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ORIGINAL ARTICLE

Karyometric analysis of nasal polyps

Nazal poliplerin karyometrik incelemesi

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Objectives: Nuclear characteristics of epithelial cells in nasal polyps were analyzed.

Patients and Methods: The slides of 35 patients who underwent surgery for nasal polyposis and 18 slides of normal mucosa were examined. The specimens were stained with hematoxylin-eosin. Tissue sections were analyzed by a CCD camera and the Karyotype Program of Human Cytogenetics Nomenclature System on a Macintosh computer (OS 9). Fifty epithelial cells in each slide were randomly selected and transferred to the automated karyotyping system and photographed. The diameters of the nuclei were measured and some peculiar nuclear features were examined, including unsmooth appearance of the nuclear membrane, anaphase-bridge, and binucleation.

Results: There were significant differences between nasal polyp and normal mucosa groups in terms of nuclear diameter, perimeter, and volume, with all variables being greater in the nasal polyp group (p=0.001). Unusual nuclear features differed significantly (unsmooth nuclear membrane appearance, p<0.001; anaphase-bridge, p=0.041; binucleation, p=0.018), as well.

Conclusion: Epithelial cells of nasal polyps exhibit nuclear instability.

Key Words: Karyometry; nasal polyp.

Amaç: Bu çalışmada nazal polip epitel hücrelerinin çekirdek yapıları incelendi.

Hastalar ve Yöntemler: Nazal polipozis nedeniyle ameliyat edilen 35 hastanın kesitleri ve 18 normal mukoza kesiti çalışıldı. Kesitler hematoksilen-eozin ile boyandı. Doku bölümleri CCD kamera ve Mac OS 9 Karyotype Program of Human Cytogenetics Nomenclature System yardımı ile incelendi. Her bir kesitten 50 epitel hücresi rastgele seçildi ve otomatik karyotip sistemine aktarılarak fotoğraflandı. Çekirdek çapları ölçüldü. Ayrıca, sıra dışı çekirdek yapıları olan düzensiz çekirdek membranı, anafaz köprüsü ve çift çekirdekli hücre oranları incelendi. Veriler nazal polip ve normal mukoza grupları arasında karşılaştırıldı.

Bulgular: Nazal polip epitel hücresi çekirdeği ile normal mukoza çekirdeği arasında çekirdek çapı, çevresi ve hacmi açısından anlamlı farklılık bulundu; bu değişkenlerin hepsi polip grubunda artış gösterdi (p=0.001). Sıra dışı çekirdek yapıları bakımından da iki grup arasında belirgin farklılık vardı (düzensiz çekirdek membranı, p<0.001; anafaz köprüsü, p=0.041; çift çekirdekli hücre, p=0.018).

Sonuç: Nazal polip epitel hücresinde çekirdek düzensizliği görülmektedir.

Anahtar Sözcükler: Karyometri; nazal polip.

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One morphometric approach that determines alterations in cell nuclear features such as shape and volume is karyometry. There is a strict correlation between nuclear volume and chromatin volume and condensation or the number of the chromosomes. This correlation was named ploidy by Rigaut et al.[1] Nuclear enlargement, usually due to the existence of abnormally high DNA (ploidy) values, is one of the alterations most often encountered in pathologic epithelia, especially in cancerous and precancerous states. The concomitant diagnosis of polyposis and neoplasia has been reported in different reviews of the literature, with a 1.17% prevalence of neoplasia being observed by Busuttil et al.[2] in 1978 and 1.8% prevalence by de la Cruz Mera et al.[3] in 1989. Hasegawa et al.[4] suggested that all inflamatuary polyps of the nasal cavity contain various types of atypical mesenchymal cells, but only in rare cases does this atypia suggest malignant transformation.

Karyometric studies must take the difficult stereologic problem of spheroid sectioning into account. Rigaut et al.[5] proposed a mathematical model to describe an aggregate of separate parallel anisotropic spheroids of variable size and eccentricity.

The aim of this study was to find out the nuclear characteristics and compare the karyometric properties of normal nasal epithelium and sinonasal polyps in humans.

