the thiol-disulphide balance in patients with acute urticaria and chronic spontaneous urticaria.

Methods

Study included 53 patients with acute urticaria and 47 healthy controls plus 57 patients with chronic spontaneous urticaria and 57 healthy controls. Levels of native thiols, disulphides and total thiols were evaluated in plasma using a new and automated spectrophotometric method. Ratios of disulphides/total thiols, disulphides/native thiols and native thiols/total thiols were calculated.

Results

For acute urticaria, there was no statistical difference compared to control group in levels of native thiols, disulphides and total thiols. For chronic spontaneous urticaria, however, there was an increase in levels of native thiols, disulphides and total thiols and the ratio of thiol/disulphide in favour of disulphide.

Conclusion

Thiol-disulphide balance was not affected by acute urticaria but shifted towards to disulphide in chronic spontaneous urticaria indicating the presence of oxidative stress.

Keywords

Acute urticaria, chronic spontaneous urticaria, oxidative stress, thiol-disulphide balance

Introduction

Urticaria is a sudden and itchy skin disorder of unknown origin associated with plaques and sometimes accompanied by angioedema. 15-25% of the population develop urticaria in some point of their lives. It is classified as acute or chronic based on the duration of symptoms. Clinical type that lasts up to 6 weeks is referred to as acute urticaria (AU), and the clinical type that lasts longer than 6 weeks is called chronic urticaria (CU)^{1,2} Recently, the physical, contact, cholinergic and aquagenic forms of urticaria are collected under the term "chronic urticaria". The term "chronic idiopathic urticaria" is dismissed and recommended to be referred to as chronic spontaneous urticaria (CSU)². AU is affected mostly by the medicines, foods and local and systemic infections and the cause is not known in 45% of the cases³⁻⁵. On the other hand, CSU can be associated with various disorders such as autoimmune, viral, bacterial and haematological diseases as well as collagen tissue and thyroid diseases^{6,7}.

Balance between the oxidants and the antioxidants in the organism is essential in maintaining cellular and biochemical functions⁸. Oxidative stress (OS) causes cellular injury when the levels of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) increase above the physiological levels in the cells exposed to endogenous or exogenous oxidants^{9,10-13}. Oxidants damage the lipids, proteins and DNA in the cells, and even lead to death¹³. Increased ROS itself is a cause of illness but sometimes the levels of ROS may increase after an illness¹⁴⁻¹⁸.

The mostly and rapidly affected proteins are sulfhydryl-containing thiols. Plasma thiols are powerful antioxidants that physiologically eliminate the free radicals^{15,19}. Serum levels of protein thiols are among the indicators of antioxidant status in the body²⁰.

Total thiol groups are highly susceptible to oxidation and are the first antioxidants that are consumed in case of oxidative stress. Thiols, also known as "native thiols", are a class of organic compounds that contain a sulfhydryl group (Native thiol : [-SH], Disulfide bond : [-SS-], Total thiol : [-SH] + [-SS])²¹. Plasma thiols mainly consist of albumin, and to a lesser extent, cysteine and glutathione¹⁹. Normally, there is a balance between the thiols and the disulphides and they also play a protective role in cellular redox homeostasis. This is called dynamic thiol/disulphide homeostasis^{19,21-25}. Disruptions in this balance can be associated with various diseases²¹ More remarkable ROS have been suggested to play a role also in pathogenesis of several inflammatory and allergic diseases including urticaria^{16,20,26-28}. Some

conflicting information has been reported on ROS.^{16,20,26-28} However, the cause is still unknown^{26-27,29-30}.

The oxidative stress has been studied with different markers using some antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), nitrite oxide (NO)) in individual groups of patients with acute and chronic urticaria. No study has been conducted to investigate the oxidative stress and thiol/disulphide homeostasis in both clinical forms combined^{27,29,30}.

In this study, we aimed to investigate the thiol/disulphide homeostasis that is of vital importance with a new, automated method developed by Erel and Neselioglu by measuring both sides of the balance in patients of AU and CSU and comparing them with different control groups²¹.

