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Research Article

Growth hormone receptor and histopathological study in patients with Tonsillitis

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ABSTRACT

The goal of the present study was to investigate the relationship between tonsillitis and growth factor in children by determining the gene expression of growth hormone receptor (GHR) in tonsils using real-time PCR (RT-PCR) as well as investigating the histopathological changes that are induced by tonsillitis. The study was done via taking samples from extirpated tonsils of 20 children which are divided according to the age into two groups, under and older than 10 years old. The samples-based mRNA was subjected to RT-PCR technique to target the GHR gene. The samples were further examined to explore the histopathological changes using tissue sectioning. The histopathological changes revealed necrosis, erosion, hyperplasia, aggregation of collagenous fibers, and the presence of lobulated tonsils. These results show evidence that the activity of the GHR gene in female patients, less than 10 years of old, is higher than that in male patients, while it is higher in male than female patients of older than 10 years of old.

Keywords: Tonsillitis; growth hormone receptor; gene expression; histopathology.

INTRODUCTION

Tonsils are tissue sets that are present on both sides of the throat, and their function is to develop immunity processes until the age of two years. Tonsils also remove bacteria and viruses that get into the respiratory airway. They form antibodies to help kill infections. Removing tonsils will prevent improvement person's ability to fight infections (Brooks and Waters, 2010). Tonsillitis means inflammation of tissue of the pharyngeal tonsils. The inflammation may invade other tissues that are located in the throat such as adenoid and lingual tissues. The symptoms include a sore throat, fever, tonsils become enlarged, difficulty swallowing, and lymph nodes become larger than normal size. The inflammation could also result in complications such as abscess formation (Klug et al., 2016). In untreated pediatric patients, consequences of disordered breathing such as snoring, sleep disorders, and imbalanced mood and inattentive could be developed.

The growth hormone (GH) is secreted at night during sleep time. Decreased levels of secreted GH may lead to reduce food intake and thus slow down growth rate (Flanagan et al., 1999; Richards and Ferdman, 2000; Van Den Akker et al., 2003). GH is a protein-based hormone that is released from lobes of the pituitary gland (Forsyth and Wallis, 2002; Hall and Guyton, 2011). It is also responsible for stimulating of growth, and it plays a great role in the immune system (Clark, 1997). GH works by binding to a specific target, the high affinity of cell surface protein, and that is the growth hormone receptor (GHR) (Kalme et al., 2003). It also directs several types of tissues such as bones, liver, and tonsils to perform several functions like elongation, improvement of digestion, and immune-response promotion respectively (Ji et al., 1999). The GHR is a cytokine-hemoprotein-based receptor (Moody et al., 1995; Zhou and Zhang, 2005). Once the GH binds to its receptor, processes of signaling cascades are activated leading to subsequent transcription of certain genes in that particular cell (Frank, 2001; Herrington and Carter-Su, 2001; Jiang and Lucy, 2001).There is a strong relationship between tonsillitis and decreasing growth that has been seen for many years in children. The clinicians recorded this problem and dramatic transformation that sometimes occurred after tonsillectomy (Bate et al., 1984; Zargari and Elpern, 2009). Some studies suggest links with upper airway obstruction, suppression of appetite, and long-term oropharyngeal infection may also play a great role for decreasing growth in connection to GH action in developing immune system (Goldstein et al., 1987; Schiffmann et al., 1985; Urquhart and Starritt, 2013; Yada, 2007) . Also, others studies have revealed connections between the immune and the neuroendocrine system (Koo et al., 2001; Richards and Ferdman, 2000; Van Den Akker et al., 2003). No doubt that the GH can influence immune function because it affects several immune system components such as thymus,

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spleen, tonsil, lymph nodes, thymoma, lymphomas, and normal T and B lymphocytes. It also influences liver, muscles, adipose tissue, the regulation of metabolism, post-natal growth, immunity, cardiac and renal systems, and gut functions (Brooks and Waters, 2010; Crocker and Murray, 2003; Hattori et al., 2001; Leniček et al., 2013).

Gene expression is the process in which gene activity is seen via the ability of that particular gene to code for a product in a cell such as a specific protein. GHR-gene expression is also regulated by factors such as nutrition, growth hormone, other steroid hormones, and some diseases such as diabetes mellitus (Brueckner et al., 2009; Pérez-Ibave et al., 2014; Ranabir and Reetu, 2011; Schwartzbauer and Menon, 1998). To the best of our knowledge, this is the first tonsil-sample-based study in world and Iraq that aimed to investigate the effects of tonsillitis on GHR-gene expression and to explore any variations in this expression in males and females of under and older than 10 years of old.

