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ORIGINAL CONTRIBUTION

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Suppressor of Cytokine Signaling Proteins 3 and 5 Potentially Delineate Polarization of Th cells in Chronic Rhinosinusitis

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Background: Chronic rhinosinusitis (CRS) is an inflammatory condition classified into chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP). Th cells manage inflammatory cells in CRS. Suppressor of Cytokine Signaling (SOCS) proteins regulate Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway in Th cells by polarizing toward Th1, Th2, and Th17 cells. This study evaluated the levels of SOCS1,3,5 in CRS patients to find associations with Th cells. **Methods**: In this cross-sectional study, 20 CRSwNP patients, 12 CRSsNP patients, and 12 controls participated. The infiltration of $CD4^+$ T cells was determined using immunohistochemistry. The expression of specific transcription factors and SOCS proteins was assessed using real-time PCR. Cytokine levels were evaluated using ELISA. SOCS protein levels were investigated using western blot analysis. **Results**: The expression of SOCS3 increased in the CRSwNP group compared to CRSsNP and control groups (p <0.001). SOCS3 protein levels increased in the CRSwNP group compared to CRSsNP (p <0.05) and control (p <0.001) groups. Although there was a significant difference in SOCS5 expression between CRSsNP and control groups, SOCS5 protein levels were significantly different between CRSsNP and control (p <0.001) and CRSwNP (p <0.05) groups. **Conclusions**: Targeted therapies may be suggested for CRS by modulating SOCS3 and SOCS5 proteins that are responsible for polarization of Th cells toward Th2 or Th1 cells, respectively. JAK-STAT pathway targeting, which encompasses numerous cells, can be limited to SOCS proteins to more effectively orchestrate Th cell differentiation.

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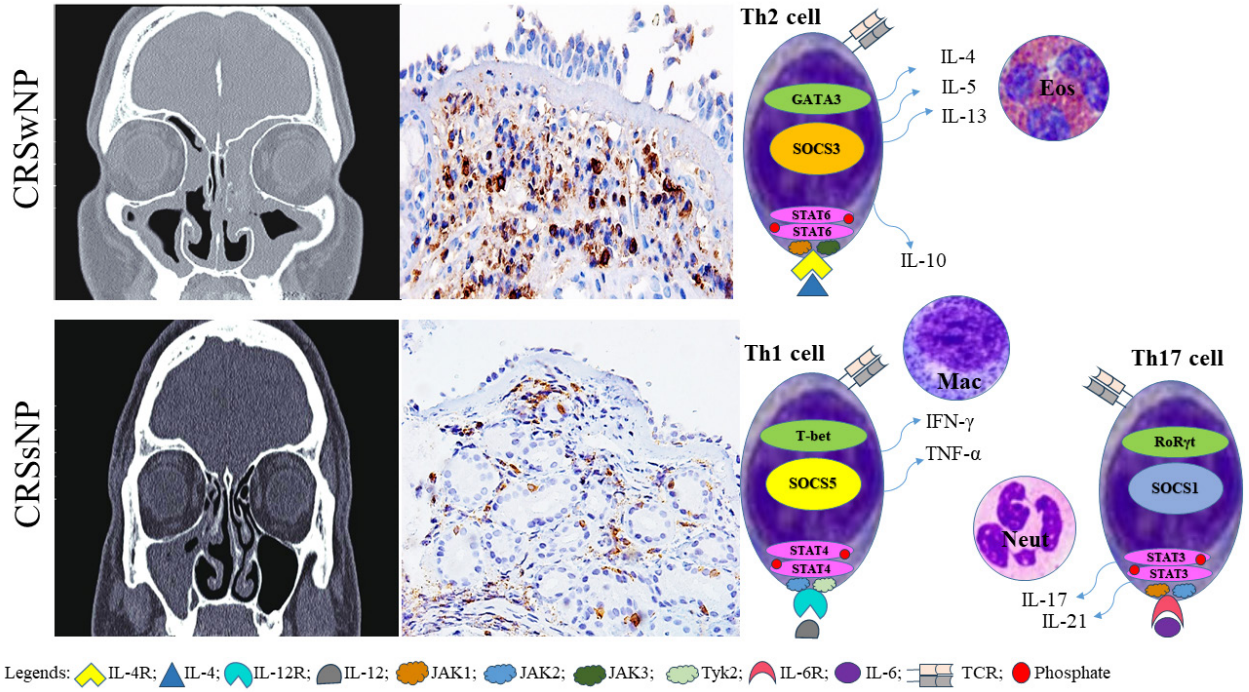
Abbreviations: CRS, Chronic rhinosinusitis; CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps; Th, T helper cells; SOCS, Suppressor of Cytokine Signaling; JAK, Janus kinase; STAT, signal transducer and activator of transcription.

Keywords: Chronic rhinosinusitis, CRSwNP, CRSsNP, JAK-STAT pathway, Suppressors of Cytokine Signaling, SOCS, Th cell, cytokine, differentiation, polarization



Graphical Abstract. **SOCS proteins inhibit JAK-STAT pathway**. SOCS3 potentially differentiates naïve Th cells into Th2 cells in CRSwNP but SOCS5 differentiates naïve Th cells into Th1 cells in CRSsNP. SOCS modulation may affect T cell polarization from Th1 to Th2 cells and vice versa.

INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory condition in which respiratory tract and paranasal sinuses are affected [1]. The GA2LEN study indicated that according to EP3OS criteria, the global prevalence of CRS was 10.9% (range 27.1%-6.9%), of which the prevalence of CRS diagnosed by specialist physicians was approximately 2%-4% [2]. Furthermore, CRS is categorized into two groups: Chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP) [3]. In adaptive immunity, T lymphocytes play a crucial role in regulating inflammatory processes at mucosal sites. The remarkable role of T helper (Th) cells ($CD4^+$) has been identified to some extent in the pathogenesis of CRS. These cells consist of IFN-γ-producing Th1 cells, IL-4-producing Th2 cells, IL-17A-producing Th17 cells, and regulatory T cells (Treg). It has been shown that CRSwNP is characterized by dominant Th2 responses, whereas CRSsNP comprises dominant Th1 responses [1].