Materials and methods

This prospective case-control study was conducted between January and August 2015. 53 patients presented with acute urticaria and 47 healthy controls as well as 57 patients with chronic spontaneous urticaria and 57 healthy controls were included in study and the patients were non-smokers who had no other disease. Subjects in control groups were also non-smokers without any other skin disorder who applied to our clinic for general health check. Food and medicine allergy, presence of infection, any systemic and neoplastic disease, inducible urticaria such as solar, aquagenic, contact, cold, physical and cholinergic, urticarial vasculitis and hereditary angioedema were selected as exclusion criteria for patients with urticaria. Patients who did not receive systemic cortisone therapy within past 1 month and antihistamine therapy within the last 10 days were included. Due to the differences in age and gender of patients, individual control groups were created for both of the groups. Approval of the ethics committee of our hospital was obtained before study. Study was conducted according to good clinical practices and Helsinki declaration. Consents of patient and control groups were obtained.

Extensiveness and severity of lesions were evaluated weekly by dermatologists to determine urticaria activation score⁸. The weekly urticaria score was determined using the following convention: 0 = no urticarial plaque, 1 = at least 20 plaques, 2 = 20 to 50 plaques, 3 = more than 50 plaques, and for the presence of itching, 0 = none, 1 = mild, 2 = moderate, 3 = severe.

Plaque and itching scores are added and this was multiplied by 7 to find activation score. Maximum daily score was 6 and the weekly score was 42. Patients were questioned for age, gender, duration of disease, food intake, medication history, history of recurrence, family history and triggering factors.

Venous blood samples were taken in tubes containing EDTA and centrifuged at 1500 rpm x for 10min and stored in the freezer at - 80 °C. The tests of thiol/disulphide homeostasis were performed using the newly developed spectrophotometric method ²¹. Native thiol of the samples were measured by 5,5'-dithiobis-(2 nitrobenzoic) (DTNB)³¹. To measure total amount, first disulphide bonds were reduced with sodium borohydride and the free functional thiol groups were exposed. The unused reduced sodium borohydride was treated with formaldehyde in order to prevent the reduction of DTNB. Total thiol groups composed of reduced and native thiol groups were determined after reaction with DTNB. The total thiol content of the sample is measured using modified Ellman reagent. Native thiol content is subtracted from the total thiol content and half of the obtained difference gives the disulphide bond amount. The disulphide parameter is a value which can be calculated automatically as half of the difference of the two measured values. The ratios of disulphide/total thiol, disulphide/native thiol and native thiol/total thiol were calculated. Total protein and albumin were measured in g/dL by colourimetric method using the automated analyser Roche-Hitachi Cobasc501. Thyroid hormones were measured by electrochemilumineszenz-immunoassay using the automated analyser Roche-Hitachi Cobasc 600 with its kits. Erythrocyte sedimentation rate were measured by on automated ESR analyser(wintrobe) and the obtained results were expressed of mm/h. **Normal values of thyroid tests are in the following ranges. Thyrotrophin stimulating hormon (TSH): 0,27-4,2 uIU/mL, free triiodothyronine (fT3):1,8-4,6 pg/mL, free thyroxine (fT4):0,9-1,7 ng/dL, Thyroglobulin antibody(AntiTG): 0-34 IU/mL, Thyroid Peroksidase antibody(AntiTPO):0-115 IU/mL.**

Shapiro-Wilk test was used to check if continuous variables in the study such as age, total protein and total thiols were normally distributed. Normally distributed variables were expressed in average $\pm$ standard deviation (AVG $\pm$ SD), non-normally distributed variables were expressed in median (IQR: Interquartile range) and the categorical variables such as gender and urticarial activation score were expressed in figures (%).

T-test and Mann-Whitney U test were used in independent groups based on the distribution of variables and the balance status of groups. When examining the relationships

between the variables and the thiol values, Pearson's r coefficient and Spearman's rho coefficient were calculated depending on the distribution of variables. Statistical significance level was considered to be $p < 0.05$.

IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) program was used for statistical analyses and calculations..

