Rhinosinusal Polyposis and Inverted Papilloma: A Morphometric Comparative Study

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Abstract

Introduction Nasal obstruction is one of the main rhinologic complaints, and two diseases must be investigated as differential diagnosis: rhinosinusal polyposis and inverted papilloma. Using traditional methods, the differential diagnosis between these diseases may be difficult. The morphometric study may be a useful tool for differential diagnosis and to define prognosis.

Objective Calculate the morphometric values of rhinosinusal polyposis and inverted papilloma and compare the average of variables obtained between the groups.

Methods The nasal mucus of 10 patients who had surgery in the Department of Otorhinolaryngology and Surgery of Head and Neck was studied; 5 had rhinosinusal polyposis and 5 had inverted papilloma. After the capture and print of corresponding data of each slide, the largest and smallest diameters of the nuclei were measured and the morphometric variables were calculated: average diameter, perimeter, ratio between largest and smallest diameter, volume, area, ratio of volume to area, form coefficient, contour index, and eccentricity.

Results We found a significant difference ($p < 0.05$) between the two groups in the following morphometric variables: largest diameter, smallest diameter, average diameter, volume, area, perimeter, and ratio of volume to area, indicating that these parameters can be useful in diagnostic differentiation between these diseases.

Conclusion We founded morphometric variables higher in patients with inverted papilloma, which can be related to the neoplastic origin of the inverted papilloma. The analysis of nuclear parameters is an instrument of great value in the differential diagnosis between rhinosinusal polyposis and inverted papilloma.

Keywords
- nasal polyps
- papilloma
- inverted
- nasal cavity
- otorhinolaryngologic neoplasms

Introduction

Nasal obstruction is one of the main rhinologic complaints, and rhinosinusal polyposis is an important cause. Among inflammatory nasal polyps, rhinosinusal polyposis is a chronic disease of the nasal mucosa and paranasal sinuses, with formation of polyps due to degeneration of the mucosa.[1] This disease affects between 1 and 4% of the population and is slightly more prevalent in men, with an incidence peaking in the fifth decade of life.[2]

This disease has a multifactorial origin and is frequently associated with salicylates intolerance (such as acetylsalicylic acid, AAS), asthma, and cystic fibrosis.[1–4] It presents macroscopically with a smooth surface and bright, jellylike appearance. It is pliable and light-colored gray or pink, with body and pedicle covered by nonkeratinized pseudostratified epithelium. It is usually located in the middle meatus, shell, and meatus superior.

The clinical presentation is widely variable, and it can manifest by the appearance of polypoid formations (usually

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bilateral), painless, and without bleeding, leading to partial or total nasal obstruction, rhinorrhea (usually serious), hyposmia or anosmia, and chronic rhinosinusitis.[1,3,4] Although the diagnosis is clinical, computed tomography is the best image test used, showing the extent of disease in paranasal sinuses.[5]

In cases of unilateral polypoid mass, the histopathology is not reliable and polyp antrochoanal and inverted papilloma may be considered as differential diagnoses.

The inverted papilloma is a benign epithelial tumor with hyperplastic epithelium. Inverted papilloma occurs predominantly in males in the fifth and sixth decades of life. It represents 0.5 to 4% of all nasal tumors, with potential for malignancy ranging from 5 to 13% and with frequent recurrence and a variant rate of 0 to 78%.[6–8]

Inverted papilloma is usually located on the nose sidewall in the inferior shell and rarely implants in the nasal septum or paranasal sinuses. Macroscopically, it is a unilateral mass, rounded, with irregular surface and color that varies from pink to gray.[1] Despite the usually unilateral location, studies have shown that inverted papilloma may appear bilaterally (0.92% of cases) and has been previously diagnosed as nasal polyps. Surgical interventions for exeresis of recurrent nasosinusal polyps showed 0.26% incidence of inverted papilloma.[7]

The etiology of inverted papilloma is not well defined, and there are several theories that attribute its onset to chronic inflammation, allergy, smoking, and infection by the Epstein-Barr virus and the human papillomavirus.[8]

The clinical presentation is a unilateral nasal obstruction, rhinorrhea, epistaxis, and anosmia. Depending on the tumor's extension and the invasion of nearby tissue, visual and central nervous system manifestations can be found.

The diagnoses should include detailed clinical and physical examination, with radiologic study and anatomopathological study.[1]

Due to the difficult differential diagnosis between rhinosinusal polyposis and inverted papilloma based on often unspecific signs and symptoms, investigation of microscopic morphological characteristics is necessary through karyometric or morphometric study, corroborating findings for an accurate diagnosis and appropriate treatment.

