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Correlation between nitric oxide levels and clinical features in patients with nasal polyposis

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Abstract

Background: Nasal polyposis (NP) is a multifactorial pathology with negative impact on quality of life. The pathogenesis of NP has not been fully elucidated. This limits the pathogenic treatment. Recent studies suggest that nitric oxide (NO) and its metabolites are involved in pathophysiological events of NP. Purpose of the study: to evaluate the tissue NO and nitrite/nitrate (NO_2^-/NO_3^-) levels in patients with and without NP and to establish the relationship between NO levels and some clinical features.
Material and methods: 86 recruited patients were divided into a case group (N_1=43), patients with NP and a control group (N_2=43), patients with septal deviations and turbinate hypertrophies. Visual Analog Scale (VAS) was used to evaluate the severity of nasal obstruction and olfactory disturbances. NO and NO_2^-+NO_3^- concentrations in tissue specimens were measured by spectrometric method.
Results: Case group had significantly lower NO (U=173.5, p<0.001) and NO_2^-+NO_3^- levels (U=123.5, p<0.001). A negative correlation was found between VAS for nasal obstruction and NO levels (r_s = -0.379, p<0.05), between VAS for olfactory disturbances and NO levels (r_s = -0.531, p<0.001), and between endoscopic score and NO levels (r_s = -0.758, p<0.05).
Conclusions: Our results corroborate the previous findings. This underlines that NO levels depend on the patency of sinus ostium and the state of osteomeatal complex. Further studies, which take into account the role of NO in different rhinosinusitis endotypes, are needed to be performed in order to improve the NP management.
Key words: nasal polyposis, nitric oxide, nasal obstruction, olfactory dysfunction, endoscopy score.

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Introduction

Nasal polyposis (NP), one of the clinical phenotypes of chronic rhinosinusitis, is recognized as being a major health problem, frequently encountered in otorhinolaryngological practice. NP is a chronic inflammatory condition of the upper respiratory tract with incompletely understood etiology [1-5]. NP is characterized by formation of nasal polyps, benign formations, arising from the mucosa of the paranasal sinuses or nasal cavity, and often manifested by a high tendency of recurrent growth even after surgical excision [1, 5-7]. NP is responsible for affecting the patient's quality of life (QoL) more than other chronic diseases, resulting in enormous socioeconomic consequences [1, 5, 8]. Olfactory dysfunction developed in NP can be debilitating with substantial impact on QoL [9]. Patients with NP express varying sleep disturbances in high proportion leading to an impairment of cognitive function and depression [10]. NP is still generating significant healthcare costs: the direct and indirect cost of lost working days [8, 11-13].

NP is attracting considerable interest due to its misunderstood pathogenesis. NP is widely considered a multifactorial pathology, inflammation playing one of the most important roles. Nevertheless, the causes that determine the persistence of chronic inflammation with nasal polyps formation, have not yet been fully established [1-3, 13-15]. There is a considerable amount of literature on the role of multiple factors (infectious and noninfectious inflammation, anatomic and genetic abnormalities, oxidative stress, aspirin intolerance/sensitivity, environmental factors) involved in the pathogenesis of NP [1, 16-18]. Therefore, pathogenic treatment of NP remains a challenging issue. Approximately 20% of patients are facing an uncontrolled pathology despite adequate medical therapy and modern sinus surgery [7, 19].

In recent years there has been considerable growing interest regarding the involvement of nitric oxide (NO) in the pathogenesis of NP [20-24]. Current understanding supports that NO and its counterpart reactive nitrogen species may participate in pathophysiological events in a variety of inflammatory diseases, including NP [20, 25]. The primary source of NO in the respiratory tract seems to be the paranasal sinuses. NO is involved in upper airway homeostasis

and immunity by modulating blood flow, regulating mucociliary clearance and acting as an antiviral and antimicrobial agent. Notwithstanding the fact that NO protects, it may expresses toxic effect under certain condition [20]. The exact role of NO in respiratory homeostasis and pathophysiology is still unclear. The purpose of this study was consequently to further current knowledge of involvement of NO in NP pathogenesis and to determine relationship between NO level and some clinical features.

