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Does procalcitonin have a role in the pathogenesis of nasal polyp?

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Abstract

Purpose The aim of this study is to investigate serum and tissue procalcitonin (PCT) levels in patients with nasal polyps.

Methods The study was designed to be prospectively controlled and included 26 patients chronic rhinosinusitis with nasal polyp (CRSwNP) endoscopically diagnosed and as a control group 25 chronic rhinosinusitis without nasal polyp (CRSsNP). NP specimens, nasal mucosal tissue and venous blood samples of both groups were collected and PCT levels determined by Elisa method. The results were compared statistically.

Results Serum PCT values were 1319.5 pg/mL in the NP group and 818.8 pg/mL in the control group. The difference between the groups was statistically significant ($p=0.0001$). In the NP group, the average PCT value of the polyp tissue was 1521.5 pg/gr, while the mean PCT value of the control group in the nasal mucosa was 414.6 pg/gr. There was a statistically significant difference between the groups ($p=0.0001$). The tissue cut-off value of PCT 750 was significant [area under curve 0.940 (0.863–1.00)]. Serum PCT 950 cut-off value was significant [area under curve 0.860 (0.748–0.972)] activity (CI: 95%).

Conclusions This is the first study of its kind to objectively examine PCT in the polyp and serum of CRSwNP patients. PCT may serve as a diagnostic biomarker in nasal polyps.

Keywords Nasal polyps · Procalcitonin · Pathogenesis · Sinusitis

Introduction

Nasal polyp (NP) is a chronic inflammatory disease of the sinonasal mucosa characterized by extrinsic extracellular edema, eosinophil, neutrophil, lymphocyte or plasma cell infiltration, increased vascular permeability and glandular changes histologically [1, 2]. Nasal polyposis (NPs), a subgroup of chronic rhinosinusitis, affects 4% of the general population and 25–30% of chronic rhinosinusitis patients [3]. NPs can clinically cause nasal obstruction, nasal discharge and anosmia [4]. Infection, allergy and immunological diseases have been suggested as possible factors in the etiology of NP and also the existence of genetic predisposition has been revealed [1]. Glutathione-S-transferase genetic polymorphism, increased chemokines, metalloproteinases, growth factors, gene expression, and increased leukotriene receptor gene expression are considered responsible for the pathogenesis of nasal polyps [4–7].

Many cytokines and inflammatory mediators have been shown to increase in NP. Tumour necrosis factor alpha (TNF-α) and interleukin 1 (IL-1) act as pro-inflammatory cytokines in the signal pathway of the nasal polyp pathogenesis. There is generally an increase in gene expression of Th2-derived cytokines such as interleukin 4 (IL-4) and interleukin 5 (IL-5). An increase in interleukin 8 (IL-8) release is also revealed. Interferon gamma and transforming growth factor beta (TGF-ß) have an antagonistic effect on Th2-induced inflammation in the nasal polyp [8–10].

Procalcitonin (PCT) is the prohormone precursor of calcitonin that is expressed primarily in parafollicular C cells of the thyroid gland, intestinal neuroendocrine cells and lung. Transformation of PCT to calcitonin is prevented by various cytokines and bacterial endotoxins [11]. PCT is an important mediator in the role of peptide in acute inflammation. Consecutive PCT measurements in emergency and intensive care

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units are a specific biomarker that helps in monitoring sepsis and antibiotic therapy in bacterial infections clinically. However, the level at the bottom in normal individuals is too low to be detected. There is a rapid increase in release in severe infections. In addition, its release increases independently of infection in some neuroendocrine tumors [12, 13]. Viral and non-infectious causes do not significantly alter PCT levels. PCT has been reported to be an accurate variable in distinguishing bacterial and non-bacterial infections [14].

It is known that TNF-α plays a role in the NP pathogenesis and is a strong stimulant of PCT production. Bacterial endotoxins and inflammatory mediators in bacterial infection activate PCT production in parenchymal tissues [15]. Demonstration of PCT expression in the nasal polyp may help in understanding possible infectious mechanism in the development of NP. The purpose of this article is to measure serum and tissue PCT levels in patients with nasal polyps and controls to determine the applicability of PCT as a potential biomarker for chronic rhinosinusitis with nasal polyp (CRSwNP).

