

Original Research

Relationship between plasma cyclin-dependent kinase 5 levels and local immunoglobulin levels in patients with nasal polyps

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Abstract: Nasal polyps are the most common benign nasal tumors that can lead to nasal obstruction and other annoying problems for the patient. Several hypotheses have been proposed as the basic mechanism of nasal polyps. In order to investigate one of the possible causes that can be a disorder in the regulation of systemic immune responses, the present study was designed to investigate the relationship between plasma cyclin-dependent kinase 5 (CDK5) levels and local immunoglobulin levels in patients with nasal polyps. A cross-section study was used to evaluate concentrations of local immunoglobulin levels (IgE, IgM, IgA, and IgG) on blood and polyp specimens from 60 patients with nasal polyps, and 60 control groups. Western Blot Analysis was done for CDK5 in plasma cells. IgA, IgG and IgE concentrations were significantly higher in polyp tissue specimens, but not in blood, of nasal polyp patients compared to the control group. Furthermore, plasma CDK5 levels were significantly higher in nasal polyp tissue compared with control. The difference in IgA, IgE and IgG expression between nasal polyp tissue and blood, supported by increased numbers of plasma cells, suggests a local production of these local immunoglobulins in nasal polyps in response to chronic antigens. Among local immunoglobulins, only there was a significant correlation between CDK5 with IgG (positive correlation) and IgE (negative correlation). The exact explanation for the relationship between plasma CDK5 and local immunoglobulins in nasal polyps needs further studies.

Key words: Local immunoglobulin; Nasal polyps; Plasma CDK5; Systemic immune responses.

Introduction

A polyp is a mucosal edematous membrane that appears as a bulge. This protrusion is attached to its bed by a narrow or wide base. Nasal polyps, in the upper respiratory tract, arise around the openings of the ethmoid sinuses (1). Polyps enter the nasal cavity and block the airflow to the olfactory bulb. The stroma of the polyp is very edematous and is filled with inflammatory cells of various densities (2).

Numerous hypotheses have been proposed as the underlying mechanism for nasal polyps, including the role of chronic infections, aspirin intolerance, trapping of contaminants due to aerodynamic changes, or aerodynamic changes, or changing in Sodium-ion uptake (3-5). Some hereditary factors also seem to play an important role (6, 7).

The high level of inflammatory mediators, which is common and evident in nasal polyps, indicates that, regardless of the etiology of the polyp, there is no doubt that chronic inflammation is a major factor in the development of polyps (8). This chronic inflammation triggers immune reactions that stimulate local immunoglobulin (9). Immunoglobulins are divided into five main groups based on physical, chemical and immunological properties: IgA, IgM, IgG, IgD, IgE (10). Also, it was observed that the expression and activity of many kinases (i.a. CDK5, GSK-3 β , JNK, p38, PKA, PKB,

PKC) can be effective in chronic inflammatory diagnosis (11-14).

Cyclin-Dependent Kinases (CDKs) are known as cell motors, which are involved in the passage of cells from one phase to another (15). Cyclin-Dependent Kinase 5 (CDK5) is one of the best CDK candidates for the diagnosis of inflammation, as it is responsible for the aberrant phosphorylation of inflamed cells (16). During inflammation, the amount of these kinases increase in blood plasma (17).

Therefore, in order to investigate one of the possible underlying causes in patients with a nasal polyp that may be disturbed in the regulation of individual systemic immune responses, the present study was designed to investigate the association of plasma CDK5 levels and local immunoglobulin levels among these patients.

Materials and Methods

Patients

This study is a cross-sectional (descriptive-analytical) type and an easy sampling method was used to select the samples. In this study, 60 patients aged 16 years and older and 60 individuals as control were examined by an ENT specialist. After clinical and Para clinical tests, the patients with nasal polyp were finally admitted to the polypectomy with a diagnosis of polyps. Prior to surgery, a blood sample was taken to measure local

immunoglobulin levels. Questionnaires were prepared to be completed by the relevant physician to collect project information.

Polyp tissue and blood serum

After surgery, polyp tissue specimens were immediately kept frozen in liquid nitrogen. To homogenize polyp tissue, they were weighed and 1mL of 0.9% NaCl solution supplemented with a protease inhibitor cocktail (Sigma Aldridge, USA) was added to each 0.1g tissue. The tissue was then homogenized with a mechanical homogenizer (B. Braun Melsungen, Germany) at 1200r.p.m. for 5 min on ice. Then the suspensions were centrifuged at 1500 g for 10 min at 4°C, and the supernatants were separated.

To separate the serum, blood samples were clotted at 25°C for 30 min and then centrifuged at 1500 g for 10 min at 4°C. All samples were stored in aliquots at 25°C for analysis.

Immunoglobulins determination

Different local immunoglobulins such as IgA, IgE, IgG, and IgM were measured by fixed-time immuno-nephelometry on a BN II analyzer (Dade Behring, Germany) using reagents containing specific polyclonal antibodies (18). Assays were calibrated against the international CRM 470 reference material (19). Both polyp tissue and blood serum samples were assayed for all immunoglobulins by commercially available ELISA kits (kit Sanquin, the Netherlands) according to the manufacturer's instructions.

