BioGecko Vol 12 Issue 01 2023

ISSN NO: 2230-5807

Immunological and Molecular study of Human Papilloma Virus in nasal polyps

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Abstract
This study was completed in laboratories of Biology Department in Faculty of Science. It explains Immunological and Molecular study of human papilloma virus in nasal polyps.Total of specimens88 (100%), Demographic characteristics for (60) patients attending to Al-Sadr Hospital and Al-Hakim General Hospital in AL-Najaf Governorate revealed that male were 42 (47.72)% and female were 18 (20.46)% and healthy group (28) that male were 16 (18.19)% and female were 12 (13.63)% , blood sample was taken from patients and control group , 5 ml was transferred into Gel tube for serum separation, for determination of IL-2 and IL-10, In the current study, the result suggested that the serum level concentration of IL-10 in nasal polyp patients (87.42 ± 32.97) pg/ml was significantly higher ($p \leq 0.05$) than healthy controls (28.57 ± 2.85) pg/ml , also, The result illustrated that serum level of IL-2 significantly decreased in nasal polyp patients (12.64 ± 35.77) pg/ml compare to healthy group (28.57 ± 2.85) pg/ml at ($p \leq 0.05$) . The results of RT-qPCR technique showed 18 /60 from tissue specimens were positive for the L1 gene.

Keywords: Molecular study, Human Papilloma Virus, Nasal polyps.

Introduction

Nasal polyps (NP) are noncancerous growths within the nose or sinuses, symptoms include trouble breathing through the nose, loss of smell, decreased taste, post nasal drip, and a runny nose , nasal congestion, sinusitis, loss of smell, thick nasal discharge, facial pressure, nasal speech, and mouth breathing , recurrent sinusitis can result from polyps , long-term, nasal polyps can cause destruction of the nasal bones and widening of the nose , the growths are sac-like, movable, and no tender, though face pain may occasionally occur , they typically occur in both nostrils in those who are affected , complications may include sinusitis and broadening of the nose (1,2). Human papillomaviruses (HPV) are small non-enveloped particles which infect skin and mucous membranes, double-stranded DNA, covered by a viral capsid composed of L1 and L2 proteins, based on such similarity, HPVs are divided into five genera (α, β, γ, μ, and ν), where α-papillomaviruses predominantly contain HPV types that infect epithelial mucosa , in addition, HPVs are classified into high-risk (HR) or low-risk (LR) types based on their oncogenic potential , approximately 20 high-risk types can be found in HPV-related cancers (3).

Materials and Methods

Samples collection and

Total of specimens 88 (100%) , demographic characteristics for (60) patients attending to Al-Sadr Hospital and Al-Hakim General Hospital in AL-Najaf Governoraterevealed that male were 42 (47.72)% and female were 18 (20.46)% and healthy group (28) that male were 16 (18.19)% and female were 12 (13.63)% , three ml of blood sample was taken from patients and control group , 5ml was transferred into Gel tube for serum separation, the blood left for about 30 minutes in room temperature for clotting and then centrifuged at 4000 rpm for 5 minutes, then the serum was collected in sterile appendrofe tube in two repeaters and kept frozen at -20 C˚for determination of IL-2 and IL-10,

Immunological study

The Enzyme-Linked Immunosorbent Assay (ELISA)

Human IL-10 and IL-2 are enzymes immunoassay for in vitro specific and quantitative determination of these cytokines in human sera, plasma and other body fluids. This test has been achieved according to the manufacturing company (Solarbio, China).