Results

Mean ages were calculated to be 36 years (IQR: 20) and 38 years (IQR: 22) respectively for 53 patients with acute urticaria (AUP) and 47 healthy controls (AUC), and no statistically significant difference was observed between the groups in age ($t=-0$, $p=0$, Table 1). Mean ages of 57 patients with chronic spontaneous urticaria (CSUP) and 57 healthy controls (CUC) were calculated to be 40 years (IQR: 25) and 40 years (QIR: 26), respectively. No statistically significant differences were found in age between the controls and both the groups of AUP and CSUP. Male patients were accounted for 35.8% ($n=19$), 29.8% ($n=14$), 43.9% ($n=25$) and 42% ($n=24$) in AUP, AUC, CSUP and CUC groups, respectively. Gender distribution was determined to be similar in both of the groups ($\chi^2=0.185$, $p=0.667$, $\chi^2=0.0227$, $p=0.634$).

Demographic and clinical characteristics of patients are provided in Table 1.

Median scores of daily urticarial plaques and itching and the weekly urticaria activity score were calculated to be 1.0 (IQR = 1.0) and 21.0 (14.0) for both groups, respectively. Urticarial plaques could be localized or extensive. There was also no difference in plaques, itching and activation scores between two groups. In this study, a significant relationship was found between disulphide levels and daily urticaria activity score, despite the unimpaired thiol-disulphide balance in acute urticaria.

Distribution of biochemistry and clinical findings of the patients are provided in Table 2. There was no difference between biochemical findings among patients. There was no statistically significant difference in total protein, albumin, sedimentation levels between AU and CSU groups

Distribution of values for native thiols, disulphides and total thiols by groups are given in Table 3. While thiol-disulphide balance didn't change in AU, it was found to change in CSU.

TSH level was higher in one AU patient and 5 CSU patients, fT3 value was higher in one AU patient and one CSU patient, fT4 value was higher in one AU patient and 2 CSU patients. Furthermore, elevated anti TPO level was observed in 10 AU patients and 8 CSU patients. Elevated anti TG level was observed in 5 AU patients and 7 CSU patients.

The relationship between the clinical and laboratory findings and the thiol-disulphide levels of acute and chronic spontaneous urticaria patients is provided in Table 4. **There is a significant relationship with, fT3 and fT4 values that is stronger in chronic urticaria. Relationship with TSH was found to be insignificant in AU but significant in CSU.**

A negative, moderate relationship was observed between native thiols and total thiols in both the AU and CSU groups ($\rho=-0.493$, $\rho=-0.459$ for AU group, respectively, and $\rho=-0.436$, $\rho=-0.430$ for CSU group, respectively, $p < 0.001$, Table 4). **There was not statistically significant relation between TSH, AntiTG, AntiTPO and thiol levels in acute urticaria group ($p > 0.05$). There was not significant relationship between AntiTG, urticaria score and thiol levels in chronic spontaneous urticaria group ($p > 0.05$).**

There was significant relationship between the age. Our study was the decrease observed in native and total thiol levels with the advanced age both in acute and chronic spontaneous urticaria.

Discussion

There is a balance between the production of free radicals and the antioxidant system which suppresses the increase in ROS in the body. Oxidative stress occurs if this balance is disrupted^{10, 32}.

Free radicals resulting from the normal metabolism or pathological processes cause an impairment of structures and functions of thiol-dependent enzymes and a change in thiol/disulphide ratio in the cell medium. Decreased plasma thiol concentration indicates the increased production of free radicals²¹.

Previous studies on oxidative stress have rather measured the levels of enzymatic antioxidants such as malondialdehyde (MDA), GSH PX and CAT and attempted to calculate the oxidative stress index.^{29,30,30} We assumed that increased disulphide levels indicated increased OS.

Recently, it became possible to obtain information on oxidative stress by determining the total ratio of plasma thiols/disulphides using a new method developed by Erel and Neselioglu. This method is both easier and allows for measurement of both sides of the balance²¹.

Previous studies have investigated the enzyme systems that may cause etiopathogenesis of urticaria. However, we tried to detect the presence of oxidative stress by examining thiol/disulphide values which, to our knowledge, had never been studied in any skin disorder. We investigated the thiol-disulphide balance in patients with urticaria. Comparison of native thiol, total thiol and disulphide values of patients with disulphide/native thiol(index1) and the disulphide/total thiol(index2), and native thiol/total thiol(index3) ratios showed that the native thiol, total thiol and disulphide values were not changed in AU, whereas an increase was observed in disulphide/total thiol ratio in CSU. This indicates that the thiol-disulphide balance system shifts towards to disulphide formation. These findings suggested the presence of OS in CSU.