MATERIALS AND METHODS

Ethics and patient's specimens

The samples were taken only from extirpated tonsils after tonsillectomy. Extirpated-tonsil samples were collected from patients with tonsillitis after tonsillectomy, 9 children under 10 years of old and 9 children older than 10 years of old, from Al-Diwaniyah Teaching Hospital, Al-Diwaniyah, Iraq and were placed in sterile-collection tubes preloaded with diethyl pyrocarbonate (DEPC). Then, the samples were transported to a laboratory and stored in a deep freezer.

Total RNA extraction

Total RNA was extracted from tonsil tissue using Accuzol® reagent kit (Bioneer, South Korea) and was done according to the company instructions as follow; 100mg of tonsil tissue was placed in a sterile 1.5ml Eppendorf tube, and 1 ml of Accuzol reagent was added. Then, the tissues were homogenized using a micropestle. After that, the tubes were shaken vigorously for a minute. Later, 200µl chloroform was added to each tube and shaken vigorously for 15 seconds. Then, a 5min-incubation period in ice was performed that was followed by a 4̊C-centrifugation process at 12000rpm for 15 minutes. The supernatant was transferred into a new Eppendorf tube, and 500µl isopropanol was added. Then, the contents were well-mixed via inverting the tube 4-5 times. An incubated period at 4˚C for 10 minutes was followed. Then, a 4̊C-centrifugation process at 12000 rpm for 10 minutes was done. After the supernatant was discarded, 1ml of 80% ethanol was added followed by vortexing. Then, a 4̊C-centrifugation process at 12000 rpm for 5 minutes was completed. The RNA pellet was exposed to an air-dry process after the supernatant was discarded. Finally, a dissolving process of this pellet was performed by adding 50µl of DEPC to be later stored at -20˚C. A NanoDrop spectrophotometer (THERMO, USA) was used to evaluate the resulted RNA for quality and quantity purposes. DNase I enzyme was applied to the extracted RNA using DNase I enzyme kit® (Promega Company, USA) and following the manufacturer's protocol.

Synthesis of cDNA

AccuPower® RocktScript RT PreMix Kit (Bioneer Company, South Korea) was used to convert RNA into cDNA and following the manufacturer's protocol. Briefly, mastermix containing 10µl of 100ng/µl RNA, 1µl of 10pmol random hexamer primer, and 9µl DEPC water was prepared. Then, the mix was placed in specific tubes provided with the kit that contained lyophilized reverse transcriptase enzyme and was followed by short periods of vortexing and spinning down. A thermocycler was utilized to perform the reaction process using conditions of 50˚C for 1 hr of cDNA synthesis (RT step) and 95˚C for 5 min of heat deactivation.

Real-time PCR

RT-PCR was performed to quantify the mRNA transcript levels of GHR. A relative gene expression analysis was carried out using $2^{-\Delta\Delta CT}$ Livak Method (1). The RT-PCR reaction was done using a Real-Time PCR system (BioRad, USA), and SYBER Green dye qPCR master mix was utilized to detect the amplification of the target gene and β-actin, a housekeeping gene for normalizing the results. Primers were designed using the primer3 plus website, and they are F: TTTAGTGCGTGCAGATGGTG and R: GCAAAACCAAGTTGGGTGTG for the GHR gene and F: TCGTGCGTGACATTAAGGAG and R: TTGCCAATGGTGATGACCTG for the β-actin gene. The product sizes are 77bp for the GHR gene and 133bp for the β-actin gene. These primers are found in the NCBI Websites under the numbers, NM_001242406.2 and NM_001101.3 respectively. The RT-PCR master mix was prepared for GHR-target gene and the β-actin gene according to AccuPowerTM 2XGreen Star qPCR master mix kit® (Bioneer Company, South Korea). The mastermix contained 5µl of 10ng cDNA, 2µL of 10pmol from each primer, 25µL of 2X green star master mix, and 16µl of DEPC water. Then, the mix was placed in RT-PCR strips. After that, the strips were briefly vortexed and centrifuged for 3 minutes. Then, the strips were placed in Miniopticon Real-Time PCR System (BioRad, USA) under the following thermocycler conditions of 1 cycle of initial denaturation at 50˚C for 1min, 40 cycles of denaturation and annealing/extension detection (scan) at 95˚C for 20s and 60˚C for 30s respectively, and 1 cycle of melting at 60-95˚C for 0.5s.

