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A Comparative Study of IL-10 Polymorphism between the Mucosa of Inferior Turbinate and Nasal Polyps by PCR Technique

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Abstract: *Nasal polyps are benign tissue swellings in the nose that originate from the paranasal sinuses, frequently from the anterior ethmoid complex. IL-10 produced predominantly by T regulatory cells, but also produced by B cells, macrophages, mast cells, monocytes and keratinocytes . IL-10 had significant anti inflammatory processes, particularly allergic inflammatory processes. (58) Patients undergoing nasal surgery were selected for this study to compare the levels of IL-10 between normal people & those with NP using PCR technique. PCR results for IL-10 showed positive results in the NP group, while all control samples were negative for IL-10. Depending on results, a difference in the pathogenesis of nasal polyps between Iraqi patients compared with Chinese patients was suggested.*

I. Introduction

Nasal polyps are benign tissue swellings in the nose that originate from the paranasal sinuses, frequently from the anterior ethmoid complex **[** Andrews AE et al.,2005**].**

Lesions were, round, soft, semitranslucent, pale and glistening that could descend between the lateral nasal wall and the middle turbinate into the nasal cavity causing symptoms such as nasal obstruction, rhinorrhea, nasal congestion, facial pressure and hyposmia **[**Fokkens WJ et al.,2005**].**

Interleukins are group of cytokines (signaling molecules), primary expressed from white blood cells **[**Brocker C et al.2010**].** The function of the immune system depends mostly on interleukins, and any deficiency of one of them could causing immune deficiency or autoimmune diseases. The greater part of interleukins are synthesized by helper CD4 T lymphocytes, in addition to monocytes, macrophages, and endothelial cells, they support the differentiation of T and B lymphocytes**[**Ofra BM et al.,2010**].**

One type of interleukins is IL-10 which is produced predominantly by T regulatory cells, but also produced by B cells, macrophages, mast cells, monocytes and keratinocytes **[**Moore KW et al.,2001**].**

IL-10 had significant anti inflammatory processes, particularly allergic inflammatory processes **[**Williams LM et al.,2004**].** Myoung SJ et al (1999) investigated the expression of (IL-10) and other cytokines in patients with nasal polyps by using PCR. They studied & compared the expression of IL-10 between control group and a study group with allergic and non-allergic nasal polyps. IL-10 was highly expressed in non-allergic nasal polyps than in the control group **[**Myoung SJ et al.,1999**]**. Pitzurra L et al, study the expression of IL-10 by using real time –PCR and found that IL-10 mRNA was highly expressed in NP- patients with fungal infection. The study showed that microbial infection is associated with high production of IL-10 **[**Pitzurra L et al.,2004**],** was because IL-10 decrease the antifungal activity of phagocytes **[**Romani L.,2004**].** The level of IL-10 was found to be decreased in nasal polyps by using enzyme-linked immunosorbent assay (ELISA) in Chinese patients. This study suggested that there was a difference in the pathogenesis of nasal polyps between Chinese patients compared with Western patients **[**Shi J et al.,2009**]**. Peric A ,et al study had shown high concentration of IL-10 in patients with an asthmatic nasal polyp than in patients with non-asthmatic nasal polyp by using PCR & flowcytometry. These findings suggested that there was a different ways of pathogenesis in nasal polyp **[**Perić A et al.,2010b**;** Perić A et al.,2010c**].**

No significant difference was found in the concentration of IL-10,when compared between allergic and non-allergic nasal polyp-patients using flow cytometry**[**Perić A et al.,2010a**].**

II. Patients And Methods

Sample collection

(58) Patients undergoing nasal surgery were selected for this study from the Department of Otolaryngology, Gazi Al-Hariri Teaching Hospital ,Baghdad; Iraq from May 2013 to January 2014.they were divided as follows:

1. **Control group:** (22) Samples of mucosa of inferior turbinate were obtained from patients undergo rhinoseptoplasty intended for septal deviation, (16) of them were females and (6) were males as shown in table

(1), all cases were in 20th were used as normal controls in this study. The inferior turbinate mucosa was grossly normal, with no evidence of infection or inflammation.