PATIENTS AND METHODS

Polyp tissue was obtained from 35 (16 males, 19 females; mean age 38±17 years) patients with nasal polyposis during endoscopic sinus surgery. The normal mucosa was obtained from 18 patients (8 males, 10 females; mean age 34±11 years) free of chronic sinusitis and allergic rhinitis and other well known rhinological diseases whom have been performed turbinate surgery for massive concha bullosa. The specimens are immediately fixed at 4 °C in a solution of 6% paraformaldehyde, phosphate buffer 0.1M, PH 7.4, for 10 hours, followed by washing for 48 hours in distilled water at 4 °C. After paraffin embedding, a parallel, approximately 4 µm thick sections are made. Slides stained with hematoxylin-eosin technique. Tissue section was analyzed in light microscope (ZEISS Axioskop 2 plus) with a 100 X immersed objectives supported with CCD camera and Mac OS 9 Karyotype Program of Human Cytogenetics Nomenclature Systems (MacK type 5.5.4). Fifty epithelial cells nuclei of each patient were analyzed and transferred to the automated karyotyping system and photographed (Fig. 1a-c). Nuclear axis was measured by an ocular micrometer (Carl Zeiss Jena, GF-P10X).

The nuclear diameters were measured X and Y. X was the axis parallel to the parent nucleus equatorial plane and Y was the principal axis perpendicular to X. Mathematical assumptions of perimeter (P) and nuclear volume (V) were formulized as $P=2\pi[(X/2)^2+(Y/2)^2)/2]^{1/2}$, and $V=\pi X^2.Y/6$ (F). Then, the patient's average of the variables of X, Y, P, V were statistically compared with Mann-Whitney test between nasal polyp group and normal mucosa group.

We also analyzed the ratio of unexpected nuclear features such as distinct un-smooth nuclear membrane appearance, anaphase-bridge and binucleated cell (Fig 1a-c). We performed chi-square test to compare the ratios of unexpected nuclear features between nasal polyp and control groups.

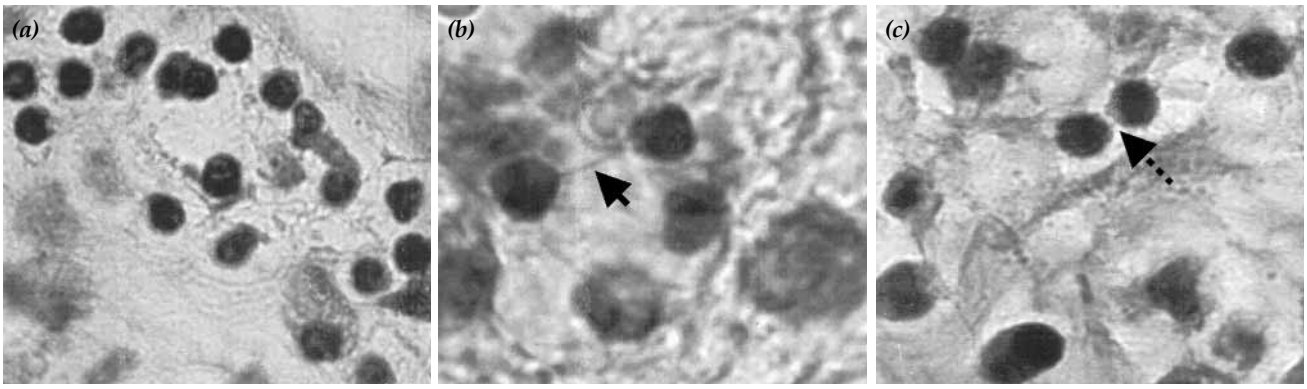

Fig. 1. *Photographs of epithelial cells nuclei of nasal polyps.* ***(a)*** *unsmooth nuclear membrane appearance,* ***(b)*** *anaphase-bridge (straight arrow),* ***(c)*** *binucleated cell (dotted arrow).*

RESULTS

Table 1 shows the results of the variables of X (equatorial axis), Y (axis perpendicular to equatorial axis), P (perimeter), V (nuclear volume) of both nasal polyp and normal mucosa groups. All the mean variables of nasal polyp group were greater than those of normal mucosa group. There were statistically significant differences (p=0.001) between nasal polyp and normal mucosa groups for all variables of X, Y, V, P.