Material and methods

This study involved eighty-six patients from the Otolaryngology Department of *Timofei Mosneaga* Republican Clinical Hospital (Chisinau, the Republic of Moldova). Prior to subject recruitment, the study protocol was reviewed and approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (SUMPh), the Republic of Moldova (Report No 30 of 29.03.2016). Written informed consent was obtained from all patients at the beginning of the study. From 2016 to 2019, eighty-six patients with NP, septal deviations and middle concha hypertrophies were recruited for the study. Patients were divided into two groups: group 1 – Case (N_1= 43) consisted of patients with NP, and group 2 – Control (N_2= 43) consisted of patients with septal deviations and middle concha hypertrophies. Patients with septal deviations and middle concha hypertrophies have nasal obstruction, comparable according to VAS to that of NP. The diagnosis was based on history, anterior rhinoscopy, endoscopic examination and paranasal sinus computed tomography (CT), CT was done by all the patients with NPs and some cases of middle concha hypertrophies. Endoscopy findings were scored according to the Lund and Kennedy scoring system [26]. The parameters, such as presence or absence and extent of nasal polyps, edema, and discharge were graded. For nasal polyps, 0 was given for the absence of polyps, 1 for polyps present within the middle meatus, and 2 for polyps beyond the middle meatus.

Routine blood sampling, blood biochemistry and urinalysis were performed to verify the presence of any illness that would be the exclusion criteria. The exclusion criteria included the following: age <18 years; pregnancy; severe chronic diseases (liver, kidney, cardiovascular, respiratory, malignancy, diabetes mellitus); chronic alcoholism; recent use (last four weeks) of topical or systemic glucocorticoids; refusal to participate in the study.

The Visual Analog Scale (VAS) was used to evaluate the severity of the nasal obstruction and severity of olfactory disturbances by scoring them on a continuous 10-cm horizontal line on which 0 cm represented no complaints and 10 cm – serious complaints, associated with nasal obstruction and olfactory disturbances, respectively. The SinoNasal Outcome Test-22 (SNOT-22) test was used in order to evaluate the severity of nasal symptoms and their influence on the QoL.

Tissue specimens were collected from all patients involved in the study. Polyp specimens were taken from all patients who underwent endoscopic surgery for NP. Control specimens (nasal mucosae) were obtained from patients who underwent an operation for septoplasty or concha hypertrophy. The freshly obtained tissue samples were immediately transferred to laboratory and stored at -70° C until needed (laboratory evaluation of NO levels). It is well known that NO rapidly degrades to nitrite and nitrate (NO_2^- and NO_3^-) in aqueous solution. Therefore, the nitrate and nitrite levels were estimated, to provide an index of NO production. Concentration of NO and its metabolites (NO_2^- and NO_3) was measured by spectrometric method in homogenized polyp and control specimens. Laboratory analyses were performed in the Scientific Biochemistry Laboratory of *Nicolae Testemitanu* SUMPh.

All statistical analyses were performed using Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0). The Kolmogorov-Smirnov test was used to test the normality of data distribution. The data were expressed as mean and standard deviations. The chi-square test was used to compare categorical variables between groups. The independent sample T-test and Mann Whitney-U test were used to compare continuous variables between the two groups. Spearman's rank correlation analyses were used to examine the association between variables. Correlations were considered negligible if $r_s < 0.3$, low if $0.3 < r_s < 0.5$, moderate if $0.5 < r_s < 0.7$, high if $0.7 < r_s < 0.9$, and very high if $0.9 < r_s < 1.0$. Differences were considered statistically significant at p value of less than 0.05.

Results

The group 1 (case) was composed of 43 patients with nasal polyposis: 26 men and 17 women, with a mean age of 48.09±13.56 years (range, 22 to 76 years). The group 2 (control) was composed of 43 patients with septal deviations and middle concha hypertrophies: 30 men and 13 women, with a mean age of 33.67±11.44 years (range, 19 to 65 years). Characteristics of the study populations are given in tab. 1. There were no statistically significant differences between the two groups regarding gender, residence, use of nasal decongestants and VAS score for nasal obstruction ($p > 0.05$), except in terms of distribution of the age. In addition, patients with NP had a greater VAS score for olfactory disorders and SNOT-22 score ($p < 0.05$), (tab. 1).

Comparison of NO and NO_2^-+NO_3^- concentrations

There were found significant differences between mean NO_2^-+NO_3^- and NO concentrations in polyp tissues and control specimens. Compared to group 2 (control), group 1 (case) had significantly lower NO_2^-+NO_3^- concentration in tissue specimens ($U=123.5$, $p < 0.001$) (tab. 2, Fig. 1B). The mean levels of NO were significantly lower in NP tissues than in control specimens ($U=173.5$, $p < 0.001$) (tab. 2, fig. 1A).