RESULTS

The results showed expressing high levels of GHR gene in the tonsillitis-patient children of under 10 years of old especially in females more than that in males. The value of fold changes in those children was 10.546 for females and 6.150 for males as shown in (table 1) and (figure 1).

Table 1: The relative gene expression analysis of growth hormone receptor (GHR) gene in tonsillitis-patient children of under 10 years of old by the 2-ΔΔCT Livak Method.

Group	CT		ΔCT	Fold change expression	Fold change Mean±SE
	(GHR)	(B-actin)			
Males	30.54	33.31	2.78	6.84	6.150±0.976
	31.12	33.47	2.35	5.10	
	30.45	33.54	3.10	8.54	
	31.30	33.34	2.04	4.11	
Females	29.73	33.15	3.42	10.68	10.546±0.438
	29.93	33.53	3.60	12.12	
	29.33	32.61	3.28	9.73	
	30.43	33.71	3.28	9.72	
	29.79	33.18	3.39	10.48	

Fold change is a measure describing how much a quantity changes going from an initial to a final value.

Table 2: The relative gene expression analysis of growth hormone receptor (GHR) gene of the tonsillitis-patient children of older than 10 years of old by the 2-ΔΔCT Livak Method

Group	CT		ΔCT	Fold change expression	Fold change Mean±SE
	(GHR)	(B-actin)			
Males	31.25	33.39	2.14	4.41	4.081±0.521
	31.14	33.21	2.08	4.22	
	32.24	33.63	1.39	2.62	
	31.17	33.51	2.35	5.08	
Females	32.18	33.41	1.24	2.35	2.129±0.153
	32.59	33.55	0.95	1.94	
	32.32	33.59	1.27	2.41	
	32.54	33.23	0.69	1.61	
	32.23	33.45	1.22	2.33	

While the results of the expression levels in the tonsillitis-patient children of older than 10 years of old revealed little expression when compared with the younger ages with high expression in the males than female. Fold change values were 4.081 for males and 2.129 for females as shown in (table 2) and (figure 2). Histopathologically, figure 3, A, and B, includes all the changes that occurred in the tissues of the sampled tonsils such as necrosis, erosion, and hyperplasia. While figure 4 shows several changes in this tissue such as aggregation of collagenous fibers and the presence of lobulated tonsils.

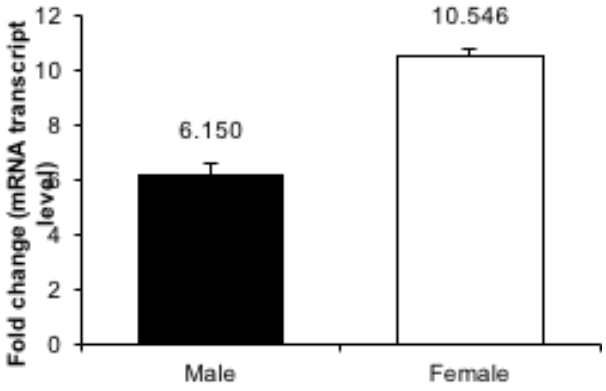

Figure 1: Growth hormone receptor (GHR)-mRNA expression in tonsillitis-patient children of less than 10 years of old. Bars display the mean and the standard error (Mean±SE). Significant values are at p<0.05.

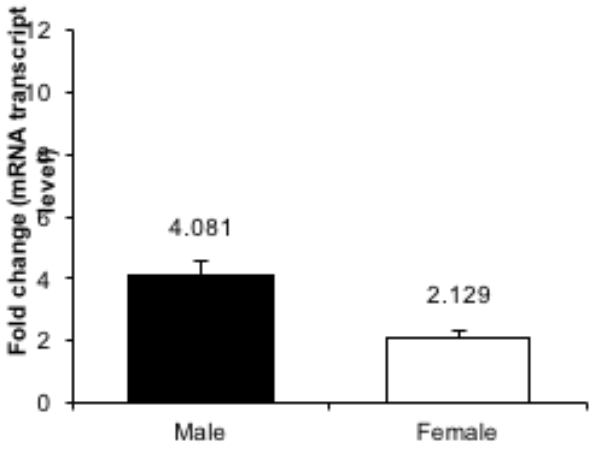

Figure 2: Growth hormone receptor (GHR)-mRNA expression in tonsillitis-patient children of older than 10 years of old. Bars display the mean and the standard error (Mean±SE). Significant values are at p<0.05