These Th cells are strictly regulated by various cytokines. The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway can regulate Th cells through cytokine receptors [4]. On the other hand, JAK-STAT pathway is extremely regulated by several regulator proteins such as Protein Inhibitors of Activated STATs (PIAS), Protein Tyrosine Phosphatases (PTPs), and Suppressors of Cytokine Signaling (SOCS). In this regard, SOCS proteins have key roles in downregulation of cytokine signaling. The SOCS family of proteins contains eight members, including SOCS1-SOCS7 and cytokine-inducible SH2 domain protein (CIS or CISH). SOCS1 induces Th17 differentiation while inhibiting Th1 and Th2 differentiation. SOCS3 induces Th2 differentiation, whereas it suppresses Th1 and Th17 differentiation, and finally, SOCS5 induces Th1 differentiation while inhibiting Th2 and Th17 differentiation. Dysregulation of the JAK-STAT pathway in Th cells may result in various immune disorders [5]. Therefore, new therapeutic strategies to target SOCS proteins will broaden promising horizons toward treatment of immune disorders, especially chronic rhinosinusitis. In this study, we aimed to evaluate the mRNA expression and protein levels of SOCS1, SOCS3, and SOCS5 in CRSwNP and CRSsNP patients and their potential association with differentiation of Th1, Th2, Th17, and Treg cells.

MATERIALS AND METHODS

Patients and Controls

The diagnosis of CRS follows the guidelines set by the European Position Paper on Rhinosinusitis and Nasal Polyps, which include symptoms such as blockage, congestion, nasal discharge, facial pain or pressure, and reduced or absent sense of smell persisting for at least 4

Table 1. Characteristics of Controls and CRS Patients

Parameters		Controls	CRSsNP	CRwNP	*p* value
Number		12	12	20	
Gender	Female	6	6	10	0.99
	Male	6	6	10	
Age (y) Mean ± SD		33.6±13.4	27.3±10.3	29±7.14	0.9
Age (y) Median (IQR)		31 (10.1)	28 (12.0)	27.0 (8.0)	
Asthma		0	0	4	-
CT score*		0	8.35 (4.2-12.75)	16 (12-19)	<0.0001
Endoscopic score*		0	2 (0-2)	3 (2-4)	<0.001
VAS score*		0	6 (5-8.35)	9 (8-11)	<0.050

*Data are expressed as median (IQR). IQR: Interquartile range; SD: Standard deviation; VAS: visual analogue scales.

weeks. To confirm the presence of nasal polyps, CT scans and endoscopic scores were utilized. Clinical scores for each CRS patient were determined using the Lund-Kennedy nasal endoscopy scores, preoperative Lund-Mackay CT scores, and both preoperative and postoperative 22-item Sinonasal Outcome Test (SNOT-22) scores [1]. Patients diagnosed with CRS were confirmed by specialist clinicians at the ENT and Head and Neck Department of Hazrat Rasoul Akram Hospital, affiliated with Iran University of Medical Sciences. The study included 20 patients with CRSwNP and 12 with CRSsNP. Additionally, healthy individuals who underwent septoplasty due to nasal septum deviation, without a history of CRS or asthma, served as the control group, providing 12 inferior turbinate tissue samples. Demographic data of the participants are presented in Table 1. The study received approval from the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1401.1042), and all participants provided written informed consent before sample collection. The research adhered to the ethical standards set by the national and institutional guidelines on human experimentation and conformed to the Helsinki Declaration of 1975, revised in 2008. Exclusion criteria for the study included CRS patients with (1) immunodeficiencies, cystic fibrosis, bronchiectasis, chronic obstructive pulmonary disease, diabetes mellitus, neoplasia, or fungal rhinosinusitis; (2) those who were pregnant or lactating; and (3) those who had upper respiratory infections within the past month. Additionally, none of the patients had used local or systemic corticosteroids, antibiotics, antihistamines, decongestants, or anti-leukotrienes in the 4 weeks prior to sample collection.

Histological and Immunohistochemistry (IHC) Analyses

To assess the pathological features of the sinonasal tissues, paraffin-embedded sections (5 mm) were stained with Hematoxylin & Eosin (H&E) for 60 minutes at room temperature. Subsequently, the sections were dehydrated, embedded in paraffin, and then rehydrated through a series of xylene and ethanol washes. Antigen retrieval was performed by immersing the sections in a low pH Target Retrieval solution (Dako, Glostrup, Denmark) and autoclaving at 121°C for 20 minutes. To inhibit endogenous peroxidase activity, sections were treated with 3% hydrogen peroxide in methanol. After a washing step, nonspecific binding was minimized by incubating the tissue sections for 30 minutes in a blocking solution (Phosphate-buffered Saline, pH 7.4, containing 2% bovine serum albumin (Sigma-Aldrich, Darmstadt, Germany), 0.1% sodium azide, and 0.1% Triton X-100) at room temperature. Sections were then incubated with Monoclonal Mouse Anti-Human CD4 (1:100, Clone 4B12, Dako) for 1 hour at room temperature to identify CD4+ Th cells. Following incubation, the sections were rinsed with Tris-buffered saline (TBS) for 10 minutes and incubated for 45 minutes at 30°C with EnVision™ (Dako) using an Autostainer (Dako). The sections were counterstained with Mayer's hematoxylin, mounted in Faramount Mounting Medium (Dako), and examined under light microscopy. A pathologist, unaware of the study groupings, evaluated an average of five random high-power fields (HPF) at 400× magnification using an Olympus CX-40 microscope (Olympus, Tokyo, Japan) to determine the frequency of T cells.

Enzyme-linked Immunosorbent Assay (ELISA)

The tissue samples were first homogenized at 2000×g for 10 minutes, followed by centrifugation at 3000×g for 20 minutes. After centrifugation, the supernatants were collected and preserved at -80°C until analysis. The concentrations of IFN-γ, TGF-β, IL-4, and IL-17A in the tissue samples were measured using ELISA kits (IBL, Germany) as per the manufacturer's guidelines. All data are expressed in pg/mL. The assay for each sample was performed in duplicate.