Similar results may also be observed in conditions other than oxidative stress. Decreased native thiol levels are not necessarily associated with increased disulphide levels and similarly, increased native thiol levels are not necessarily associated with decreased disulphide levels. The important factor here is the change in ratios that indicates overall balance. This may be associated with immune response of thiol in inflammation^{33,34}. Decreased plasma thiol concentration is an evidence for production of free radicals. Decreased thiol levels are also associated with leukocyte activation. Urticaria is accompanied by immunologic activation and inflammation by the activation of mast cells, basophils and eosinophils, and ultimately, oxidative stress along with increased ROS^{26,29,33,34}.

In this study the method developed by Erel and his colleagues was used to measure thiol-disulphid balance. While thiol-disulphid balance did not change in AU, it was found to change in CSU. In previous studies OS was measured in only AU or in only CU. However we compared both AU and CSU with control groups separately and also with each other. For CSU, thiol balance may be disrupted because of the long course of the event. Therefore we think that the results of our study could be important. These results may show that OS could be present in CSU while it is not present in AU.

The limitation of this study was that the patients did not give any past history about their diseases and we found out various diseases after their laboratory tests. These diseases might also trigger the OS.

There are various studies about the OS in AU and CSU which were performed in blood samples.. Some studies showed an increase OS, whereas some studies showed no change in OS.³⁵⁻⁴⁰

Raho et al. obtained samples of skin with and without lesions from chronic urticaria patients and healthy subjects, and found markedly elevated levels of SOD, GSH and MDA in skin with lesions. Interestingly, they reported no difference between the normal skin and the control group. Authors, therefore, suggested that the oxidative stress occurs secondarily to inflammation^{29,26}. This may be explained by the fact that increased activity score have led to an increase in thiol-disulphide balance in favour of disulphide in our study. We have not measured the thiol-disulphide values in skin tissue. It would be better to perform concomitant measurements in both skin and plasma.

There are studies on dynamic thiol/disulphide homeostasis in different disease processes.⁴²⁻⁴⁵ Eren et al. demonstrated that the total thiols markedly decreased in patients with migraine as compared to control group⁴². Ergin et al. determined that the thiol/disulphide balance shifted towards to oxidative status in patients with hyperemesis gravidarum as compared to healthy pregnant women⁴³. Kundi et al. found lower levels of native and total thiols and higher levels of disulphide in patients with acute myocardial infarction compared to control group and stated that the thiol/disulphide homeostasis might be used as a new marker of oxidative stress in patients with acute myocardial infarction⁴⁴. Yılmaz et al. investigated the thiol/disulphide homeostasis in asphalt workers exposed to polycyclic aromatic hydrocarbons and found that the balance shifted towards to disulphide⁴⁵.

In our study, a significant relationship was found between disulphide levels and daily urticaria activity score, despite the unimpaired thiol-disulphide balance in acute urticaria. Urticaria activity score is associated with the severity of disease. Disulphide levels that increase with the increase in urticaria activity score may be important in disease activation. This shows that the activation of urticaria has effects on thiol-disulphide but more detailed studies with a large sample set are needed to explain the mechanism of activation. Dilek et al. have found similar findings in their study⁴¹.

Another finding of our study was the decrease observed in native and total thiol levels with the advanced age both in AU and CSU. This supports the relationship between the advanced age and the oxidative stress^{46,47}

In this study, relationship between thyroid tests and thiol values in patients with urticaria was also evaluated. As can be seen in Table 4, There is a significant relationship with, fT3 and fT4 values that is stronger in CSU. Relationship with TSH was found to be insignificant in AU but significant in CSU. From these results, it can be concluded that urticaria, particularly CSU, may be associated with thyroid disorder. Conducted studies reported that thyroid disorder was observed with a two-fold higher frequency in patients with CSU than normal community⁴. There are studies indicating that the oxidative stress also occurs in patients with thyroid disorder^{48,49}. Oxidative stress appears to be further increased in urticaria patients with thyroid disorder. Further studies are needed to gain a clear understanding whether the changes in thiol values in our study were caused by the activation of disease itself or causes such as accompanying thyroid disorder.