We also found the ratios of all unexpected nuclear features were greater in nasal polyp than in the control group (Table 2). There were statistically significant differences between the two groups, regarding the ratios of distinct un-smooth nuclear membrane appearance (p<0.001), anaphase-bridge (p=0.041), and binucleated cell (p=0.018).

The present study revealed a significant difference between nasal polyp and normal mucosa groups in term of nuclear diameters, nucleus perimeters, volume, and unexpected nuclear features.

DISCUSSION

Karyometry has gained space popularity over the last few years, especially in the field of experimental pathology where such studies in the area of histopathological diagnosis are lacking. This increasd reliability was also caused by the fact that science depends on accurate observation and karyometry involves the application of mathematical models that are based on geometric and statistical probabilities, thus providing statistically assessable and reliable data.[6]

Rigaut et al.[5] documented nuclear alterations in the nasal epithelia of workers exposed to nickel in whom the prevalence of metaplastic and dysplastic alterations were high, with a consequently higher incidence of malignant neoplasias of the nose and sinuses. de la Cruz Mera et al.[3] and Busuttil et al.[2] observed an incidence of malignant neoplasia of 1.8 and 1.17%, respectively in patients with rhinosinus polyposis, but did not report any direct or transitional correlation between the etiopathogenesis of the disease and the occurrence of neoplasia. They did not question a possible malignant transformation or the causal occurrence between the two pathologies. Pereira et al.[7] reported the occurrence of anaplastic carcinoma in paranasal sinus of a patient with typical clinical signs and symptoms of nasal polyps and questioned whether polyps might undergo malignant transformation. The authors suggested that the occurrence of polyps results in a chronic inflammatory reaction in the rhinosinus mucosa, leading to alterations in the epithelium accompanied by metaplasia, and eventually, to dysplasia and carcinoma. The authors also reported that 15% of neoplasias can present as typical rinosinusitis because of anatomic

TABLE I

THE RESULTS OF THE VARIABLES OF X (EQUATORIAL AXIS), Y (AXIS PERPENDICULAR TO EQUATORIAL AXIS), P (PERIMETER), V (NUCLEAR VOLUME) OF BOTH NASAL POLYP GROUP AND NORMAL MUCOSA GROUP

Group	Number	X µm	Y µm	V μm^3	P µm
Nasal polyp	35	9.9±1.9	13.8±2.4	793,8±539,3	37.81±6.7
Normal mucosa	18	7.0±1.1	9.0±1.5	251.9±72.0	25.3±17.3
		p=0.001	p=0.001	p=0.001	p=0.001

TABLE II

THE PERCENTAGES OF THE UNEXPECTED NUCLEAR FEATURES

Group	Un-smooth nuclear membrane	Anaphase-bridge	Binucleated cell
Nasal polyp	31.66%	4%	10.66%
Normal mucosa	16.4%	1.2%	4.8%
	p<0.001	p=0.041	p=0.012

and functional characteristics that postpone the occurrence of the neoplastic symptoms.

Coste at al.[8] found that the percentages of S-phase cells were significantly higher in nasal polyps than in mucosa, and proliferating cell nuclear antigen indexes were significantly higher in nasal polyps than in the suprabasal area and full height of the mucosal epithelium. de la Cruz Mera et al.[3] have suggested that the observed stromal atypias, epithelial alterations, and granulomas might be related to etiopathogenesis of rhinosinus polyposis. Fernandes et al.[9] declared a significant difference between glandular epithelium of normal nasal mucosa and rhinosinus polyposis in nuclear diameters, nuclear area, nuclear volume, and no difference in nuclear shape.

We observed significant differences for nuclear diameters, perimeter, volume, and also for unexpected nuclear features between nasal polyp and normal mucosa.

There is a nuclear instability in nasal polyp tissue.

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