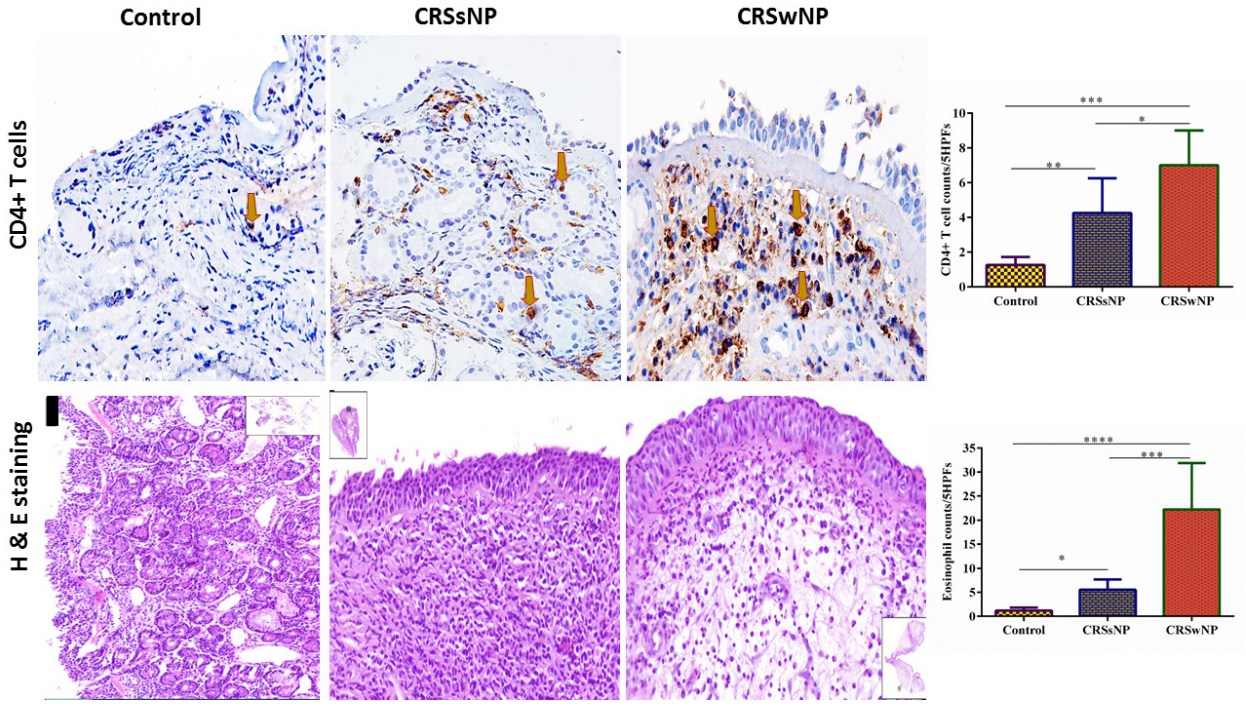


Figure 1. **The infiltration CD4⁺ T cells (helper T cells) to the sinonasal tissues of healthy controls, CRSsNP and CRSwNP patients using IHC staining is illustrated**. Moreover, the infiltration of eosinophils is represented using H&E staining. Scale bar 111 μm (▬).

Quantitative Real-time PCR

According to the manufacturer's instructions, total RNA was extracted from sinonasal tissues using Trizol (Invitrogen, USA). The integrity, quality, and quantity of the RNA were evaluated using PCR amplification of glyceraldehyde-3-phosphate dehydrogenase, a 2% denaturing agarose gel, and NanoDrop™ 2000/c Spectrophotometers by Thermo Fisher Scientific. To eliminate genomic DNA, samples were treated with an RNase-free DNase Set (Qiagen, Chatsworth, CA, USA). Subsequently, 500 ng of total RNA was used for cDNA synthesis employing the PrimeScript™ RT reagent Kit (TaKaRa, Korea). The reverse transcription reaction was performed in a 20 μL mixture containing 2.5 U of MML-V reverse transcriptase (GIBCO BRL, Grand Island, NY) and 50 pmol of random hexanucleotides, incubated at 42°C for 60 minutes and then at 85°C for 5 seconds to deactivate the reverse transcriptase enzyme. Likewise, real-time PCR was conducted in a 20 μL volume including 10 μL of 2× SYBR Green Real-time PCR Master Mix (TaKaRa, Korea), 1 μL of cDNA, and 1 μL each of forward and reverse primers at 200 nM concentration. Nuclease-free water was used in place of cDNA for negative controls. The PCR targeted the expression of transcription factors for Th1 (*T-bet*), Th2 (*GATA3*), Th17 (*Rorγt*), and Treg (*FoxP3*) cells, as well as *SOCS1*, *SOCS3*, and *SOCS5* genes. The primer details are listed in Table 2. The PCR cycles, performed using Rotor-Gene Q (Qiagen, Hilden, Germany), included 40 cycles at 95°C for 5 seconds for denaturation, and 60°C for 30 seconds for annealing and extension. Expression levels were normalized to *β-actin*, and relative mRNA levels of the target genes were calculated using the $2^{-\Delta\Delta CT}$ method, reported as relative expression.

Western Blot Analysis

Sinonasal tissue samples were homogenized and subjected to vigorous vortexing in RIPA buffer, supplemented with PMSF and a protease inhibitor cocktail (both in DMSO from Santa Cruz, California, USA). The protein extracts, measuring 25 μg each, were prepared in SDS sample buffer and boiled for 5 minutes. Subsequently, the proteins were separated using 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto Polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 1 hour at room temperature using PBS-Tween-20 with 5% skim milk before being incubated with specific antibodies: anti-SOCS1 (ab62584) at a 1:1500 dilution, anti-SOCS3 (ab16030) at a 1:2000 dilution, and anti-SOCS5 (ab97283) sourced from Abcam, Cambridge, UK. Protein bands were visualized using 3,3' diaminobenzidine (DAB) as the substrate. All data were normalized against β-actin levels to ensure consistency in protein quantification. Reprobing technique was uti-