Correlation analysis between NO levels and some clinical features in study groups

The statistical analysis, performed using Spearman's coefficient, showed significant correlations between NO level and VAS for nasal obstruction, VAS for olfactory disorder

Table 1

Comparison of the demographic and clinical characteristics of all patients

Characteristics	Value		p
	Group 1 *(case)* (N_1=43)	***Group 2*** *(control)* (N_2=43)	
Age (years), Mean±SD	48.09±13.56	33.67±11.44	< 0.001[a]
Gender (male/female), N	26/17	30/13	0.249[b]
Residence (urban/rural), N	17/26	18/25	1.0[b]
Nasal decongestants (naphazoline/ xylometazoline/ oxymetazoline/ combined/ several types), N	9/18/2/0/14	5/15/2/1/20	0.482[b]
VAS score for nasal obstruction, Mean±SD	8.28±1.054	7.81±0.982	0.051[a]
VAS score for olfactory dysfunctions, Mean±SD	8.35±1.744	1.47±2.25	< 0.001[a]
Lund-Kennedy endoscopic score, Mean±SD	9.28±2.529	-	
SNOT-22 score, Mean±SD	61.79±15.875	50.30±15.26	0.001[c]

Note: SD, Standard deviation; N, number; VAS, Visual Analog Scale; SNOT-22, SinoNasal Outcome Test-22; – Mann-Whitney-U test; [b] – Chi-square test; [c] – Independent sample T-test.

Table 2

Comparison of NO and NO_2^-+NO_3^- concentrations in tissue specimens taken from patients

Characteristics	Value		p
	Group 1 *(case)* (N_1=43)	***Group 2*** *(control)* (N_2=43)	
NO_2^-+NO_3^- (µM/g.prot.) Mean ±SD	1.15±0.54	2.89±1.02	< 0.001[a]
NO (µM/g.prot.), Mean±SD	0.79±0.49	1.99±0.85	< 0.001[a]

Note: SD, Standard deviation; [a] – Mann-Whitney-U test.

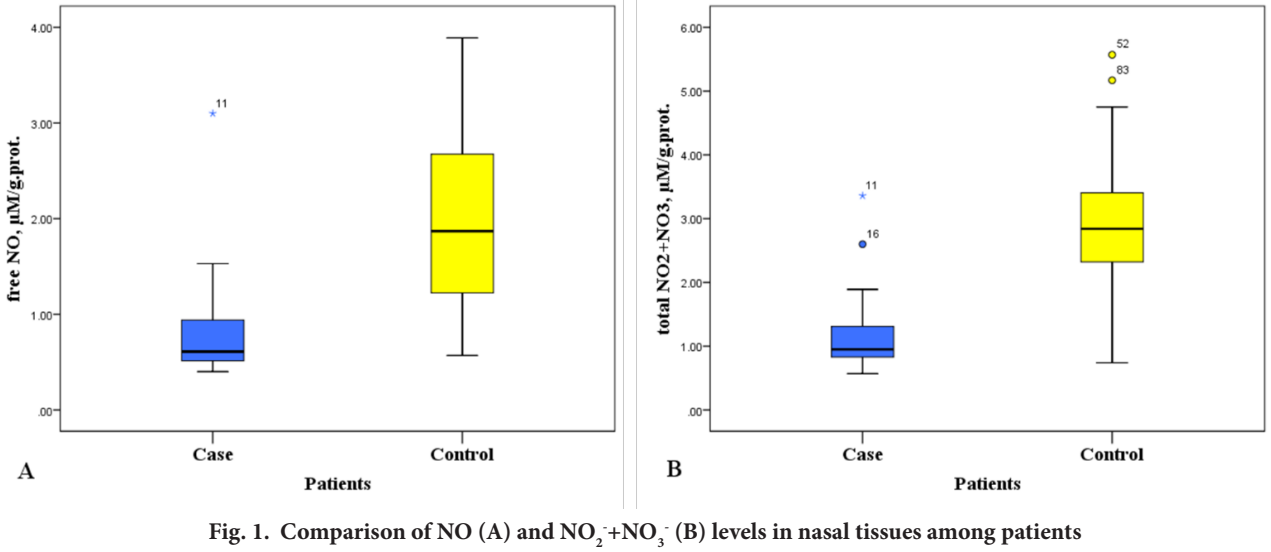

Fig. 1. Comparison of NO (A) and NO_2^-+NO_3^- (B) levels in nasal tissues among patients

and Lund-Kennedy endoscopic score in case group (tab. 3). However, no significant correlations were observed between NO level and nasal obstruction, and olfactory disorder in control group ($p > 0.05$ for all) (tab. 3). Furthermore, correlations were found negligible.