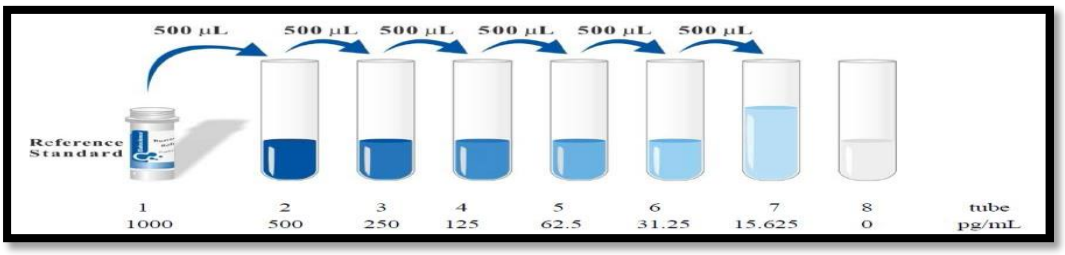

Figure 1: Standard curve for specimen diluents

Principle of the Test

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL- 17 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL- 10 and IL-2 present is captured by the coated antibody after incubation. Following extensive washing , a biotin - conjugate antibody specific for IL- 2 and IL-10 are added to detect the captured IL-10 and IL-2 protein in sample. For signal development , horseradish peroxidase (HRP) -conjugated Streptavidin is added, followed by Tetramethyl-benzidine (TMB) reagent . Following a wash to remove any unbound combination , and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm.

Standard Curve

For IL-10 and IL-2 antigens, the standard curve was made as illustrated in (Figure 2); they graphed by a plotting knew standard concentration against their correspondent optical densities, they were used to quantify the test samples for these entire assay.

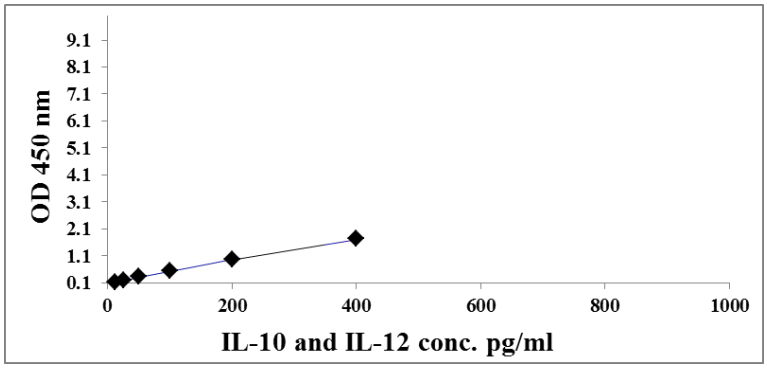

Figure 2: Standard Curve for IL-10 and IL-2

Genomic DNA Extraction

Genomic DNA from paraffin embedded block tissue samples were extracted by using G-spin TM Total DNA Extraction Kit (Fixed tissues protocol) and done according to company instructions.

Genomic DNA estimation

The extracted genomic DNA was by using Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm)

Real-Time PCR

Real-Time PCR techniquewas performed for detection Human papillomavirus from polyps biopsy tissue samples.

Real-Time PCR master mix preparation

PCR master mixwas prepared by using (RealMODTM Green SF 2X qPCR mix)and this master mix done according to companyinstructions as following Table (1).

PCR Master mix	Volume
DNA template (5-50ng)	5µL
HPV Forward primer (10pmol	1µL
HPV Reveres primer(10pmol)	1µL
HPV probe (10pmol)	2µL
GoTaq® qPCR Master MixProbe	10µL
Nuclease free water	1 µL
Total volume	20 µL

After that, these PCR master mix component that mentioned in table were transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in Real Time PCR Thermocycler (MiniOpticon . BioRad. USA).

qPCR Thermocycler Conditions

qPCR thermocycler conditions were done by using Real-Time PCR thermocycler system as following Table (2).

PCR step	Temp.	Time	repeat
Initial activation	95C	10min	1
Denaturation	95C	15sec.	40 cycle
Annealing and extension	60C	30sec	

Real-Time PCR Data analysis:

Real-Time data analysis was performed by analysis of threshold cycle number (CT value) that presented the positive amplification in Real-Time PCR cycle number.

Result and Discussion

Demographical distribution of nasal polyp patients and control

Demographic characteristics for (60) patients attending to Al-Sadr Hospital and Al-Hakim General Hospital in AL-Najaf Governorate revealed that male were 42 (47.72)% and female were 18 (20.46)% and

healthy group (28) that male were 16 (18.19)% and female were 12 (13.63)% the difference between male and female according to gender and age shown in Table (3).

