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Original Research Article

Expression of the nestin marker in the mucosa of human nasal inverted papillomas using immunohistochemical technique

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Abstract: Inverted papilloma is a tumour which arising from the lateral wall of the nasal cavity. It is common in males in 4th to 6th decades and is fairly uncommon neoplasm of the nasal region. The epithelium of this tumour consist of many layers, typically squamus or ciliated columnar cells. Sometimes transitional epithelium enclosed by layer of columnar or squamous cells. A peroxidase-labeled streptavidin-biotin technique was used in Immunohistochemical staining. The staining strength of epithelial cells in nasal papilloma mucosa was analogous to the nasal mucosa of inferior turbinate. The staining was detained to the cytoplasm as well as the plasma membrane, which was deep in the basal part and feeble in the upper part. Total removal of the tumour should be done as final management of inverted papillomas with very long term follow-up (nearly 2 years) to notice any consequent recurrences.
Keywords: nestin marker, immuno histochemical technique.

INTRODUCTION:

Inverted papilloma is a tumour which arising from the lateral wall of the nasal cavity. It is common in males in 4th to 6th decades and is fairly uncommon neoplasm of the nasal region [1]. Although several studies have been tried to found the etiology of inverted papilloma, still undecided. Human papilloma viral infection believed to play significant roles in this tumour [2]. Inverted papilloma in addition occupies the orbits, pharynx and temporal bone. Inverted papillomas characterized by high rate of recurrences with malignant tendency. Nasal congestion or discharge and epistaxis are the main presentation of this tumor. Merely 18% of cases arise from the nasal septum whereas bilateral cases found in less than 4 % of cases [4].

Males affected more than females with ratio nearly 5 to 1 and frequently seen in whites more than blacks. The usual age mean is 50 years [5]. The epithelium of this tumour consist of many layers, typically squamus or ciliated columnar cells .sometimes transitional epithelium enclosed by layer of columnar or squamous cells [3]. Even though histologically benign, papilloma are clinically threatening lesions since their high recurrences following surgical removal (approximately 80%), with moderate risk of malignant conversion (approximately 45) [6].

The mucosa of nasal cavity is the primary corporeal barrier to unknown materials and a conditioner used for inhaled air. The respiratory epithelium which is pseudostratified columnar epithelium consist of four main kinds of cells, ciliated and secretory columnar , goblet and basal cells, the last one contributes in the conservation of nasal mucosal cells and integrity. In abundant respiratory diseases, for example sinusitis, the surface epithelium is harshly damaged and should be regenerated to re-establish its protective functions [7].

This renewal method may engage stem cells (progenitor cells) inside the nasal epithelium. Yet, nearly all studies concerning respiratory epithelium and its growth and progress have paying attention on the trachea, bronchi and further major parts of the lung [8]. Only some studies that concerned with the nasal epithelium have chiefly concentrate on epithelial response to different environmental invectives [9]. By the way, further work is required to estimate the mechanisms implicated in proliferation of tissue in nasal papillomas. Stem cells are self-replicating cells; responsible for regeneration of all cells of respiratory epithelium, and take part in repairing tissue on a variety of surface mucosa [10].

Different stem cell markers have been introduced in different new studies, together with nestin [11]. The nestin expression has been accounted as an

evidence of recognition of stem cells in diverse mucosal surfaces counting the small intestine and cornea of the eye [12]. New study proposes that the dynamic ratio between growth and proliferation was diminished in stem cells present in nasal epithelial layer taken from humans complained from nasal problems [13]. Another new study assumes that stem cells probably supportive appendage used for looking at the inflammatory progression inside the nasal papilloma and polyps [14]. This study was intended to simplify the nestin expression as prospective stem cell marker in mucosa of nasal inferior turbinate and inverted papillomas in an attempt to find out the part and the character of nestin in mucosa of normal inferior turbinate and inverted papillomas.