Western blot analysis

Plasma was centrifuged at 2μM dose for 12 hours after inhibitor treatment, and the cell pellet was washed with cold PBS and lysed in RIPA buffer containing protease inhibitor cocktail (Sigma Aldridge, USA). After determining the concentration of CDK5 by the Bradford method, a certain amount of plasma CDK5 was placed in 10% SDS page gel and then transferred to the nitrocellulose membrane using Semidry Transfer Cell (Bio Red). Plasma CDK5 was assessed using primary and secondary antibodies. The intensity of each band was calculated by ImageJ software and the CDK5 to actin ratio was normalized.

Statistical analysis

Statistical analysis was done by SPSS 12.0 software. For unpaired comparisons, analysis was performed by the Kruskal–Wallis test and the Mann–Whitney–U two-tailed test. After comparisons were made between groups, then the Kruskal–Wallis test was performed to establish significant inter-group variability. Then, the Mann–Whitney U-test was used to compare groups. To assess the relationships among parameters, the Spearman rank correlation coefficient was done, and the significance level was <0.05.

Results

60 patients with nasal polyps and 60 controls were studied. In patient group, there were 37 men (61.66%) and 23 women (38.34%) with an average age of 37 years. In the control group, there were 40 men (66.66%) and 20 women (33.34%) with a mean age of 35 years.

According to Western Blot Analysis, CDK5 was produced in both patient and control groups. But there were significant differences between concentrations of CDK5 among both groups ($P=0.029$) (Figure 1).

Also, there were high concentrations of IgA, IgE, and IgG in the polyp tissue of the patient group and these concentrations were significantly higher than controls (Table 1) (Figure 2). But, IgA, IgE, and IgG concentrations did not have a significant difference in

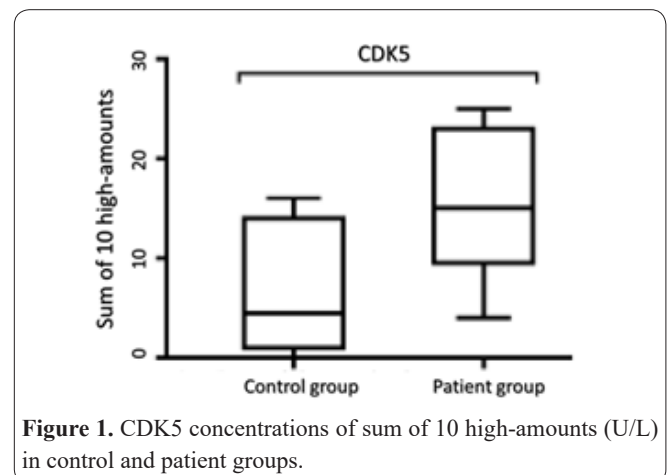


Figure 1. CDK5 concentrations of sum of 10 high-amounts (U/L) in control and patient groups.

Table 1. Concentrations of IgA, IgE, IgG, IgM, and CDK5 in control and patient groups.

	Control group	Patient group	Kruskal-Wallis	Control vs. Patient group*
Blood Serum				
IgA (g/L)	1.380	2.460	0.069	-
IgE (kU/L)	0.348	1.526	0.178	-
IgG (g/L)	9.620	11.810	0.281	-
IgM (g/L)	0.870	0.650	0.392	-
Polyp Tissue				
IgA (g/L)	0.487	1.199	<0.0001	0.001
IgE (kU/L)	0.149	0.532	0.002	0.001
IgG (g/L)	2.115	5.012	<0.0001	<0.0001
IgM (g/L)	0.899	0.898	0.8.15	-
CDK5 (U/L)	5	15	0.005	0.029

*Mann–Whitney U-test.

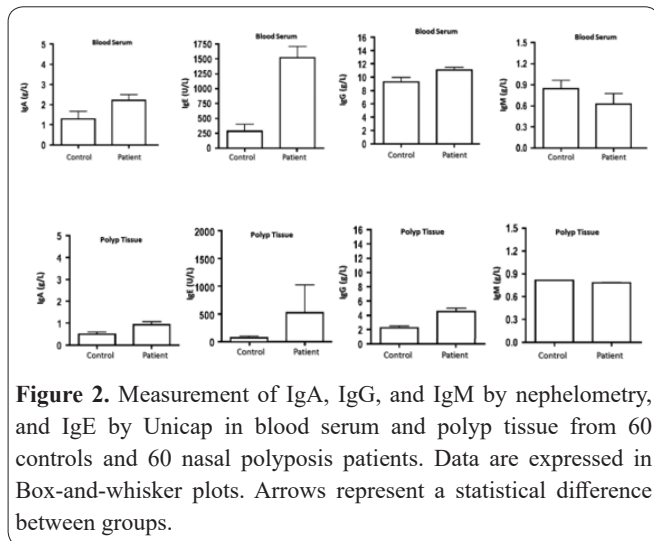


Figure 2. Measurement of IgA, IgG, and IgM by nephelometry, and IgE by Unicap in blood serum and polyp tissue from 60 controls and 60 nasal polyposis patients. Data are expressed in Box-and-whisker plots. Arrows represent a statistical difference between groups.

blood serum of controls and patient groups. About IgM, there was no statistical difference in both polyp tissue and blood serum between the two groups.