Table 3: Distribution of samples according to gender and age

Variable	Group	Patient group	%	Healthy group	%
gender	**Male**	42	47.72	16	18.19
	Female	18	20.46	12	13.63
Age (years)	**10-19**	5	8.33	2	7.14
	20-29	8	13.33	9	32.14
	30-39	19	31.66	4	14.28
	40-49	14	23.33	5	17.85
	50-59	8	13.33	5	17.85
	60-69	4	6.66	2	7.14
	70-79	2	3.33	1	3.57

Nasal polyposis is a disease described since antiquity, many of its aetiopathogenic aspects are still to be discovered , According to data published in the many countries , it affects 2 - 5% of the general population and accounts for 5% of otolaryngology consultations , it is a disease that affects men more frequently than women by a ratio of 2-3:1, and often appears in the middle ages of life (4). In local study, (5) founded that the percent of males 54.7 % was more than females 23.4 % in Al-Najaf province also , (6) they found these masses were more commonly seen in males 47 (88.7%) than in females 6 (11.3) , age group (30-39) was the predominant group in this study, In other studies , (7) they showed the majority of non-neoplastic masses occurred in the age group 11–30 years , also (8) they reported ,the age of patients ranged from 12 to 78 years were in the age group of 21-40 years, followed by patients in the 41-60 age group .

Immunological study

Evaluation IL-10 levels in patients and healthy controls

In the current study, the result suggested that the serum level concentration of IL-10 in nasal polyp patients (87.42 ± 32.97) pg/ml was significantly higher ($p \leq 0.05$) than healthy controls (28.57 ± 2.85) pg/ml as shown in Figure (3).

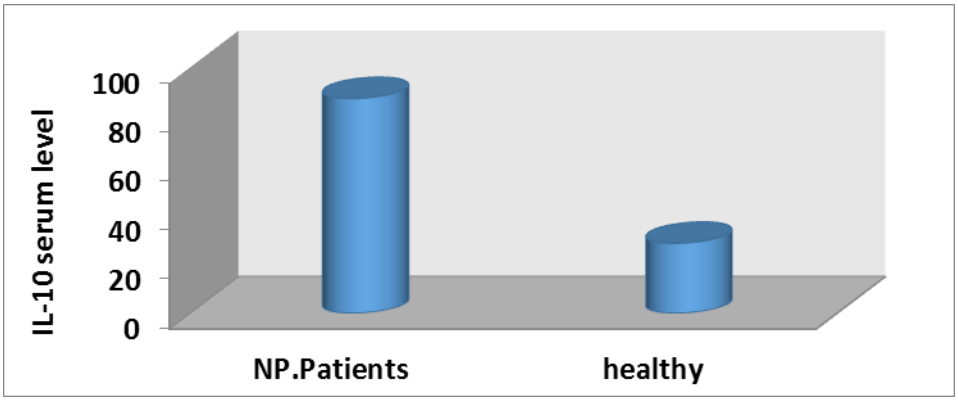

Figure 3: Serum level of IL-10 in healthy and nasal polyp patients

IL-10 is a potent anti-inflammatory cytokine that protects the host from excessive tissue damage during the host's defense against pathogens and has a pivotal role in the development and maintenance of immune tolerance and homeostasis (9). Many previous studies identical with this study (10) and (11) they found that IL-10 are highly expressed in patients withnasal polyp , while (12) they reported that the serum level concentration of IL-10 in nasal polyp patients was significantly lower than healthy groups .

Evaluation IL-2 levels in patients and healthy control

The result illustrated that serum level of IL-2 significantly decreased in nasal polyp patients (12.64 ± 35.77) pg/ml compare to healthy group (28.57 ± 2.85) pg/ml at ($p \leq 0.05$).