MATERIALS AND METHODS:

Eighty (80) samples were selected for this study from the Department of Otolaryngology, Teaching Hospital in Baghdad city from December 2014 to July 2015. Thirty (30) Samples of inferior turbinate mucosa were obtained from patients undergoing nasal septoplasty, twenty (20) of them were males and ten(10) were females, approximately the total cases were in 40^{th} age were used as normal controls in this study. The mucosa of inferior turbinate was abhorrently normal, with no evidence of inflammation or infection. Fifty (50) Samples of nasal inverted papillomas were obtained from patients undergone nasal excision of papilloma , thirty five (35) males and fifteen (15) females, the total cases were in range 40^{th}-50^{th} age old (Table &Figure(1)).

Table 1

Group	Females		Males		Total number
	Number	%	Number	%	
Control	10	33.33	20	66.66	30
Papilloma	15	30	35	70	50

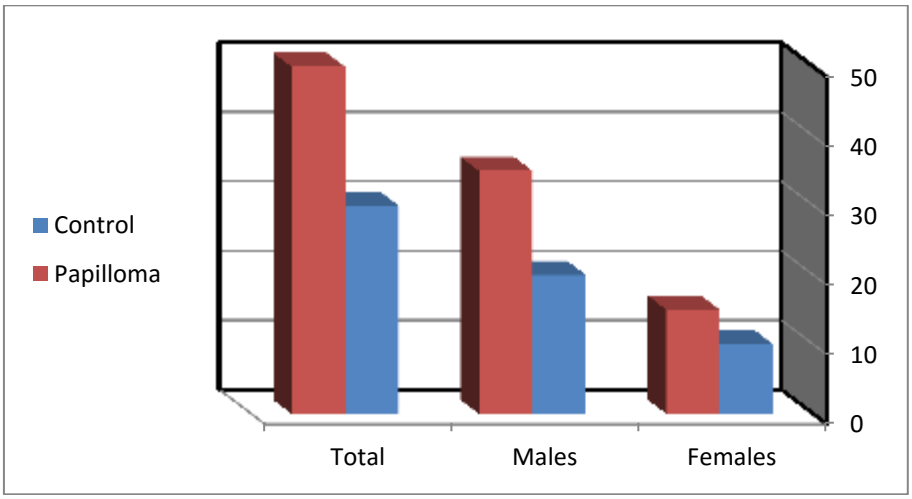

Fig 1: study sample

For all patients there was no history of chronic diseases, respiratory allergy or asthma. For immunohistochemical technique, all samples (nasal inferior turbinate mucosa and nasal papillomas) were engrossed during the night in a 4% paraformaldehyde within phosphate-buffered saline (pH 7.5). Then samples were dehydrated in ethanol (a graded succession) then in xylene and finally embedded in paraffin wax. After that, the expression and allocation of nestin in nasal inferior turbinate mucosa and nasal papillomas was evaluated by using immunohistochemical technique.

A peroxidase-labeled streptavidin-biotin technique was used in Immunohistochemical staining. In brief, paraffin sections (3-5 mm) were sliced and sited on charged glass slides. Then slides were autoclaved in 15 mM citrate buffer (pH 6.5) for 10 min. On the road to satisfy endogenous peroxidase activity, slides were placed in solution of 4% H2O2 for 20 min. Following washing in PBS, slides were after that incubated during the night at 50 C with diluted anti-nestin antibody (*Geneaid, USA*).

RESULTS:

In typical nasal mucosa of inferior turbinate, nestin was limited mainly to the superficial epithelium and the cells of glandular epithelium. Staining was typically strongest in the lower part of the epithelial layer, diminished on the way to the upper part, and frequently limited to the cytoplasm of the cells. In a few

parts, deep staining was infrequently detected in the whole epithelial layer (Figure 2&3).

The staining strength of epithelial cells in nasal papilloma mucosa was analogous to the nasal mucosa of inferior turbinate with minor difference. The staining was detained to the cytoplasm as well as the plasma membrane, which was deep in the basal part and feeble in the upper part. Some parts of the epithelial lining, there was deep staining detected in the whole layer (Figure 4&5).