While analysing the correlation between the VAS for nasal obstruction and NO levels in case group, it was observed a low, negative correlation (r_s = -0.379, $p < 0.05$) (tab. 3). From the scatterplot (fig. 2A), which relates the VAS for nasal obstruction to the NO levels, it may be concluded that the increase of nasal obstruction corresponds to the decrease of NO values. A more severe nasal obstructive symptoms corresponded to the lower levels of NO. A moderate, negative correlation was found between VAS for olfactory dysfunctions and NO levels (r_s = -0.531, $p < 0.001$) (tab. 3). From the scatterplot (fig. 2 B) it may be concluded that patients reporting the worst olfactory status were associated with the lowest mean of NO. A Spearman's correlation run to determine the relationship between Lund-Kennedy endoscopic score and NO levels showed a high, negative correlation between endoscopic score and NO levels (r_s = -0.758, $p < 0.05$), (tab. 3 and fig. 2C).

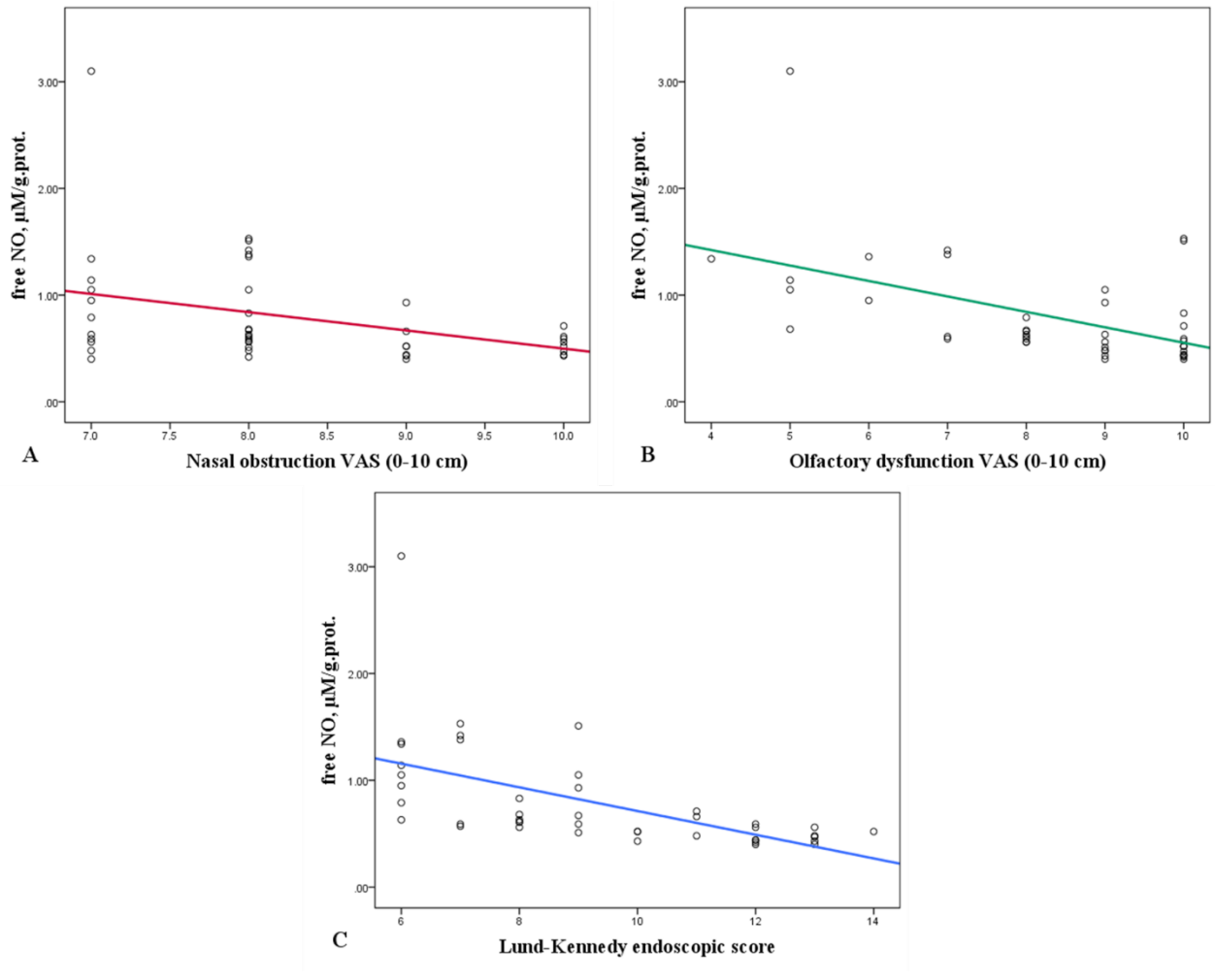

Fig. 2. Correlation between NO levels and some clinical findings: VAS for nasal obstruction (A), VAS for olfactory dysfunctions (B), and Lund-Kennedy endoscopic score (C)

Discussion

The main findings of this study are summarized as follows: (1) NO and NO_2^-+NO_3^- levels in tissues of patients with NPs were lower than in patients without NP; (2) Patients who had lower NO levels reported a more increased VAS score for nasal obstruction and olfactory dysfunction, and a more increased endoscopic score.

NO is a reactive molecule with physiological and pathological effects, mediated by its metabolites rather than the NO molecule itself, being involved in a variety of vital processes, such as antimicrobial/antiviral activity, blood flow regulation, cell metabolism, neurotransmission, immunity, inflammation control, platelet function, etc. [16]. NO production is catalysed by nitric oxide synthase (NOS). Once synthesized, NO acts as an intracellular or extracellular messenger. There are known three isoforms of NOS: NOS-1 (nNOS) and NOS-3 (eNOS) are constitutive isoforms, found in neurons and endothelial cells, and NOS-2 (iNOS) is in-

Table 3

Correlations between variables using Spearman's coefficient