Materials and methods

The study was designed prospectively. Fifty-one patients aged 18 years or older were eligible to participate in this study and two different groups of patients were selected: NP and control. Endoscopic examinations and radiological evaluations with paranasal sinus computed tomography (CT) scans were performed on all patients before intervention. CRSwNP was defined as presence of endoscopically visible bilateral polyps growing from the middle nasal meatus and affecting the ethmoidal and maxillary sinuses, according to a CT scans of the paranasal sinuses. The severity of NP was defined as middle meatus only (grade 1), beyond middle meatus but not blocking the nose completely (grade 2), completely obstructing the nose (grade 3). Patients with chronic rhinosinusitis without nasal polyps (CRSsNP) were used as a control group. The study was approved by the Ethics Committee of Istanbul Education and Research Hospital and written informed consent was obtained from each patient. Exclusion criteria for the study groups included the following: age < 18 or > 80 years, patients with a diagnosis of allergic rhinitis and positive prick test, cystic fibrosis, asthma, aspirin intolerance, antrochoanal polyps, fungal sinusitis, immunodeficiency and autoimmune diseases. NP specimens were collected endoscopically from the middle meatal segment under local anesthesia and venous blood samples were taken on the same day. The nasal mucosal tissue samples were taken from the middle meatus endoscopically and venous blood samples were taken at the same time from the patients in the control group. PCT was measured from tissue and venous blood samples taken from both groups and it was investigated whether there was any difference between the two groups.

Evaluating specimens

Tissue samples were homogenized using 0.1M phosphate buffer (pH: 7.4) and Next Advance Homogenizer on ice (Bullet Blender Storm, Next Advance, Inc. Troy, NY 12180, USA). To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates were centrifuged at 1500*g*, +4 °C for 10 min, and the supernatant fractions were separated. Serum and supernatant stored at − 80 °C until analysis. PCT levels of tissue and serum were determined by Sandwich-ELISA method. We used a commercial kit (Elabscience, USA, Human PCT ELISA Kit) to measure PCT, following the instruction provided by the manufacturer. This assay employs an antibody specific for human PCT coated on an appropriate micro ELISA plate. Centrifuge the standard at 10,000×*g* for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 2000 pg/mL. Then make serial dilutions as needed. The dilution gradient is as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL Standards and samples are added to plate wells and combined with the specific antibody. The optical density (OD) can be measured with spectrophotometry at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of PCT. The concentration of PCT in samples can be calculated by comparing the OD of the samples with the standard curve. The minimum detection limit of the "Human PCT" is 31.25 pg / mL, and the coefficient of variation is < 10%.

Statistical analysis

Mean, standard deviation, median, maximum, minimum, frequency and ratio values were used in the descriptive statistics of the data. The distribution of the variables was measured by the Kolmogorov–Simirnov test. Mann–Whitney *U* test was used in the analysis of quantitative independent data. Chi-square test was used in the analysis of qualitative independent data. Kruskal–Wallis procedure was used to compare polyp and serum PCT levels among subgroups. The effect level and cut-off value were investigated by ROC curve. SPSS 22.0 program was used in the analyzes. A value of $p < 0.05$ was considered statistically significant.

Results

Twenty-six patients with CRSwNP and 25 patients with CRSsNP met the inclusion criteria for this study. Table 1 summarizes the demographic features for each group. There

Table 1 Patients demographics and PCT levels

	Control group			NP group			*p*
	Mean ± SD/*n*-%		Median	Mean ± SD/*n*-%		Median	
Age	41.6 ± 11.3		47.0	46.8 ± 14.2		48.5	0.157[m]
Gender							
Female	6	24.0%		7	26.9%		0.811[χ2]
Male	19	76.0%		19	73.1%		
Serum PCT (pg/mL)	818.8 ± 393.6		739.6	1319.5 ± 297.6		1243.5	0.0001[m]
Tissue PCT (pg/gr)	414.6 ± 284.1		377.1	1521.5 ± 359.6		1519.8	0.0001[m]