Another result of our study was the fact that about half of the urticaria patients in both groups had emotional stress based on patients past history. Emotional stress itself can trigger urticaria but urticaria can also cause emotional stress and thus, increased oxidative stress⁵⁰. This is supported by the significant relationship between the disulphide/total thiol ratio and the history of stress factor.

Conclusion

Urticaria is a disease of which the exact cause is not known. Studies on oxidative stress have been conducted as part of the studies on its etiopathogenesis. Based on the data of our study on urticaria and oxidative stress, there is a balance in homeostasis in acute urticaria but the dynamic thiol/disulphide homeostasis is impaired in patients with chronic spontaneous urticaria. Further studies are needed to support these findings. This relationship will be able to be cleared by the furtherly designed studies in larger groups measuring concominantly thiol levels in both plasma and tissue.

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Table 1. Demographic and clinical characteristics of patients

	AU (n=35)	AUC (n=47)	CSU (n=57)	CUC (n=57)	Test statistical	p
Age					Z=0.290 Z=0.323	0.772 ¹ 0.747 ²
MEAN(IQR)	36.0 (20.0)	38.0 (22.0)	40.0 (25.0)	40.0 (26.0)	Z=1.182	0.237 ³
Min – Max	19.0 – 81.0	19.0 – 74.0	18.0 – 73.0	18.0 – 75.0		
Gender[n (%)]					$\chi^2=0.185$ $\chi^2=0.000$	0.667 ¹ 1.000 ²
Male	19 (35.8)	14 (29.8)	24 (42.1)	25 (43.9)	$\chi^2=0.227$	0.634 ³
Female	34 (64.2)	33 (70.2)	33 (57.9)	32 (56.1)		

Duration of illness	AU	CSU
0-1,5 months	53 (100.0)	0 (0.0)
1.5-3 months	0 (0.0)	18 (31.6)
3-6 months	0 (0.0)	7 (12.3)
6 months longer	0 (0.0)	32 (56.1)

Stres story	AU	CSU
+	23 (43.4)	28 (49.1)
–	30 (56.6)	29 (50.9)

AU: Acute Urticaria AUC :Acute Urticaria control

,CSU: Chronic Spontaneous Urticaria,CUC: Chronic Spontaneous Urticaria control,

Table 2. Distribution of biochemistry and clinical findings of the patients

	AU MEDIAN(IQR) Min-Max	CSU MEDIAN(IQR) Min-Max
Total protein(g/dl)	7.32±0.41	7.45±0.39
	7.37(0,50)	7.44±0.55
Albumin(g/dl)	4.69±0.38	4.71±0.30
	4.69±0.39	4.70±0.39
fT3*(pg/dl)	2.83±0.69	3.06±0.60
	1.12 – 4.43	1.03 – 4.52
fT4(ng/dl)	1.22 (0.19)	1.27 (0.28)
	0.87 – 4.15	0.94 – 2.79
TSH(µU/ml)	1.69 (2.01)	1.98 (1.90)
	0.22 – 5.51	0.10 – 8.17
AntiTPO(IU/ml)	10.48 (2.01)	12.26 (10.87)
	5.00 – 340.60	0.95 – 600.00
AntiTG(IU/ml)	16.93 (14.15)	19.00 (12.45)
	10.00 – 2997.00	6.30 – 570.40
Sedimentation(mm/h)	11.0 (13.0)	11.0 (12.0)
	1.0 – 47.0	1.0 – 55.0
Plaques	1.0 (1.0)	1.0 (1.0)
	0.0 – 3.0	0.0 – 3.0
Itching	2.0 (2.0)	2.0 (1.0)
	0.0 – 3.0	0.0 – 3.0
Urticaria activation score	21.0 (14.0)	21.0 (14.0)
	0.0 – 42.0	0.0 – 42.0
Acute urticaria and Chronic spontaneous urticaria groups comparison TSH= thyrotrophin stimulation hormon fT3= free triiodothyronine, fT4 =free thyroxine, Anti TG=Thyroglobulin antibody AntiTPO=Thyroid Peroxidase antibody .		