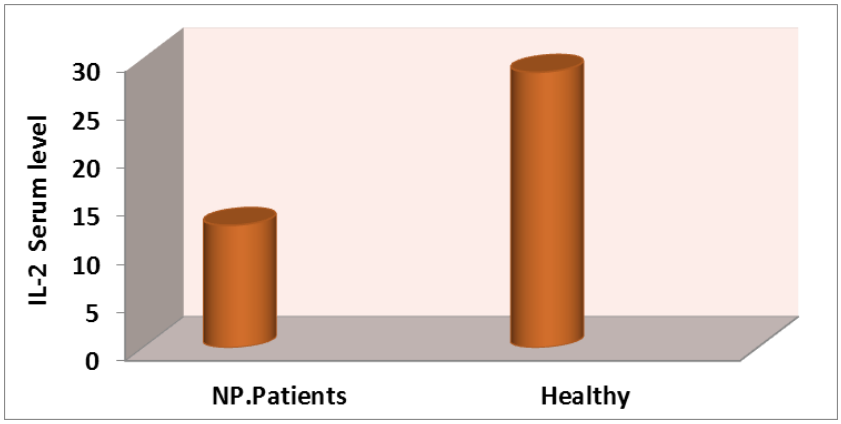

Figure 4: Serum level of IL-2 in healthy and nasal polyp patients

IL-2 plays a central role in T and B cells cooperation. It induces T-cell proliferation and secretion of IFN-γ and TNF-α , the levels of IL-2 measured by ELISA are decreased in nasal polyps compared to control tissue , there is a positive correlation between levels of IL-2 and proportion of Treg cells in nasal polyps, indicating that the decreased number of Treg cells in nasal polyps may result from the downregulation of IL-2 signaling pathway (13) .

Molecular study

Use RT-qPCR to estimate the expression of the for detection HPV in PFET sample

The results showed 18 /60 from tissue specimens were positive for the L1 gene by RT-qPCR technique figure (4-3). In Human papillomavirus (HPV), two late proteins are involved in capsid formation : a major (L1) and a minor (L2) protein , in the approximate proportion 95:5% , L1 forms a pentameric assembly unit of the viral shell in a manner that closely resembles VP1 from polyomaviruses. Intermolecular disulphide bonding holds the L1 capsid proteins together , L1 capsid proteins can bind via its nuclear localisation signal (NLS) to karyopherins Kapbeta (2) and Kapbeta (3) and inhibit the Kapbeta(2) and Kapbeta (3) nuclear import pathways during the productive phase of the viral life cycle , Surface loops on L1 pentamers contain sites of sequence variation between HPV types **(**14).

The current results reported the HPV is considered one of the causes of the nasal polyp simillarty with other studies , (5) she found 3/13 from tissue specimens of nasal polyp were positive for the L1 gene , also (15) they showed 27/60 from tissue specimens of nasal polyp were positive for the L1 gene . While other finding ,(16) they stated that, there are many differences among studies reporting the prevalence of HPV in sinonasal polyposis, including different techniques, tissue fixation methods, numbers of samples, and selection of controls that make comparisons challenging.

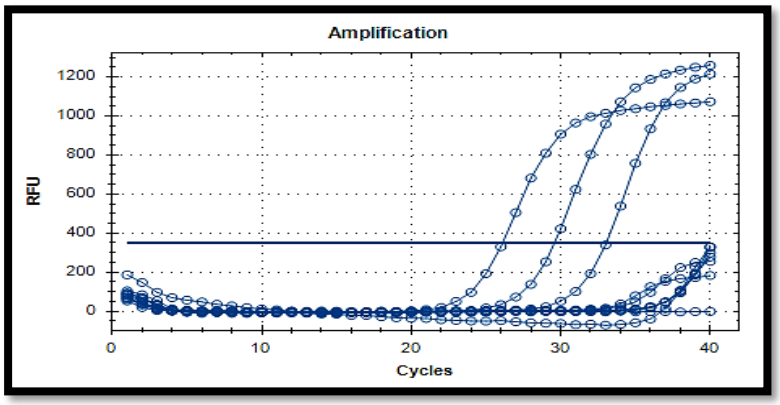

Figure 5: Real-Time PCR amplification plot of L1 gene for detection HPV in PFET sample.

Conclusions: The result illustrated that serum level of IL-2 significantly decreased in nasal polyp patients compare to healthy group at ($p \leq 0.05$). The results of RT-qPCR technique showed 18 /60 from tissue specimens were positive for the L1 gene.

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