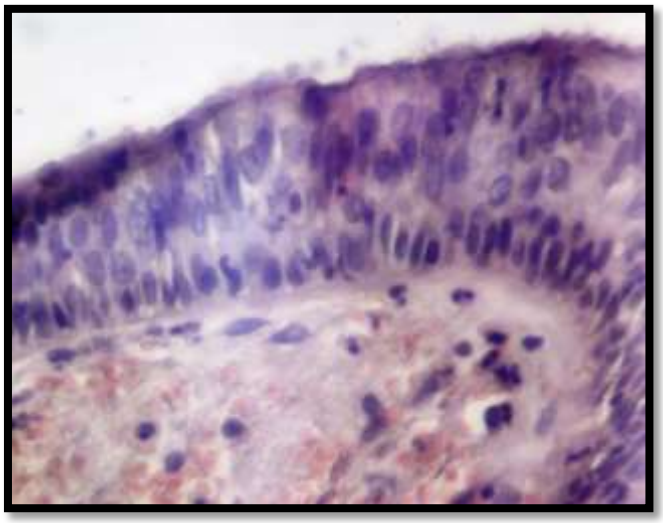

Fig 2: Immunohistochemical localization of nestin in normal human nasal mucosa in 38 years old female(X400) .

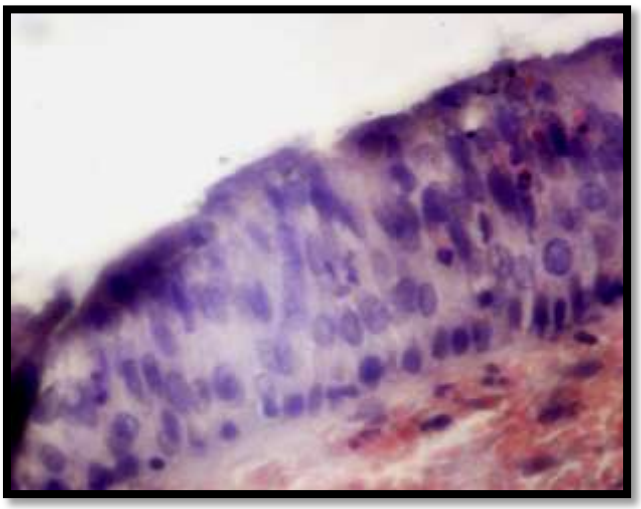

Fig 3: Immunohistochemical localization of nestin in normal human nasal mucosa in 46years old male(X400)

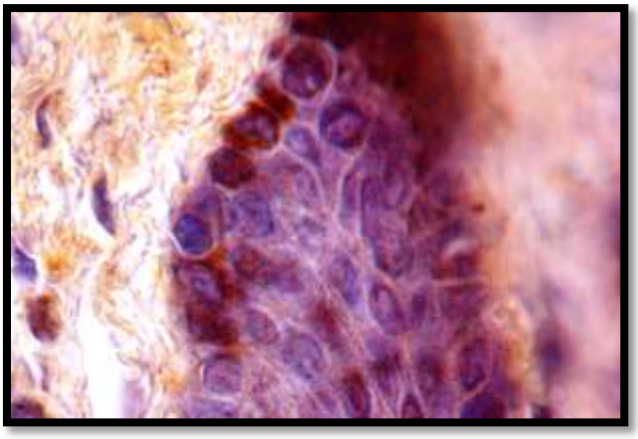

Fig 4: Immunohistochemical localization of nestin in nasal papilloma in 45 years old female (X1000)

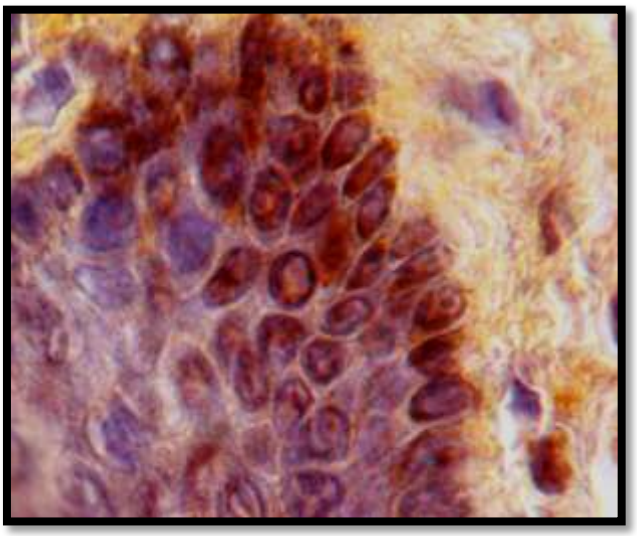

Fig 5: Immunohistochemical localization of nestin in nasal papilloma in 51 years old male (X1000)