Table 3. Distribution of values for native thiols, disulphides and total thiols by groups

	AU	AUC	CSU	CSUC	Test Statistic	p
Native Thiol					t=0.687	0.494 ¹
Mean	453.50±43.39	445.71±67.81	448.68±44.53	454.67±43.4	t=0.710	0.479 ²
Median(IQR)	453.20 (58.45)	454.90 (90.40)	448.70 (58.10)	453.65 (65.85)	t=-0.269	0.789 ³
Disulphide					t=0.186	0.853 ¹
Mean	19.17±8.22	19.47±7.78	18.45±6.61	19.76±7.69	t=0.957	0.341 ²
Median(IQR)	19.00 (9.70)	18.55 (11.48)	18.15 (7.75)	20.25 (8.15)	t=0.741	0.460 ³
Total Thiol					t=0.600	0.550 ¹
Mean	491.76±45.58	484.62±71.15	485.57±49.43	494.24±45.25	t=0.954	0.342 ²
Median(IQR)	493.40 (65.05)	488.00 (104.90)	485.30 (70.45)	500.50 (67.38)	t=-0.081	0.936 ³
Index-1 Disulphide /native thiol					Z=0.633	0.527 ¹
Mean	0.04±0.02	0.05±0.07	0.05±0.08	0.06±0.13	Z=1.445	0.148 ²
Median(IQR)	0.04 (0.03)	0.05 (0.03)	0.04 (0.02)	0.00 (0.10)	Z=1.217	0.224 ³
Index-2 Disulphide /total thiol					Z=1.025	0.305 ¹
Mean	0.04±0.05	0.05±0.04	0.06±0.10	0.05±0.12	Z=3.370	0.001 ²
Median(IQR)	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	0.00 (0.10)	Z=1.569	0.117 ³
Index-3 Native thiol /total thiol					t=0.005	0.996 ¹
Mean	0.92±0.03	0.92±0.03	0.92±0.03	0.92±0.04	Z=0.850	0.396
Median(IQR)	0.92 (0.04)	0.92 (0.06)	0.92 (0.04)	0.90 (0.00)	t=-0.913	0.363 ³

¹ Acute urticaria and control groups comparison

² Chronic urticaria and control groups comparison

³ Acute urticaria and Chronic spontaneous urticaria groups comparison

* Pearson r coefficient, [†] point double series correlation coefficient, others Spearman rho coefficient. ¹, ² p<0.05; ³ p<0.001

Table 4. The relationship between the clinical and laboratory findings and the thiol-disulphide tests

	ACUT ÜRTICARIA GROUPS							CRONIC SPONTAN ÜRTICARIA GROUPS					
	Native Thiol	Disulphide	Total Thiol	Index 1	Index 2	Index 3		Native Thiol	Disulphide	Total Thiol	Index 1	Index 2	Index 3
Age	-0.537 ³	-0.141	-0.544 ³	0.089	0.014	-0.052		-0.436 ³	-0.092	-0.430 ³	0.027	0.049	-0.102
fT3	-0.008*	0.059*	0.006*	0.130	0.124	-0.077*		0.367 ^{2,*}	-0.007*	0.334 ^{1,*}	-0.241	-0.142	0.184
fT4	0.241	0.093	0.273	0.086	-0.005	-0.085		0.280 ¹	0.202	0.317 ¹	0.161	0.156	-0.108
TSH	-0.059	0.038	-0.032	0.099	0.134	-0.046		-0.246	0.172	-0.181	0.318 ¹	0.276 ¹	-0.278 ¹
ANTI TG	0.078	-0.073	0.059	-0.133	-0.087	0.102		0.029	-0.028	0.023	-0.046	-0.055	0.048
ANTI TPO	-0.127	-0.172	-0.148	-0.086	-0.094	0.108		-0.021	0.241	0.024	0.270	0.244	-0.279 ¹

¹ Acut urticaria and control groups comparison, ² Chronic urticaria and control groups comparison

* Pearson r coefficient, † point double series correlations coefficient, ‡ Spearman rho coefficient. ¹ p<0.05, ² p<0.01, ³ p<0.001