[i] Mann–Whitney *u* test, χ^2 Chi-square test, *SD* standard deviation

p value < 0.05 statistically significant

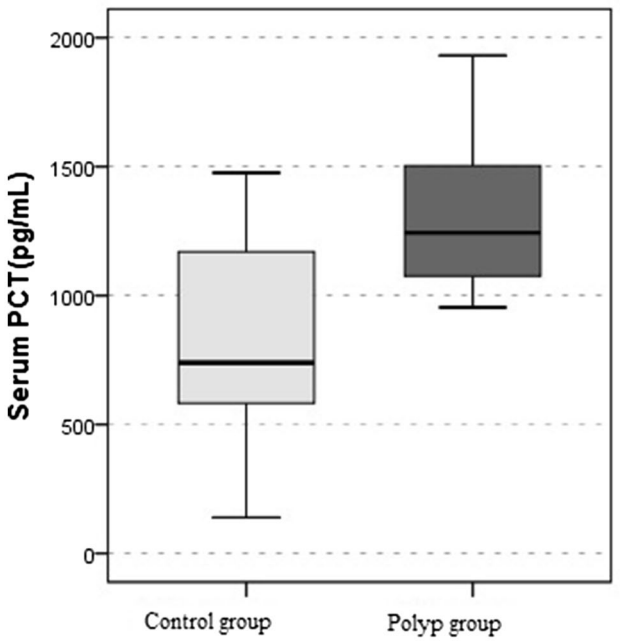

Fig. 1 Comparison of serum PCT levels between NP group and control group

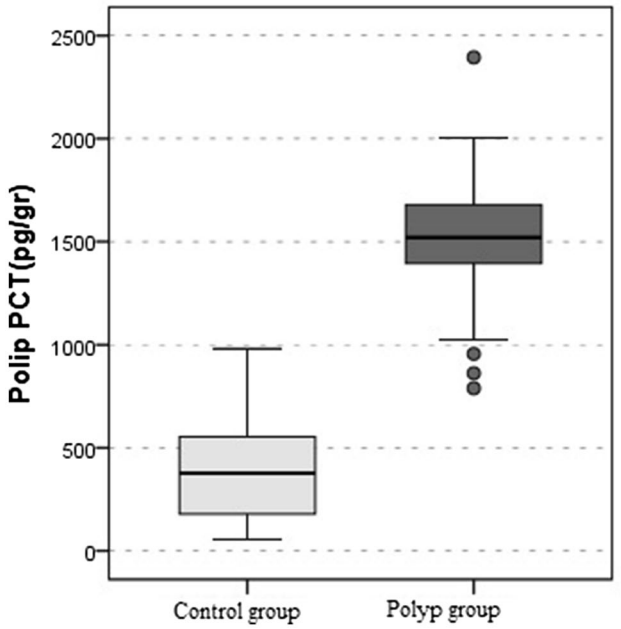

Fig. 2 Comparison of tissue PCT levels between NP group and control group

was no statistically significant difference regarding age ($p = 0.157$) or gender ($p = 0.811$) between the NP and control groups.

Tissue PCT levels in the NP group were significantly increased ($p = 0.0001$) compared with the levels in the control group. In addition, serum PCT levels in the NP group were significantly increased ($p = 0.0001$) compared with the levels in the control group (Table 1; Figs. 1, 2).

The significant efficacy of serum PCT was observed between NP patients and control group in case of differentiation of the patients.[area under the curve: 0.856 (0.704–0.948)]. Serum PCT 950 cut-off value was significant [area under the curve 0.860 (0.748–0.972)] (Table 2).

The significant efficacy of tissue PCT was observed between NP patients and control group in case of differentiation of the patients.[area under the curve: 0.986 (0.964–1.00)]. Tissue PCT 750 cut-off value was significant [area under the curve: 0.940 (0.863–1.00)] (Table 3).

The majority of the patients were grade 2 and 3. There was no statistically significant difference between the severity of NP and serum & polyp PCT levels, respectively ($p = 0.442$, $p = 0.472$) (Table 4).

Table 2 Serum PCT cut-off value in determining of patients in polyp and control groups

	Area under curve	% 95 CI	*p*
Serum PCT (pg/mL)	0.856	0.704–0.948	0.0001
Cut Off 950	0.860	0.748–0.972	0.0001

CI confidence interval, *PCT* procalcitonin

$p < 0.05$ statistically significant

Table 3 Tissue PCT cut-off value for determining patients in polyp and control groups

	Area under curve	% 95 CI	*p*
Polyp PCT(pg/gr)	0.986	0.964–1.000	0.0001
Cut Off 750	0.940	0.863–1.000	0.0001

CI confidence interval, *PCT* procalcitonin

$p < 0.05$ statistically significant

Discussion

The result of the present study indicated that PCT levels in serum and polyp tissue were significantly higher in the NP patients compared to the nasal mucosal tissue of participants in the control group, however, PCT levels did not correlate with severity of NP. The high PCT levels in the NP tissues may increase the tendency to bacterial infections by suppressing ciliary movement in patients with nasal polyposis, which may lead to elevated serum PCT levels via systemic effect.

Primary diagnostic utility of PCT is thought to be in establishing the presence of bacterial infections. Immunohistochemistry studies suggest that it may be up-regulated in conditions such as acute inflammation and bacterial infection [16–18]. To the authors' knowledge, this is the first published study that has objectively quantified PCT levels in the NP tissue and serum of patients with NP.