2. Study group: (36) Samples of nasal polyposis were obtained from patients who had undergone nasal polypectomy, (22) females and (14) males, all cases were in 20th age old except one female was 10 years old (Table 1).

Table (1): The study sample

Group	Females		Males		Total number
	Number	%	Number	%	
Control	6	27.3	16	72.7	22
Study	22	61	14	39	36

Extraction of DNA

Process of extraction was according to kit (**Geneaid. USA**): Small sections were sliced (up to 25 µg) from blocks of paraffin-embedded tissue and transferred to a 1.5 ml microcentrifuge tube.1 ml of absolute ethanol was added to wash the sample pellet. Then 200 µl of GBT buffer was added and mixed by vortex for 5 seconds. After that, 200 µl of absolute ethanol was added to the sample lysate and vortexed immediately for10 seconds. Then 400 µl of W1 Buffer was added with 600 µl of wash buffer (ethanol added) to the GD column. After added 100 µl of preheated elution buffer, Centrifugation was done at 14-16,000 x g for 30 seconds to elude the purified DNA. Measurement of the concentration and purity of DNA by using Nanodrop was done. The concentration was (1.84-1.98) mg/ml and the purity of samples were in between (1.56-1.84). (The dependable range of purity of Nanodrop was (1.8-2 the ratio of 260/280).

Agarose gel electrophoresis

DNA extraction was confirmed by agarose gel electrophoresis **[**Sambrook J et al.,1989**]**. Agarose gel was prepared by dissolving 1.5gm of agarose powder in 100 ml of TBE buffer (PH.8) in boiling water bath, allowed to cool to 50°C and ethidium bromide at the concentration of 0.5µg/ml was added. The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was poured gently into the tray, allowed to solidify at room temperature for 30 min. The comb was then removed gently from the tray. The tray was fixed in an electrophoresis chamber filled with TBE buffer that covered the surface of the gel. 3µg of loading buffer and 10µg of DNA sample was transferred into the wells in agarose gel, and to one well there is 10µg of DNA ladder. The electric current was allowed at 70 volt for 50min.The gel was removed from the tank, and the excess liquid was drained .The gel was placed in the dark room, and visualized at UV beam at 480nanometer**.** The product band differentiated and was compared it with ladder band as a control. Image for the gel was captured by digital camera connected to imaging system.

Primers of IL-10

Primers were prepared by adding 100 µl of (not deionised water) to the powder of primer in concentration 100 nM to obtain a concentration of 100µM. Then the primers were vortex thoroughly. The primer ID number was written on the lid of the vial. Primers were then stored in the refrigerator at a degree(-20°C). Primer sequence for IL-10 was**:**

Table (2): Primer sequence for IL-10

Forward	**F- CCT AGG TCA CAG TGA CGT GG .3**
Reverse	**R-GGT GAG CAC TAC CTG ACT AGC .3**

Polymerase Chain Reaction

The process was according to kit (AccuPower ® ProFi Taq PCR PreMix) as a follow: Template DNA and primers of IL-10 were thawed before use. Template DNA and primers were added into the Accu Power® Pro Fi Taq PCR Pre Mix tubes. Distilled water was added into the Accu Power® ProFi Taq PCR PreMix tubes to a total volume of 20 µl (K-2631, K-2632).The lyophilized blue pellet was dissolved completely and spin down by pipetting up and down several times and then briefly spinning down. The reaction was performed under the certain conditions as shown in table (3). The reaction was maintained at 4°C after the completion of amplification and the sample was stored at a degree (-20°C) until use.5 µl of the reaction mixture was loaded directly on agarose gel without adding a loading dye to analyze the PCR
products.