		Nasal obstruction VAS (cm)	Olfactory dysfunctions VAS (cm)	Lund-Kennedy endoscopic score
Case group (patients with NP)				
NO, µM/g.prot.	Spearman's correlation	-0.379*	-0.531**	-0.758*
	P value	0.012	< 0.001	< 0.001
Control group (patients without NP)				
NO, µM/g.prot.	Spearman's correlation	-0.084	-0.038	
	P value	0.59	0.80	

Note: * - Correlation is significant at the 0.05 level; ** - Correlation is significant at the 0.01 level.

ducible isoform, found in activated cells during infection or inflammation [20, 21]. A number of studies have found that exhaled NO is mainly produced in the upper airways [20, 25], where NO is generated by the paranasal sinus epithelium and then diffuses into the nasal cavities. Therefore, the paranasal sinuses appear to be the primary source of NO in the respiratory system [21, 27-29].

In this study, there were estimated the NO and NO_2^- +NO_3^- levels in tissue specimens, finding the lowest NO and NO_2^-+NO_3^- levels in patients with NP. These results are in line with some previous results. Frendo et al. [30] reported that nasal NO was significantly lower in patients with NP compared with controls. Torretta et al. [31] also showed that median nasal NO levels were lower in NP than in controls. Arnal et al. [29] demonstrated that NO concentration in nasal nonallergic polyposis was significantly decreased compared with both controls and polyposis with allergy. These findings contrast with previous results reported by Karlida et al. [23], showing an increased tissue NO levels in patients with NP. They suggested that inflammatory cells cause more excessive free oxygen radicals (FORs), including NO, and that natural antioxidants are inadequate for detoxification of these FORs. Therefore, undetoxified FORs may cause tissue damage and NP [23].

The NO_2^-+NO_3^- concentrations are hardly distinguishable from those of Bugdayci et al. [32], who demonstrated the change in NO_2^-/NO_3^- levels of nasal polyp patients, reporting a higher level of NO_2^- and NO_3^- than in normal tissue ($p<0.05$). They mentioned that further studies are required concerning the significance of changes in lipid peroxidation and nitrite levels in pathogenesis of NP [32].