Although infections, allergic and immunological diseases are responsible for pathogenesis of NP, the precise underlying pathogenesis of it is not exactly known [1]. Mucosal inflammation with eosinophil predominance is likely the most significant factor in its pathogenesis. The findings of previous studies have perpetually demonstrated that NP stroma include different important molecules such as cytokines, chemokines, mediators, growth factors, antimicrobial and anti-inflammatory peptides and adhesion molecules and expressed in mucosal tissue of chronic sinusitis with NP [19–23]. Maxfield et al have shown that serum levels of periostin, a mediator of fibrosis, have increased significantly with chronic sinusitis in patients. In addition, they have been reported to be useful biomarkers for polyposis in sinonasal diseases [20].

Furthermore, Matsusaka et al found a correlation between high periostin concentration and nasal disorders, such as chronic rhinosinusitis with nasal polyp and olfactory dysfunction [21]. In another study, Psaltis et al. reported a reduction in the expression of lactoferrin, an antimicrobial peptid, at both the mRNA and protein level, in mucosal tissue specimens taken from affected sinuses of patients with nasal polyps were lower than those in patients without polyps [22]. In China, Yue et al showed that YKL40, a secreted mammalian glycoprotein, is highly expressed in patients with NPs and that IL-4 may play a significant role in the YKL 40 signaling pathway in NP patients [23]. Lechapt-Zalcman et al. reported that matrix metalloproteinase (MMP) 9, a metalloproteinase also called gelatinase B, expression is primarily increased in nasal polyps [24]. In contrast, Lee et al. found that MMP-2, a metalloproteinase also called gelatinase-A, is the predominant form of MMPs in nasal polyps [25]. Bhandari et al. showed that MMP-2 expression is characteristic of nasal polyps [26].

Production of PCT is controlled by the calcitonin 1 gene (CALC-1) on chromosome 11. Transcription and translation of CALC-1 gene is normally confined to the thyroid C-cells and, to a lesser extent other neuroendocrine cells. Chemokines, interleukins, growth factors, TNF-α, anti-inflammatory cytokines are proteins in the pathway where PCT is involved. Production is, however, activated in all parenchymal tissues in response to bacterial infection, especially intervened by cytokines IL-6, TNF-α and IL-1β [12]. TNF-α a strong stimulant of PCT production may, along

Table 4 Comparison of polyp severity and tissue and serum PCT levels

	Grade I ($n=5$)		Grade II ($n=11$)		Grade III ($n=10$)		*p*
	Mean ± SD	Med	Mean ± SD	Med	Mean ± SD	Med	
Serum PCT (pg/mL)	1337 ± 392	1159	1419 ± 336	1345	1201 ± 156	1188	0.442[K]
Polyp PCT (pg/gr)	1479 ± 104	1446	1461 ± 480	1534	1610 ± 290	1551	0.472[K]

[K]Kruskal–Wallis

with other cytokines, further support the already high PCT levels [27–29]. For this reason, in the development of nasal polyps, the production of PCT may be induced by mediators produced in response to bacterial infections, TNF-α especially. The upregulation of a vast number of genes associated with proinflammatory chemokines suggests that PCT participates in the inflammatory response not only by stimulating local production of cytokines but also by triggering a chemotactic response that further augments its toxic effects [30]. Some interleukins such as IL-3, IL-15, IL-11a, Bmp4 growth factor and IL-13 (anti-inflammatory cytokine) are downregulated in PCT pathway.

Araujo et al reported that PCT decreased mesangial cells viability by 36% compared to control cells and induced significant apoptosis [30]. Similarly, PCT may serve probably by stimulating the apoptosis of sinonasal mucosal cells.

A high serum and tissue levels of PCT in patients with nasal polyp may aid in the design of new therapeutic strategies, especially local and systemic forms of antibiotic agents, for improving the clinical outcomes of patients with NP.

Our study has several limitations. There was a relatively small sample size within each group. Standalone findings of increased PCT expression makes it hard to comment on the relevance of the study. Furthermore, examining either up or downstream mediators in PCT signaling to better understand the story and mechanisms of these findings would be suggested. We think that there is a need for studies that can show how molecular mechanisms are.

Conclusion

The present study revealed that a high tissue and serum levels of PCT are strongly associated with the presence of nasal polyps in patients with CRS. These findings show that PCT may serve as a diagnostic biomarker in nasal polyps but advanced investigations are needed to reveal on infectious causes of NP pathogenesis.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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