Within the Patient group, the concentrations of IgG4 were positively correlated with CDK5 ($r=0.831$, $P<0.0001$), while there was a negative correlation between IgE and CDK5 in patient group ($r=-0.598$, $P=0.001$). In the control group, these correlations did not have significant differences (Table 2).

Discussion

Polyps are patho-physiologically referred to as adenomatous masses in the nasal tissue and its surrounding cavities that can lead to airway obstruction, increased mucus secretion, loss of sense of smell, and decreased sense of health. In most patients, polyps are associated with increased sensitivity to a variety of drugs, fungi, and other environmental inhalants. Out of 60 selected patients, 56.7% with IgE concentrations above 100 were selected as patients with allergies. In all patients, the level of CDK5 in female patients was higher than in men ($P=0.034$), but more studies are needed to confirm this in both females and males.

Concentrations of IgA, IgE, IgG, and IgM in nasal polyp tissue were independent of patients' atopic status. High concentrations of immunoglobulins indicate that they leave the polyp tissue and enter the blood serum. Gevaert *et al.* (2005) also showed that the IgE/albumin ratio was present in the polyp tissue and in the blood serum, which confirms the high concentration of IgE in the tissue caused IgE to act not only locally (20). Smurthwaite *et al.* (2001) also examined the presence of IgE protein in nasal polyp tissue and blood plasma through local de novo synthesis of IgE in the nasal explant model (21). Their results showed that the concentration of immunoglobulins is also enhanced by the local synthesis in nasal polyp tissue. On the other hand, the current results showed that there was no significant increase in the concentration of IgA, IgE, IgG and IgM in the blood serum of patients with nasal polyps, which indicates that these immunoglobulins act locally.

The increase of plasma CDK5 in the patient group than the control showed that this inflammatory response plays an important role. When T cell is stimulated, memory B cells start to produce phenotypic markers in

Table 2. Correlation of IgA, IgE, IgG, and IgM vs. CDK5 in both control and patients group.

	Control Group	Patient Group
CDK5 vs. IgA		
Correlation coefficient	-0.273	-0.294
<i>P</i> -value	NS	NS
CDK5 vs. IgE		
Correlation coefficient	0.279	-0.598
<i>P</i> -value	NS	0.011
CDK5 vs. IgG		
Correlation coefficient	0.076	0.831
<i>P</i> -value	NS	<0.0001
CDK5 vs. IgM		
Correlation coefficient	0.189	-0.435
<i>P</i> -value	NS	NS

blood plasma via activating CDK5 (22, 23). The interesting point about CDK5 is its different correlation with different immunoglobulins. As mentioned in the result part, there was no significant correlation between CDK5 with IgA and IgM. This non-correlation showed that there is a little amount of IgA and IgM in blood serum and therefore these two immunoglobulins are acted as local immunoglobulins. On the other hand, there was a significant positive correlation between CDK5 and IgG. Immunoglobulin G is the most abundant antibody in serum and extracellular fluids and plays an important role in defense against pathogens (24). There is a relationship between serum IgG levels and the severity of some diseases such as immunodeficiency, infectious diseases and autoimmune diseases (25). Since both CDK and immunoglobulin G play an effective role in defense against pathogens, the positive correlation between them is quite justified. Despite IgG, there was a negative correlation between CDK5 and IgE. Immunoglobulin E is naturally present in small amounts in the blood. The amount increases when the body overreacts to the allergen or fights the parasitic infection (26). Because this immunoglobulin overreacts to allergens, it decreases CDK5 levels.

Therefore, in general, the increase in IgA, IgG and IgE concentrations in nasal polyps is due to the response to microbial-host interaction, and high IgA levels in nasal polyps are due to the IgA-independent function of T cells or secondary lymphatic organs (27). A genome-wide association study (28) can identify candidate genes in this regard. In previous research, several reports have been reported on the relationship between the expression of some enzymes and some human disorders and diseases (29-32), and in this article, the relationship between plasma cyclin-dependent kinase 5 levels and local immunoglobulin levels in patients with nasal polyps were investigated.

In nasal polyps, there is a high level of immunoglobulins, which indicates the presence of many antigens in this tissue. Also, differences in the expression of IgE, IgG and IgA between nasal polyp tissue and blood serum confirm the local function of these immunoglobulins in nasal polyps. This production is reflected locally in the immune response to antigens. On the other hand, high levels of CDK5 in blood plasma in patients with nasal polyps indicate the importance of this enzyme in the

immune response. Among all studied local immunoglobulins, only there was a significant correlation between CDK5 with IgG (positive correlation) and IgE (negative correlation). How these immunoglobulins affect CDK5 is somewhat clear, but more cellular and molecular studies are needed to obtain more accurate information.

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