Table 2. Primer Sequences used in Real-time PCR

Gene	Size (bp)	Forward and Reverse primers
T-bet	115	F: 5'- CTGGAGGTGTCGGGGAAAC -3' R: 5'-ATGGGAACATCCGCCGTCC-3'
GATA-3	107	F: 5'-TCATTAAGCCCAAGCGAAGG-3' R: 5'-GTCCCCATTGGCATTCCTC-3'
Rorγt	144	F: 5'- AGACTCATCGCCAAAGCA -3' R: 5'- CCTTGTAGAGTGGAGGGAAA -3'
FoxP3	124	F: 5'-ATTCCCAGAGTTCCTCCACAAC-3' R: 5'-ATTGAGTGTCCGCTGCTTCTC-3'
SOCS1	109	F: 5'-CGCCCTTAGCGTGAAGAT-3' R: 5'-CTCGAAGAGGCAGTCGAAG-3'
SOCS3	83	F: 5'-TTCAGCTCCAAGAGCGAGTA-3' R: 5'-GTCACTGCGCTCCAGTAGAA-3'
SOCS5	91	F: 5'-ATCTGGAGACAGCCATACCCA-3' R: 5'-CAAATCAGGCACGAGGCAGT-3'
β-actin	131	F: 5'-TCCCTGGAGAAGAGCTACG-3' R: 5'-GTAGTTTCGTGGATGCCACA-3'

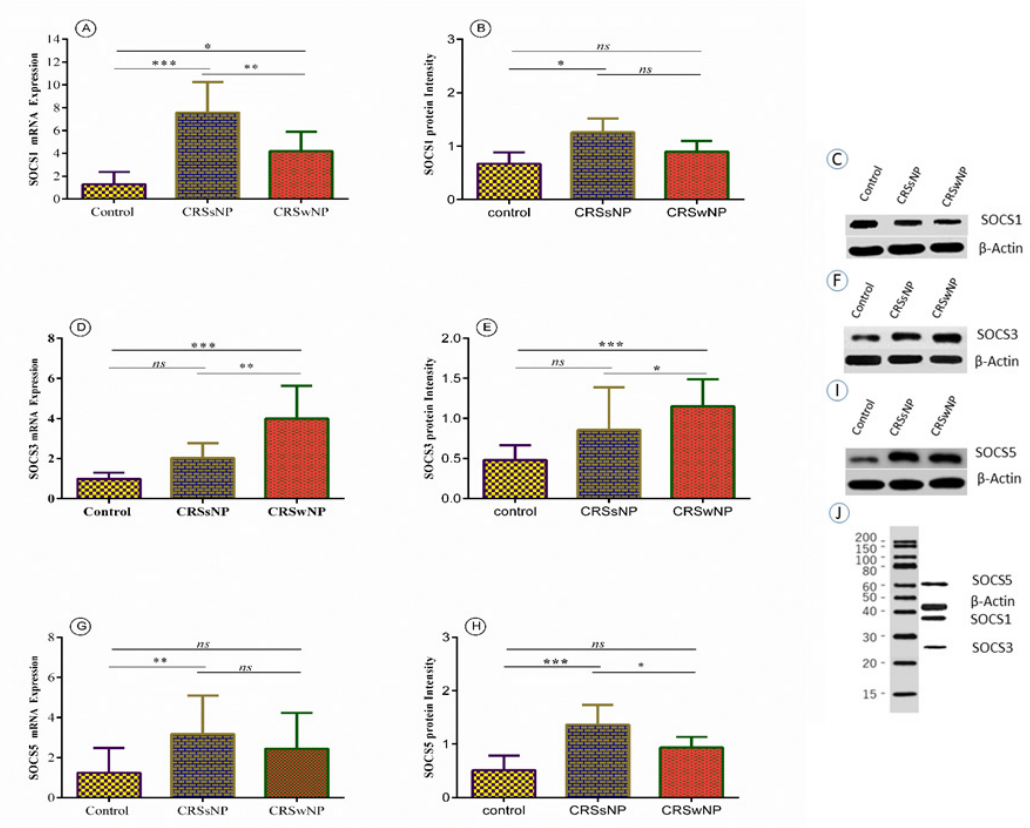


Figure 2. **The mRNA expression of *SOCS1*** (**A**), *SOCS3* (**D**), *SOCS5* (**G**) using real-time PCR and the protein levels of *SOCS1* (**B**,**C**), *SOCS3* (**E**,**F**), *SOCS5* (**H**,**I**) in CRSsNP and CRSwNP patients and healthy controls using western blotting analysis, respectively. (**J**) Represents bad sizes of the proteins in comparison to a ladder (15-200 Kd). All western blot bands were normalized to β-actin.

lized to facilitate the detection of SOCS1 and β-actin on a single membrane. Initially, after immunodetection, the membrane was washed with TBS-T to eliminate excess detection agents and then treated with a warm stripping buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, and 100 mM β-mercaptoethanol) at 50°C for 15-30 minutes with gentle shaking to displace bound antibodies. The membrane was then extensively rinsed in TBS-T to completely remove any residual stripping buffer. Following this, it was re-blocked using 5% non-fat dry milk in TBS-T for 1 hour at room temperature to minimize non-specific interactions.

Statistical Analyses

Statistical analyses were conducted using SPSS version 23.0 (SPSS Inc., Chicago, Illinois, USA) and visualized with GraphPad Prism software version 6.1 (GraphPad, La Jolla, California). The Kolmogorov-Smirnov test was utilized to assess the normality of the data. Intergroup differences were examined using the Kruskal-Wallis test. For the analysis of real-time PCR data, an independent sample T-test was employed. The Mann-Whitney U test was used to analyze differences between the two groups. Correlations between cell counts were evaluated using the Spearman correlation test, while Pearson correlation analysis was applied to assess variations in gene expression. The Receiver Operating Characteristic (ROC) regression model helped estimate the optimal cut-off values for gene expression levels to distinguish CRS patients from controls, with the best cut-off values identified using the Youden index (J) method. Statistical significance was set at a P-value of less than 0.05.

RESULTS

Demographic Data

Twenty patients (10 female and 10 male) with CRSwNP, 12 patients with CRSsNP (6 female and 6 male), and 12 healthy controls (6 female and 6 male) participated in this study. The mean± standard deviation (SD) age of the population study was as follows: 29±7.14 for CRSwNP, 27.3±10.3 for CRSsNP, and 33.6±13 for controls. Demographic data, CT score, endoscopic score, and the VAS score of CRS patients and healthy controls were presented in Table 1. The number of $CD4^+$ T cells increased in the CRSsNP (p <0.01) and CRSwNP (p <0.001) groups compared with the control group. Also, there was a significant difference between CRSwNP and CRSsNP patients (p <0.05). In addition, the number of eosinophils was higher in the CRSsNP (p <0.05) and CRSwNP (p <0.0001) groups than the control group. Also, there was a significant difference between CRSwNP and CRSsNP patients (p <0.001) (Figure 1).