Table (3): Reaction conditions

No.	Steps	Temperature	Time	Cycles
1	Pre-Denaturation	95 °C	5 min	1 cycle
2	Denaturation	94 °C	15-20 sec	25-35cycles
3	Annealing	60°C	45 sec	25-35cycles
4	Extension	72 °C	1min/kb	25-35cycles
5	Final extension	72 °C	10 sec	1 cycle

III. Results

Details of PCR results for IL-10 showed positive results in the NP group. while all control samples were negative for IL-10. Pearson Chi square showed significant relation of PCR results as compared to pathological groups. (table 4 & figure 1).

Table (4): study groups

PCR results	Group				Total number
	Control		Nasal polyp		
	Males	Females	Males	Females	
Positive	0	0	6	15	21
Negative	6	16	8	7	37
Total	6	16	14	22	58
P value	0.03				

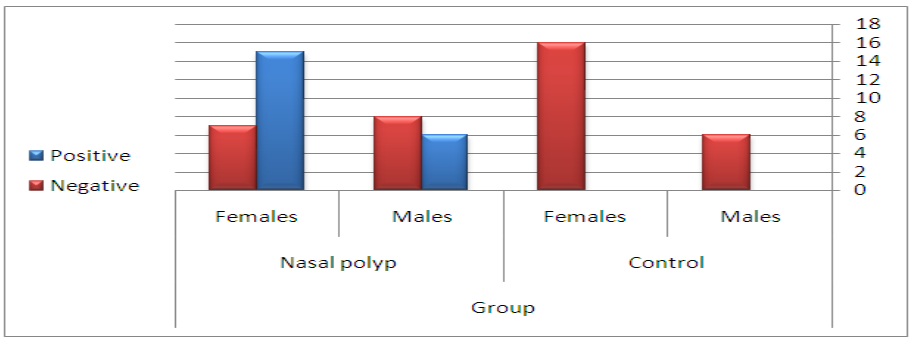

Figure (1): PCR results with sample groups

The DNA electrophoresis results were shown in the figure(3).in lane1 DNA marker (Lambda DNA\ EcoR+ Hind 111) which had 4bands(21.226,5.148,4.268,2.027bp), (22) years old female with nasal polyp had one band(21.226) compare with DNA marker, (21) years old male with nasal polyp had one faint band (21.226) compare with DNA marker, 19) years old female with nasal polyp had one band(21.226) compare with DNA marker, (23) years old female with nasal polyp had one band(21.226) compare with DNA marker, (20) years old female with nasal polyp had one band(21.226) compare with DNA marker, (22) years old male with nasal polyp had one band(21.226) compare with DNA marker, (26) years old male with nasal polyp had one band(21.226) compare with DNA marker.

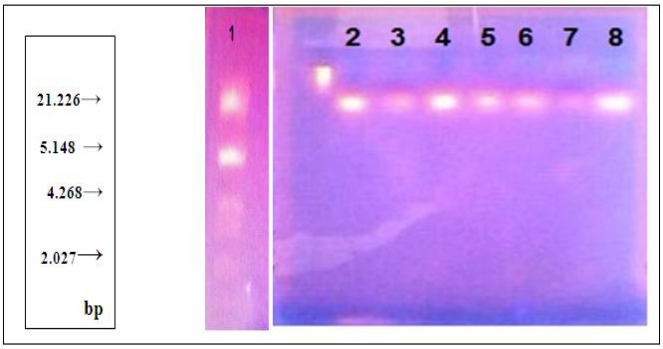

Figure (2): the pattern of Agarose gel electrophoresis of IL-10.

Lane- 1- DNA marker (Lambda DNA\EcoR+Hind 111)
Lane-2- (22) years old female with nasal polyp.
Lane-3- (21) years old male with nasal polyp.
Lane-4- (19) years old female with nasal polyp.
Lane-5- (23) years old female with nasal polyp.
Lane-6- (20) years old female with nasal polyp.
Lane-7- (22) years old male with nasal polyp.
Lane-8- (26) years old male with nasal polyp.