Morphometry is a direct method of evaluating the morphology of an injury. Karyometry is, basically, nuclear morphometric study that evaluates possible nuclear changes, such as in shape and volume.[9] In recent publications, morphometric study has been commonly used, primarily because it is statistically accurate and due to the extreme degree of objectivity, economy, accuracy, and the reduction of the usual bias inherent in subjective assessing.[10,11]

The benefits of morphometric study in relation to qualitative study are numeric results, which make them easily reproducible and confirmable by different laboratories. Moreover, the fact that they are composed of nonsubjective mathematical elements makes them very well accepted among researchers.[10] Karyometry can be studied through the use of a microscope coupled with a clear chamber. With this method, the nucleus is drawn manually, the measurements are made with millimetric paper, and, finally, statistical calculations are made. A computerized system can also be used in which the nucleus is automatically analyzed.[9]

The karyometric variables are largest diameter, smallest diameter, ratio between largest and smallest diameter, contour index, eccentricity, and shape index of the nucleus.

Objectives

1. To calculate the morphometric values of rhinosinusal polyposis and inverted papilloma.
2. To compare the average of variables obtained between the groups.
3. To provide data for differential diagnosis between these diseases.

Materials and Methods

We studied the nasal mucus of 10 patients treated with surgery; 5 had rhinosinusal polyposis and 5 had inverted papilloma. The specimens obtained during the surgical procedure were examined by the Pathological Anatomy Service of this institution for diagnostic assessment. They were fixed in formalin, enclosed in paraffin, and then stained with hematoxylin and eosin. These specimens were obtained through the authorization of the patient or those responsible for the patient, with informed consent, according to the guidelines and approval of the Research Ethics Committee of the same institution.

Methods

After approval by the Research Ethics Committee, the biopsies of 5 patients with histopathologic diagnosis of rhinosinusal polyposis and 5 patients with inverted papilloma were analyzed.

The slides were obtained from surgical specimens, stained with hematoxylin and eosin in the Laboratory of Pathology. The slides were analyzed at more than 1,000× magnification, using immersion oil, through an Olympus BX.60 (Tokyo, Japan) microscope coupled to a computer. A software program, Image-Pro Plus version 4.0—Media—Cybernetics (Media-Cybernetics, Rockville, USA), was used to capture the image for the isolation of 15 fields of each patient's slide, which contained 50 randomly selected nuclei, totaling 250 nuclei of each of the diseases studied.

The slides were individually analysed and after the capture and print of each one of them, the largest and smallest diameters of each of the 250 nuclei were measured on millimeter paper, and afterward, the morphometric variables were calculated and listed below, through the following mathematical formulas:

- $M = (D \cdot d)^{1/2}$
- $P = (\pi/2) \cdot [1 \cdot 5 \cdot (D \cdot d) - M]$
- ratio between largest and smallest diameter: D/d
- $V = \pi \cdot (M^3)/6$
- $A = \pi \cdot M^2/4$
- ratio area to volume: $2 \cdot M{:}3$
- form coefficient: $= 4\,F \cdot \pi \cdot A/P^2$
- $I = P/A^{½}$
- $E = (D + d)^{1/2} \cdot (D - d)^{1/2}/D$

where *M* is average diameter; *P* is perimeter; *D* is the largest diameter; *d* is the smallest diameter; *V* is volume; *A* is area; *F* is form; *I* is contour index; and *E* is eccentricity.

The variables were calculated for each slide. After we calculated the average of each variable in each disease, we compared the averages between groups with rhinosinusal polyposis and groups with inverted papilloma .

Statistical Analysis

We used the program GraphPad Stat (Graph Pad Inc, San Diego, USA), version 3.0 for Windows 95 (Microsoft, Redmond, Washington, United States). The nonparametric Kruskal-Wallis test was used to calculate the values of the averages of each variable in the two groups. After this analysis, the posttest multiple comparisons of Dunn were used when *p* obtained significance (when $p < 0.05$).

Results

The morphometric variables for each of the diseases studied are represented in ►**Table 1**, with minimum and maximum values and averages.

The comparisons of morphometric variables between the groups are represented in ►**Table 2**.

Discussion

Rhinosinusal polyposis presents morphologically as a mass in the nasal cavity, composed of body and pedicle, covered with nonkeratinized pseudostratified epithelium, with ciliated cells and caliciform glands with stroma of loose, richly vascularized fibrous tissue. In morphometric presentation, it can present with increased nucleus size compared with normal mucosa, but without changes in its form.[9] The inverted papilloma has hyperplastic epithelium that expands into the adjacent stroma. Morphometric data about this disease are scarce in literature, not allowing a consistent characterization of it.

Morphometric study has been used for many decades to distinguish normal and altered cells.[12,13] By the simple measure of variables such as largest diameter and smallest diameter, it is possible to calculate other variables, permitting the tridimensional analysis of cells. The analyses of shape and volume alterations, among other variables, corroborate the diagnosis and prognosis of certain diseases.