Analysis of SOCS Genes and Proteins

The mRNA expression of *SOCS1* increased in the CRSsNP group in comparison to both CRSwNP (p <0.01, FC: 1.82) and control groups (p <0.001, FC: 5.89). Interestingly, there was a significant difference between CRSwNP and control groups (p <0.05, FC: 3.85) (Figure 2A). However, western blot analysis for SOCS1 protein level showed there was only a significant difference between CRSsNP and control groups (p <0.05) (Figure 2B,C). The expression of *SOCS3* increased in the CRSwNP group in comparison to both CRSsNP (p <0.01, FC: 1.97) and control (p <0.001, FC:4.03) groups. However, no significant difference was observed between CRSsNP and control groups (Figure 2D). Similarly, western blot analysis indicated the protein level of SOCS3 increased in the CRSwNP group in comparison to CRSsNP (p <0.05) and control (p <0.001) groups. No significant difference was detected between CRSsNP and control group (Figure 2E,F). Only there was a significant difference between CRSsNP and control groups in terms of *SOCS5* expression level (p <0.01, FC: 2.56) (Figure 2G). However, western blot analysis revealed there were significant differences between CRSsNP group and control (p <0.001) and CRSwNP (p <0.05) groups (Figure 2H,I). All western blot results were normalized to β-Actin bands. Figure 2J illustrates all band sizes of the target proteins and β-actin.

Analysis of Th-specific Transcription Factors and Related Cytokines

The expression of *GATA3* increased in the CRSwNP group in comparison to both CRSsNP (p <0.01, FC: 1.81) and control groups (p <0.0001, FC: 2.84). However, no significant difference was observed between CRSsNP and control groups (Figure 3A). The concentration of IL-4 was significantly elevated in the CRSwNP group in comparison to both CRSsNP (p <0.01) and control groups (p <0.001) (Figure 3B). Although the expression of *T-bet* increased in the CRSsNP group in comparison to the control group (p <0.05, FC: 2.19), no significant difference was seen between CRSwNP and CRSsNP groups (Figure 3C). However, the protein levels of IFN-γ increased in the CRSsNP group in comparison to both CRSwNP (p <0.01) and control groups (p <0.05) (Figure 3D). The expression levels of *RoRγt* increased in the CRSsNP group in comparison to both CRSwNP (p <0.01, FC: 1.97) and control groups (p <0.001, FC: 2.83) (Figure 3E). The concentration of IL-17 increased in the CRSsNP group compared with both CRSwNP (p <0.01) and control groups (p <0.001) (Figure 3F). The expression of *FoxP3* decreased in the CRSwNP (p <0.05, FC: 0.55) and CRSsNP (p <0.01, FC: 0.43) groups compared with the control group. There was no significant difference between CRSwNP

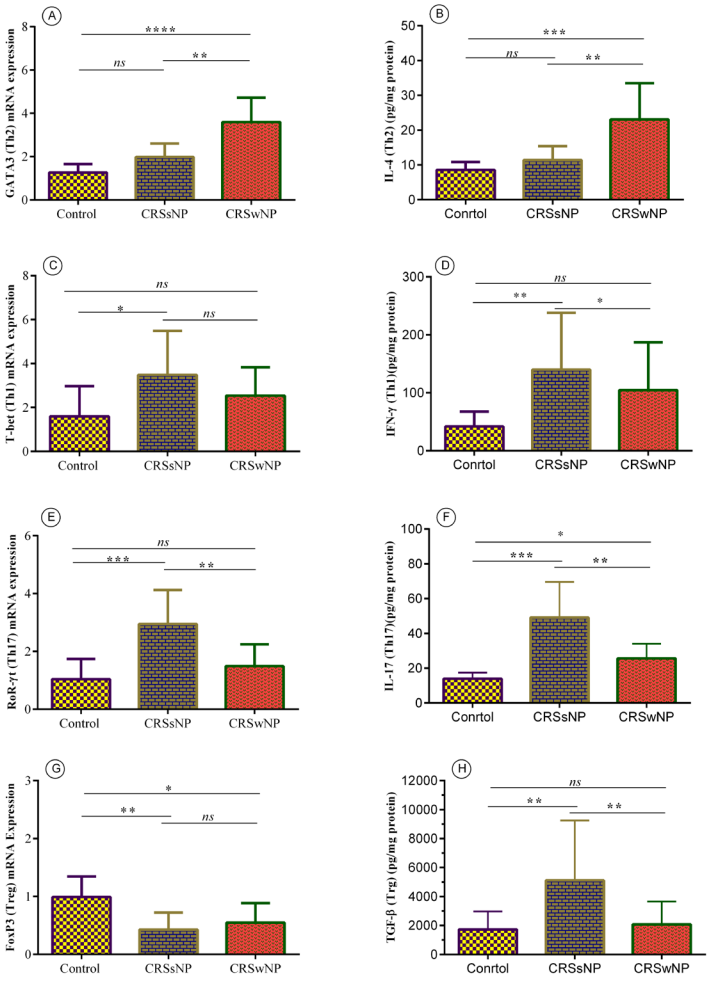


Figure 3. **The mRNA expression of T-bet** (**A**), *GATA3* (**C**), *Rorγt* (**E**), *FoxP3* (**G**) transcription factors and the concentration of IFN-γ (**B**), IL-4 (**D**), IL-17 (**F**), and TGF-β (**H**) cytokines in Th1, Th2, Th17, and Treg cells, respectively.