IV. Discussion

Patients of this study consider as non-allergic, non-asthmatic depends on medical history. Results of our study showing significant increase of IL-10 expression in nasal polyps compared with control group.

Our results agree with **[**Myoung SJ et al.,1999**;**Perić A et al.,2010a**]**, whom found IL-10 was highly expressed in non-allergic nasal polyps than in the control group.

Agreement also achieved with another Studies demonstrated that IL-10 was elevated in nasal polyp without mention the type of nasal polyps which used in these studies **[**Jager A & Kuchroo VK.,2010**;** Shen Y et al.,2011**]**.

Explanation for these results was appear in Figueiredo CR et al study, the development of polyps appears to be the end result of chronic inflammation; however, the cytokine profile of nasal polyps appears to be independent of allergic status in most studies [Figueiredo CR et al.,2007].But ,instead depends on inflammatory status which in sequence lead to increase anti inflammatory cytokines like IL-10 as immune reaction.

Disagreement was found with studies that found higher levels of IL-10 in asthmatic NP- patients than in nonasthmatic NP- patients **[**Bolard et al., 2001; Robinson DS et al.,1996; Kauffman HF& Der Heide SV.,2003; Perić A et al.,2010 b; Perić A et al.,2010c;Peric A et al.,2013; Rinia AB et al,2007.

On histopathologic examination, in asthmatic patients' polyps, epithelial layer was infiltrated by numerous eosinophils. in nonasthmatic patients' polyps, the eosinophil infiltration of the epithelium was limited[Chen YS et al.,2005]. Another study has shown that atopic nasal polyp patients have a higher level of eosinophilic inflammation than non-atopic patients with nasal polyps [Perić A et al.,2011].

The link between these two diseases(nasal polyps with asthma compared with nasal polyps without asthma) is further made reasonable by the observation that the nasal polyp eosinophilic inflammation is significantly higher in NP patients with concomitant asthma when compared with nonasthmatic NP patients (Rinia AB et al,2007) .

IL-10 is considered to inhibit the release of cytokine synthesis of monocytes, and leads to marked immunosuppression [Chen YS et al.,2005].

So IL-10 in asthmatic NP- patients will increase as a response to inflammatory and allergic state of asthmatic NP ,acting as anti inflammatory and anti allergic at the same time.

The level of IL-10 was found to be decreased in nasal polyps by using enzyme-linked immunosorbent assay (ELISA) in Chinese patients[Shi J et al.,2009].

Histologically, nasal polyps can be divided into 4 types: edematous (eosinophilic), fibrotic (non eosinophilic), glandular, and atypical [Kakoi H& Hiraide F.,1987; Davidsson A& Hellquist HB,.1993;Hellquist HB.,1996 ;Hellquist HB,.1997; Ferreira Couto LG et al.,2008].

The types of inflammatory cells that infiltrate nasal polyps differ in Asian and Western patients [Bachert C et al.,1997; Bernstein JM et al., 1997**]**. Bachert C et al, reported that eosinophilic nasal polyps were found in more than 80% of Western patients with NP[Bachert C et al.,1997], whereas Hao et al, reported that the incidence of fibrotic polyps (mainly accumulation of lymphocytes and neutrophils) was relatively higher (more than 40%) in Asian patients with NP**[**Hao J et al.,2006**]**.

Zhang et al ,reported a lower incidence of eosinophilic nasal polyps in Chinese patients than in European patients. Also found that noneosinophilic polyps were present in nearly 60% of Chinese patients with NP, while eosinophilic polyps were present in less than 40% of those patients .So, the pathogenic mechanisms of nasal polyps vary in Eastern and Western populations**[**Zhang N et al.,2006**]**.

Therefore, with less eosinophilic infiltration ,there will be less secretion of anti-inflammatory cytokine (IL-10) as explained above .

Depends on our results, a difference in the pathogenesis of nasal polyps between Chinese patients compared with Iraqi patients from histological and cytokine profile also suggested in this study**.** This could be due to different living environments and genetic conditions.

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