Karyometry also plays an important role in the experimental area, which requires exact measurements. Thus, the variables calculated by mathematical formulas become statistically reliable parameters.[10] Karyometric analysis of substances with oncogenic potential has assumed significant importance. An example is a Brazilian study that evaluated the effect of sodium cyclamate in pregnant rats that showed significant changes in the cells of fetuses exposed to the administration of this artificial sweetener.[14] Fetuses with smallest weight gains were observed, represented by smaller nucleus but without changes in its form. Another study evaluated the effect of the administration of aspartame on the exocrine pancreas of rats' fetuses, suggesting a teratogenic role.[15]

The application of karyometry in rhinosinusal diseases can be justified by significant histologic changes found in them. Rhinosinusal polyposis presents high association with asthma, patients with acetylsalicylic acid intolerance, allergic rhinitis, and syndromes such as Churg-Strauss, Young, and Kartagener, which present in common rhinosinusitis.

These inflammatory factors combined with genetic disorders may be related to the development of inflammatory polyps causing stroma swelling, infiltration of inflammatory cells with a predominance of eosinophils, and thickening of the basal membrane and local fibrosis.[9]

In inverted papilloma, there is a benign epithelial alteration originating a hyperplastic epithelium. Previous studies

Table 1 Minimum, maximum, and average morphometric variables

	Rhinosinusal polyposis			Inverted papilloma		
	Minimum volume	Maximum volume	Average	Minimum volume	Maximum volume	Average
Largest diameter (μm)	10.00	36.00	20.00	11.00	40.00	25.00
Smallest diameter (μm)	6.00	20.00	12.00	7.00	22.00	14.00
Medium diameter (μm)	7.75	22.58	15.20	9.54	26.83	19.31
Volume (mm^3)	243.35	6,030.50	1,874.20	454.53	10,116.00	3,756.80
Area (mm^2)	47.12	400.55	182.61	71.47	565.49	292.96
Perimeter (mm)	25.53	80.45	52.43	31.73	94.51	65.16
Ration of volume to area	5.16	15.06	10.16	6.36	17.89	12.87
Eccentricity	0.32	0.98	0.81	0.30	0.95	0.82
Form coefficient	0.39	1.00	0.90	0.64	1.00	0.89
Contour index	3.55	5.67	3.74	3.55	4.41	3.75
Largest diameter/smallest diameter	1.06	5.67	1.71	1.05	3.09	1.75

Table 2 Comparison between morphometric variables

	Rhinosinusal polyposis × inverted papilloma *p* values
Largest diameter	<0.001
Smallest diameter	<0.001
Medium diameter	<0.001
Volume	<0.001
Area	<.0001
Perimeter	<0.001
Ration of volume to area	<0.001
Eccentricity	>0.05
Form coefficient	>0.05
Contour index	>0.05
Largest diameter/ smallest diameter	>0.05

have shown that despite having a unilateral location, the inverted papilloma may appear bilaterally with previously diagnosed nasal polyps.[6]

Thus, the morphometric study shows a quantitative analysis of cellular changes because the changes observed in diseased cells, such as architecture, cellular level, cellularity, extension of the invasion, and nature and extent of the inflammatory reaction, exemplify characteristics of the disease quality.

After measuring the largest and smallest diameters, the other morphometric variables were calculated. Among the parameters studied, there were statistically significant differences in the variables of largest diameter, smallest diameter, average diameter, volume, area, perimeter, and relationship of volume to area, indicating that these parameters can be useful in diagnostic differentiation between these diseases.

In a previous study comparing glandular epithelium of normal nasal mucosa with epithelium of rhinosinusal polyposis, significant differences were also found in the parameters noted above, with the exception of the ratio between volume and area, reinforcing the karyometric characteristics of rhinosinusal polyposis, in which we observed an increase in the nuclear volume without any change in nuclear form.[9] These data refute the possibility of malignant degeneration of this condition and corroborate the investigation of morphometric changes related to the pathogenesis of this disease.[9]

Analyzing the parameters of eccentricity, form coefficient, contour index, and ratio between largest and smallest diameters, no significant difference was found between the groups, indicating that the cells studied were similar and cannot contribute to the differential diagnosis.

The cells of inverted papilloma have higher values in all parameters except when compared with the form coefficient, showing that the cells of the inverted papilloma are greater in all areas studied.

This finding may be related to the neoplastic etiology of inverted papilloma, when the cancer cell suffers a structural disorder, especially at the nuclear level, although this relation has been better studied in malignant neoplastic cells.[16]

Conclusion

The analysis of nuclear parameters is an instrument of great value in the differential diagnosis between rhinosinusal polyposis and inverted papilloma, as there was statistical difference in 7 of the 11 variables studied. This is a pilot study and further studies are needed that address these diseases and compare them to find accurate karyometry values, not only for differential diagnosis but also for assessment of prognosis for these diseases and their evolution.

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