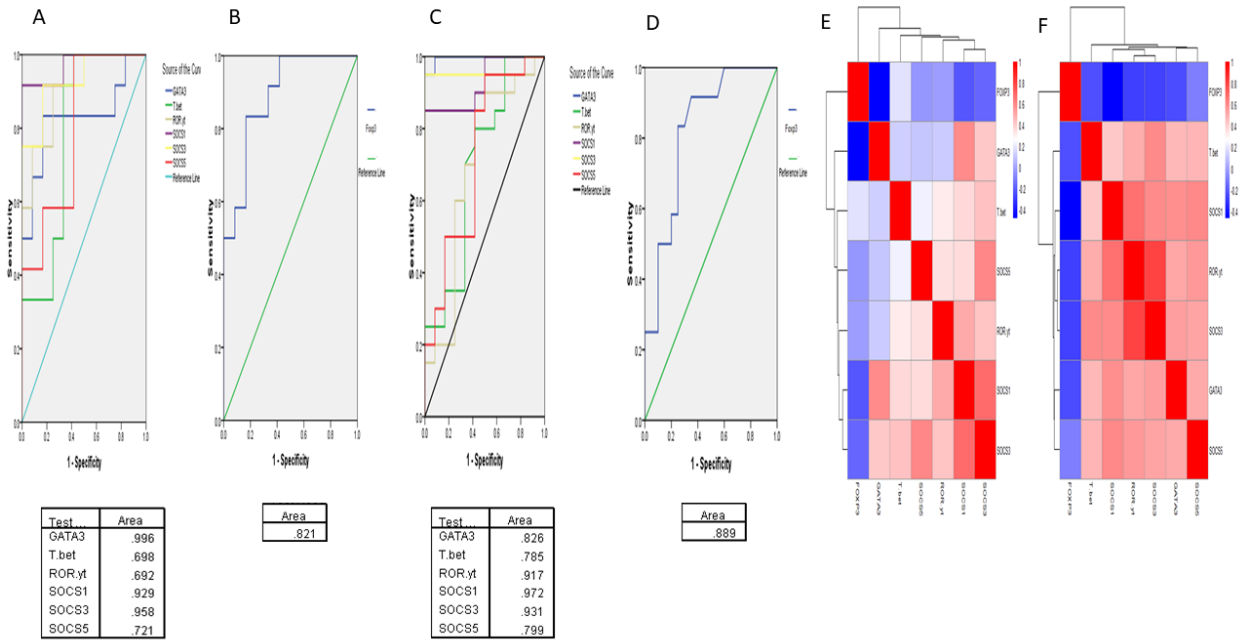
and CRSsNP groups (Figure 3G). The concentration of TGF-β increased in the CRSsNP group compared with both CRSwNP and control groups (p <0.01) (Figure 3H).

ROC Curve Analysis and Correlation for Genes

The ROC curve indicated the sensitivity and specificity of *GATA3*, *T-bet*, *Rorγt*, *FoxP3*, *SOCS1*, *SOCS3*, and *SOCS5* expression levels in CRS patients. The area under the ROC curve (AUC) for discriminating CRSwNP patients from controls were 0.996, 0.698, 0.692, 0.821, 0.929, 0.958, and 0.721, respectively (Figure 4A,B). The AUC for discriminating CRSsNP patients from controls were 0.826, 0.785, 0.917, 0.889, 0.972, 0.931, and 0.799, respectively (Figure 4C,D). Correlation analysis showed a direct correlation between *SOCS1* and *GATA3* (P=0.003) and *Rorγt* (P=0.000). Although SOCS3 had a direct correlation with *GATA3* (P=0.019), *T-bet* (0.013), and *Rorγt* (P=0.015), there was no correlation between SOCSs and transcription factors (Figure 3E). Correlation analysis also indicated a direct correlation between *Rorγt* and *SOCS1* (P=0.003), *SOCS3* (P=0.000), and *SOCS5* (P=0.005) (Figure 4F). We revealed a negative correlation between all *SOCSs* and *FoxP3* in CRSs patients. In addition, none of the genes correlated with *SOCS5* (Figure 4E,F). All correlation analyses are summarized in Appendix A.

DISCUSSION

Several groups of inhibitory proteins play a key role in modulating this signaling pathway, with the suppressor of cytokine signaling (SOCS) proteins being particularly significant. SOCS proteins are known to regulate transcription factors specific to T helper (Th) cells [5]. One notable inhibitor among these is SOCS3, which prevents



Test ...	Area
GATA3	.996
T.bet	.698
ROR.γt	.692
SOCS1	.929
SOCS3	.958
SOCS5	.721

Test ...	Area
GATA3	.826
T.bet	.785
ROR.γt	.917
SOCS1	.972
SOCS3	.931
SOCS5	.799

Figure 4. **Receiver operating characteristic** (ROC) curve for diagnosis and prognosis of CRSwNP (**A,B**) and CRSsNP (**C,D**) patients compared with healthy controls. Hierarchical clustering analysis showing the relationship between expression variation of *GATA3*, *T-bet*, *Rorγt*, *FoxP3*, *SOCS1*, *SOCS3*, and *SOCS5* in CRSwNP (**E**) and CRSsNP (**F**) patients.

the differentiation of Th cells into Th17 cells by blocking STAT3 activity. Additionally, SOCS3 suppresses the *RORc* gene, which is crucial for the differentiation of Th17 cells [6,7]. Inhibition of STAT4 also suppresses Th1 cell differentiation with IL-12 [8]. Research by Seki et al. indicated that elevated levels of *SOCS3* are linked to the severity of allergic conditions such as asthma and atopic dermatitis. This is due to SOCS3's dual role: it inhibits Th1 cell activity while promoting Th2 cell differentiation. Notably, *SOCS3* expression in Th2 cells is about 23 times higher than in Th1 cells [9]. In a study conducted by Wang et al., western blot analysis revealed that SOCS3 protein expression was significantly lower in patients with atopic CRSwNP compared to those with CRSsNP. This reduction in SOCS3 was also associated with decreased expression of *FoxP3* and a reduction in regulatory T (Treg) cells [10]. In a study by Moriwaki et al., selective translocation of SOCS3 silencing siRNAs strongly inhibited hypersensitivity and eosinophilia in a mouse model of asthma [11]. As suggested by SOCS3 inhibiting Th17 cells, a study by Wong et al. showed that deletion of SOCS3 gene increased Th17 cell differentiation and IL-17 production [12]. Shouda et al. treated a murine model of rheumatoid arthritis caused by Th17 responses through transducing the SOCS3 gene with an adenoviral vector [6]. Our study reveals that SOCS3 expression is increased in patients with CRSwNP, thereby deviating Th cell polarization toward Th2 cells. A study by Lan et al. showed that SOCS3 plays an important role in Treg cells as *FoxP3* levels in these cells decrease when SOCS3 mRNA and protein are increased. Conversely, if they switch off SOCS3, they increase *FoxP3* expression. They showed that SOCS3 could be targeted to increase *FoxP3* expression, thereby increasing the number of Treg cells [13] because SOCS3 suppresses the expression of *FoxP3* and *CTLA-4*. However, in a study on mice, Pillemar et al. reported that *SOCS3* was not expressed in mouse Treg cells [14]. In a study by Wang et al., western blot analysis revealed that SOCS3 protein expression in the group of patients with chronic atopic polyp rhinosinusitis was significantly reduced compared with patients without chronic polyp rhinosinusitis and was associated with decreased expression of *FoxP3* and low Treg cells [10].