DISCUSSION:

A stem cell in adults defined as an undifferentiated cell establish between differentiated cells in any part of tissue. A stem cells be capable of self-renew, and be able to differentiate to give up the main particular cell kinds of the tissue. The principal functions of these stem cells are to preserve, replace and repair the damaged tissue in which they are originated [15, 11].

However, only some studies have estimated the existence of stem cells in the mucosa of nasal airways, in spite of both neoplastic and immediate proliferative behavior of the nasal epithelium. This study recognized the appearance and allocation of nestin in ordinary nasal mucosa of inferior turbinate and inverted papillomas. Records propose that nestin may had a significant function in the renewal and production course of action of the cells in the epithelial layer in mucosa of inferior turbinate and inverted papillomas, maintain the integrity of nasal mucosa and participate in inverted papilloma development.

The pseudo stratified epithelium that outline human respiratory passages is gradually renewed in usual conditions, other than cells of respiratory airway epithelium can in addition proliferate widely to repair an injury [16]. Stem cells of nasal epithelial are in addition thought to exist in the basal epithelial layer of mucosa and preserve the surface mucosa by generating fleeting magnifying cells that travel, proliferate, regenerate and differentiate to repairer injured epithelial surface and substitute lost or broken epithelial cells. So, studies have estimated that nestin perhaps particularly expressed in the generating and proliferating partition of the basal layer of epithelium. This study, establish that nestin was restricted in the basal part of epithelial layer. As a result, the current outcome signifies that nestin may possibly play a significant role in differentiation of tissue within the cells of the superficial layer of epithelium in ordinary

mucosa of papilloma. These suggestions hold up the outcomes viewing that respiratory epithelium of nasal passages are regenerated, renewed and proliferated on the way to reinstate its defensive role in several acute and chronic nasal diseases [7].

The appearance of nestin within the layer of epithelium diminished quietly since the basal layer on the way to the upper one. These results are reliable with the study suggest that nestin expression is found in the basal layer in addition to the upper layer in the epithelium of skin [17]. Preceding studies that have been used Ki67 and PCNA as stem cell markers to distinguish the nasal polyp and papilloma activity on the subject of proliferation [18, 19]. PCNA is a nuclear protein with the purpose of growth directive. Ki67 is a monoclonal antibody distinguishes any type of a nuclear antigen which may be found in every phase of growth cycles in mitotic cells. These two studies verified that PCNA plus Ki67 immuno-positive cells were mostly concentrated in the basal part of the epithelial layer, in some places no immuno-positivity was observed [18, 19]. In contrast, results of this study confirmed that there was constant expression of nestin alongside the basal part of the epithelial layer of papilloma. As a whole, these results show that not all cells with nestin -positive reaction had been stained positive for Ki67 or PCNA and propose that nestin expression included not just the recognized stem cell partitions of the nasal mucosa but also fleetingly differentiated cells.

Founded on these consequences, this study supposes that nestin take part in the development of inverted papillomas and therefore contributes to proliferation of epithelial cells. On the other hand, taking into consideration the variety of cell natures expressing nestin in addition to PCNA and Ki67, , it is not obvious whether the cells with nestin immuno-positive reaction in nasal inferior turbinate mucosa and inverted papillomas reveal a higher proliferation rate or move around further willingly than the other cells with nestin-negative reaction. Prospect studies separating and propagating progenitor cells of nasal epithelial layer in vitro and investigating the consequence of these cells would assist to reply all these inquiries.

CONCLUSIONS:

Respiratory epithelium of nasal papillomas are regenerated ,renewed and proliferated on the way to restore its defensive role against several medications .Total removal of the tumour should be done as final management of inverted papillomas with very long term follow-up(nearly 2 years) to notice any consequent recurrences.

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