Sadek et al. [25] studying histological and genetical expression of iNOS to evaluate the role of NO in the pathogenesis of allergic and non-allergic NP detected that the expression of iNOS in both epithelial and stromal layers was greater in NP than in control tissues. The allergic NP group showed more iNOS expression than those of non-allergic NP group. Similarly, Kang et al. [33], studying the expression of iNOS, and the production of peroxynitrite represented by the formation of 3-nitrotyrosine (3-NT) by immunohistochemistry in nasal polyps, demonstrated that the stromal cells of the nasal polyp had higher labeling intensity for both iNOS and 3-NT.

Although our results regarding NO levels differ from those of Karlidag et al. [23], and NO_2^-+NO_3^- levels differ considerably from those of Bugdayci et al. [32], the difference could nevertheless be explained. Furthermore, several studies, for instance [20, 25, 33], performed on iNOS and the levels of NO, showed more iNOS expression and upregulation in NP despite lower nasal NO levels than in controls. These are possibly related to mechanical blockage of the ostiomeatal complex by nasal polyp tissue and failure of NO generated constitutively and inducible in the sinuses to reach the nasal airway and to diffuse into nasal tissue. Colantonio et al. [34] supported that the rise of NO after medical and surgical treatment is due to decrease of obstruction of sinus ostium. Colantonio et al. propose the following scenario. NO levels, being the result of inducible plus constitutive production, in uncomplicated allergic rhinitis with patent sinus ostia tend to be elevated, but when inflammation is sufficient to obstruct sinus ostium (as observed in NP), NO levels fall.

Patient-reported subjective measures of symptoms, such as VAS for nasal obstruction and for olfactory dysfunctions, and objective measure such as Lund-Kennedy score were assessed in order to correlate the findings with the levels of NO. There were found negative correlations that are consistent with previous results [28, 31, 35, 36].

Delclaux et al. [36] conducted a study in which statistical relationships were demonstrated between nasal NO and severity scores (clinical: p=-0.31, p=0.015; endoscopic: p=-0.57, p<0.0001; CT: p=-0.46, p=0.0005). Torretta et al. [32] found that Lund-Mackay scores (CT scores) inversely correlated with median NO levels (r=-0.31; p=0.04). Jeong et al. [28] observed a significant inverse relationship between NO levels and sinus CT scores, severity of nasal obstruction, and purulent rhinorrhea in chronic rhinosinusitis with polyps. No publications were identified that assess the correlation between NO concentrations and endoscopy Lund-Kennedy score. However, Nass et al. have found that the correlation between CT and nasal endoscopic findings in chronic rhinosinusitis diagnosis constitutes 90% [37].

Landis et al. [38] summarized in their review that both nasal NO and olfactory function are worth testing routinely in any rhinology workup. Regrettably, no evidence for NO levels in NP and loss of smell was found. Elsherif et al. [39] found that olfactory function and NO concentration correlate in chronic rhinosinusitis patients but not in healthy subjects, suggesting that both parameters do not directly influence each other. The inflammatory process present in chronic rhinosinusitis presumably affects olfaction and NO levels. Nasal NO produced by the paranasal sinuses seems not to directly influence olfactory function [39].

Our results related to NO levels in NP tissue and relationship between NO levels and clinical outcomes are in agreement with those obtained in earlier studies despite some limitations regarding the methodology of assessing the NO concentrations and endotypes of recruited patients.

Conclusions

In conclusion, our results corroborate the previous findings in the literature regarding significantly lower NO levels in NP compared to controls and negative correlation between NO levels and nasal obstruction, olfactory disturbances, and endoscopic appearance in patients with NP.

This study provides additional insight into complicated biological pathways of NO involvement in the pathogenesis of NP. Taken together, these results would seem to suggest the idea that the levels of NO, generated mainly in paranasal sinuses in normal state and pathological one, depend on the patency of sinus ostium and the state of osteomeatal complex. NP environment is characterized by abnormalities in NO metabolism. Further studies, which take into account

the role of NO in different chronic rhinosinusitis endotypes, are needed to be undertaken in order to allow an earlier and more accurate diagnosis, noninvasive follow-up monitoring, and development of new therapeutic approaches that will prevent the harmful direct and indirect effects mediated by NO.

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Author's contribution
AI conceptualized the idea, conducted literature review, collected the data, interpreted the data, wrote the manuscript, revised and approved the final text.

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Ethics approval and consent to participate
The study protocol was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Report No 30 of 29.03.2016). It was obtained an informed consent from all participants in the study.

Conflict of Interests
There is no known conflict of interests to declare.