We showed that IL-17 increased in CRSsNP patients. IL-17 plays a critical role in CRSsNP by driving robust neutrophilic inflammation, thereby intensifying mucosal defense mechanisms [15]. The interaction of IL-17 with SOCS1, which is known to enhance Th17 differentiation, presents a unique insight into the cytokine's regulatory dynamics. Understanding the modulation of IL-17 by SOCS1 within the JAK-STAT pathway reveals a potentially pivotal role in the persistent inflammatory state observed in CRSsNP. By delving deeper into these interactions, researchers could uncover novel therapeutic targets aimed at regulating IL-17 activity, thus offering

new avenues for treatment in immune-mediated mucosal disorders. Moreover, we demonstrated a higher IFN-γ in CRSsNP patients. In fact, IFN-γ is paramount in the Th1 immune response, significantly shaping the inflammatory landscape of CRSsNP. As a principal cytokine in Th1-mediated immunity, IFN-γ not only enhances macrophage activation but also orchestrates the broader immune defense against pathogens. This cytokine's role is crucial given the dominant Th1 responses in CRSsNP, distinguishing it from the Th2-skewed responses of CRSwNP [16]. The modulation of IFN-γ by SOCS1, which typically inhibits Th1 and Th2 differentiation while promoting Th17 cell responses, underscores a complex regulatory mechanism potentially exploitable for therapeutic interventions. Insights into how IFN-γ interacts with SOCS proteins, particularly in the context of the JAK-STAT signaling pathway, could illuminate new strategies for managing CRSsNP by modulating its Th1/Th17 cytokine profile. Our results were consistent with previous studies that indicated Asian CRSsNP patients exhibited a mixed Th1/Th17 immune response [17].

Another cytokine inhibitor of the SOCS family is SOCS1, which inhibits Th1 responses and inhibits IFN-γ production by specific inhibition of STAT1, thus preventing classical activation of macrophages [18]. In contrast, SOCS3 activates classical macrophages [19]. In turn, *SOCS1* expression reduces the immune system's response to Th2 responses and increases the severity of symptoms and complications [20]. In the absence of SOCS1, *FoxP3* gene expression is lost and Treg cells are transformed into Th1-like pathogen cells (with IFN-γ secretion) and Th17 cells (with IL-17 secretion) [21]. Our study showed that SOCS1 protein levels were higher in CRSsNP patients than in CRSwNP patients and controls. The study by Park et al. showed that SOCS1 and SOCS3 are also expressed in the nasal mucosa of healthy individuals, but in patients with CRSwNP and CRSsNP (both asthma and non-atopic groups), both mRNA and protein levels increased dramatically [22]. In another study by Hulse and colleagues in the US, the level of SOCS3 by western blotting was significantly reduced [23]. One of the reasons for these differences in various studies is that the content of infiltrating cells in this area and the cytokine milieu vary from population to population. However, further studies are needed to elucidate the molecular mechanisms underlying this variation. As noted, the increase in SOCS1 inhibits IFN-γ signaling, which in turn may divert immune responses to Th2 responses. The expression of *SOCS1* and *SOCS3* is high in patients with RA. SOCS3 levels are also elevated in chronic long-term infectious diseases and periodontitis, Crohn's disease, and ulcerative colitis [24]. A study by Park et al. showed that *SOCS1* and *SOCS3* mRNA was higher in CRSwNP and CRSsNP patients than in controls. However, there was no significant difference between CRSwNP and CRSsNP groups. Their study showed that IL-4, TNF-α, and IFN-γ increase *SOCS1*, but IL-6, TNF-α, and IFN-γ increase *SOCS3*. IFN-γ is the most effective cytokine in the expression of *SOCS1* and *SOCS3*. This study showed that *SOCS1* and *SOCS3* are also expressed continuously in the sinus mucosa of healthy individuals, but their levels increase after signaling of inflammatory cytokines [24].

Another inhibitor in this family is SOCS5, which specifically expresses Th1 cells and strongly inhibits IL-4 signaling (by inhibiting STAT6), thus inhibiting the differentiation of these cells into Th2 [25,26]. There is a notable gap in the research regarding the role of SOCS5 in regulating T cells in rhinosinusitis, particularly concerning its impact on Th cell responses. Our findings demonstrate an increase in *SOCS5* mRNA expression and protein levels in the CRSsNP group. Previous research, such as the study by Seki et al. on Th1 and Th2 cell lines from transgenic mice, highlights that during Th1 cell differentiation, a decline in JAK1 and IL-4 receptor (IL-4R) activity corresponds with an upsurge in SOCS5 expression. Specifically, *SOCS5* is predominantly expressed in Th1 cells and engages with the cytoplasmic region of IL-4R, independent of its tyrosine kinase phosphorylation status. This interaction leads to the inhibition of STAT6 activation, thus blocking the differentiation into Th2 cells. In transgenic mice overexpressing SOCS5, there was a significant reduction in Th2 cell development [26].

ROC curve analysis highlighted the potential of specific genes for the diagnosis or prognosis of Chronic Rhinosinusitis (CRS) patients. Correlation analyses revealed a direct association between *SOCS1* and *SOCS3* with Th, Th2, and Th17 cells across both CRS phenotypes. However, *SOCS5* was only correlated with Th17 cells in CRS without nasal polyps (CRSsNP) patients. Intriguingly, all SOCS genes exhibited a negative correlation with regulatory T (Treg) cells in CRS patients. Although the correlation analysis did not confirm a Th2 dominance in CRS with nasal polyps (CRSwNP) patients, it did indicate a mixed Th profile with a Th17 predominance. In contrast, SOCS protein expression suggested a Th2 dominance in CRSwNP patients and a Th1/Th17 dominance in CRSsNP patients. These discrepancies between gene expression and protein results underscore the need for further research to resolve these contradictions and better understand the role of SOCS proteins in the immunopathogenesis of CRS. This study proposes that SOCS proteins could be significant in developing molecular and targeted therapeutic strategies for CRS, though additional research is necessary to fully understand their functions and mechanisms without disrupting their natural roles in immune homeostasis. The study was advantageous due to its low cost and brief duration. However, it faced several limitations, including a small patient cohort and unequal

numbers of asthmatic patients in each group. Additionally, while immunohistochemistry (IHC) and qReal-time PCR techniques were employed based on prior studies, flow cytometry or immunofluorescence co-localization assays might offer more precise methods for distinguishing Th cell types.

CONCLUSION

This study explored the expression of *SOCS1*, *SOCS3*, and *SOCS5* at the mRNA and protein levels in patients with CRS. Our findings reveal distinct expression patterns between CRSwNP and CRSsNP, suggesting that these conditions may involve different regulatory mechanisms within the JAK-STAT signaling pathway. Specifically, we observed elevated levels of *SOCS1* and *SOCS5* in CRSsNP patients, which could indicate a role in promoting Th1 cell differentiation. Conversely, elevated SOCS3 in the CRSwNP group aligns with a Th2-dominant immune response. We suggested that different SOCS proteins may influence the fate of Th cell polarization. Modulating these proteins, potentially through the use of cytokine inhibitors or SOCS-targeted interventions, holds promise for the treatment of CRS. For instance, targeting SOCS3 and SOCS5 could shift Th cell polarization towards inflammatory (Th1) or anti-inflammatory (Th2) responses, respectively. These insights highlight the possibility of classifying CRS more accurately and developing targeted therapeutic strategies based on the pattern of inflammatory cell infiltration and the mechanisms influencing immune cell deviation towards either inflammatory (Th1/Th17) or anti-inflammatory (Th2) conditions. While our findings provide a foundation for potential therapeutic approaches, translating these insights into clinical practice necessitates further extensive research and validation to ensure efficacy and safety.

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Appendix A

Supplementary file. Correlation analysis between gene expression variation of *GATA3*, *T-bet*, *Rorγt*, *FoxP3*, *SOCS1*, *SOCS3*, and *SOCS5* in CRSwNP and CRSsNP patients.

CRSwNP								
Correlations								
		GATA3	*T-bet*	*RORyt*	*SOCS1*	*SOCS3*	*SOCS5*	*FOXP3*
GATA3	Pearson Correlation	1	.116	.107	.592**	.413*	.090	-.488**
	Sig. (2-tailed)		.526	.561	.000	.019	.625	.005
	N	20	20	20	20	20	20	20
T.bet	Pearson Correlation	.116	1	.315	.366*	.435*	.217	.171
	Sig. (2-tailed)	.526		.079	.039	.013	.220	.349
	N	20	20	20	20	20	20	20
ROR.yt	Pearson Correlation	.107	.315	1	.505**	.426*	.345	-.030
	Sig. (2-tailed)	.561	.079		.003	.015	.053	.871
	N	20	20	20	20	20	20	20
SOCS1	Pearson Correlation	.592**	.366*	.505**	1	.689**	.359*	-.235
	Sig. (2-tailed)	.000	.039	.003		.000	.044	.195
	N	20	20	20	20	20	20	20
SOCS3	Pearson Correlation	.413*	.435*	.426*	.689**	1	.602**	-.189
	Sig. (2-tailed)	.019	.013	.015	.000		.000	.301
	N	20	20	20	20	20	20	20
SOCS5	Pearson Correlation	.090	.217	.345	.359*	.602**	1	-.048
	Sig. (2-tailed)	.625	.220	.053	.044	.000		.792
	N	20	20	20	20	20	20	20
FOXP3	Pearson Correlation	-.488**	.171	-.030	-.235	-.189	-.048	1
	Sig. (2-tailed)	.005	.349	.871	.195	.301	.792	
	N	20	20	20	20	20	20	20
**. Correlation is significant at the 0.01 level (2-tailed).								
*. Correlation is significant at the 0.05 level (2-tailed).								

CRSsNP								
Correlations								
		GATA3	*T-bet*	*RORyt*	*SOCS1*	*SOCS3*	*SOCS5*	*FOXP3*
GATA3	Pearson Correlation	1	.470*	.516**	.587**	.542**	.510*	-.251
	Sig. (2-tailed)		.021	.010	.003	.006	.011	.237
	N	12	12	12	12	12	12	12
T.bet	Pearson Correlation	.470*	1	.502*	.423*	.603**	.477*	-.237
	Sig. (2-tailed)	.021		.012	.039	.002	.019	.264
	N	12	12	12	12	12	12	12
ROR.yt	Pearson Correlation	.516**	.502*	1	.676**	.805**	.559**	-.284
	Sig. (2-tailed)	.010	.012		.000	.000	.005	.179
	N	12	12	12	12	12	12	12
SOCS1	Pearson Correlation	.587**	.423*	.676**	1	.598**	.605**	-.468*
	Sig. (2-tailed)	.003	.039	.000		.002	.002	.021
	N	12	12	12	12	12	12	12
SOCS3	Pearson Correlation	.542**	.603**	.805**	.598**	1	.525**	-.280
	Sig. (2-tailed)	.006	.002	.000	.002		.008	.186
	N	12	12	12	12	12	12	12
SOCS5	Pearson Correlation	.510*	.477*	.559**	.605**	.525**	1	-.104
	Sig. (2-tailed)	.011	.019	.005	.002	.008		.630
	N	12	12	12	12	12	12	12
FOXP3	Pearson Correlation	-.251	-.237	-.284	-.468*	-.280	-.104	1
	Sig. (2-tailed)	.237	.264	.179	.021	.186	.630	
	N	12	12	12	12	12	12	12
*. Correlation is significant at the 0.05 level (2-tailed).								
**. Correlation is significant at the 0.01 level (2-tailed).								