DISCUSSION

Tonsillitis is a serious health problem that applies challenges to the health of the children. Rapid detection of the inflammation etiology can prevent the occurrence of complications such as growth failure, rheumatic fever, and dyspnea (Egeli and Inalkaç, 1997). The failure of growth in children is a risky issue that is caused by several factors such as malnutrition, indigestion, malabsorption, and decrease or absent of GH and/or GHR that

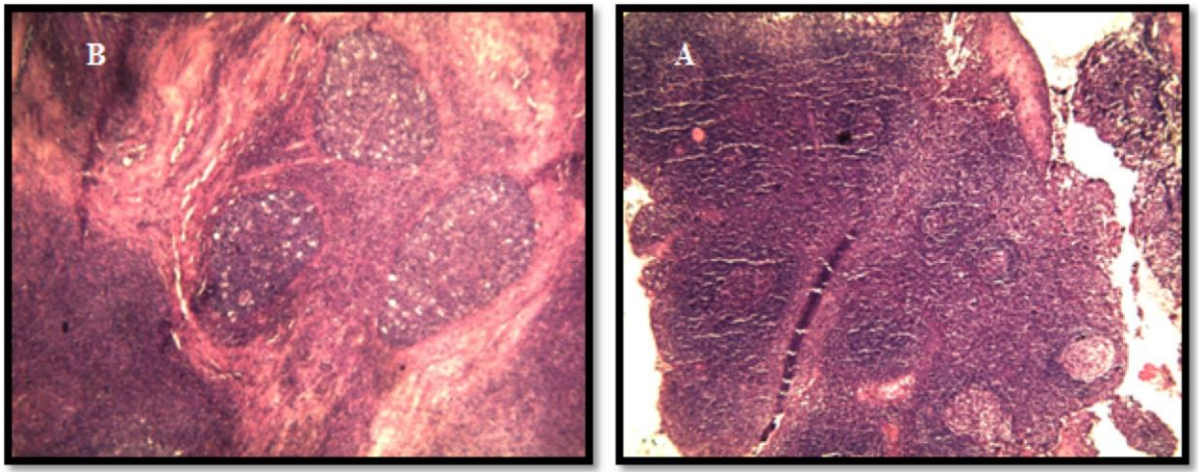

Figure 3: The Histopathological sections of tonsils. A. necrosis, erosion, hyperplasia and lymphoid follicles. B. There is some hyperplasia with the extreme clearness of germinal center. 200X (H&E stain).

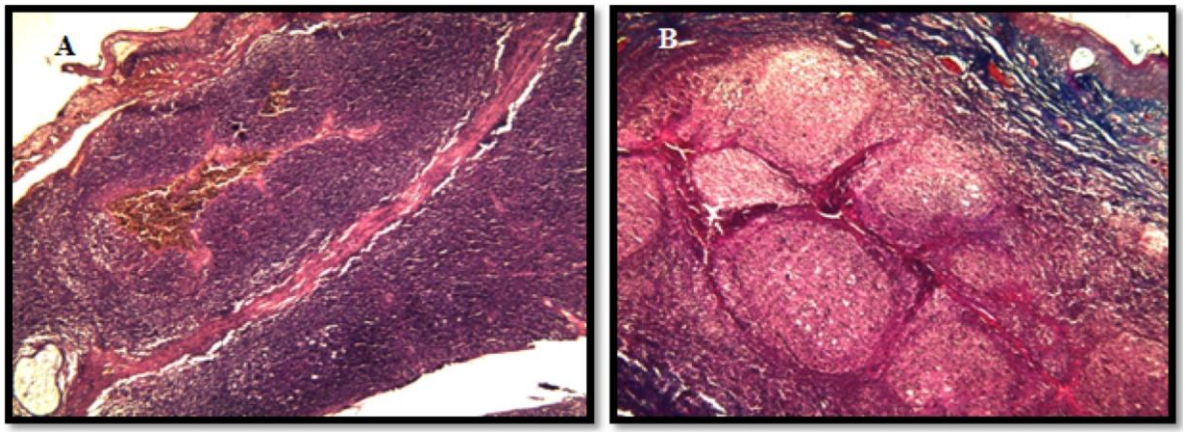

Figure 4: The Histopathological section of tonsils. A. Excessive collagenous fibers with conjunctive stroma. B. There are strong fascicles with clear division tendency into lobule formation. 200X (H&E stain).

could be affected in some diseases such as tonsillitis (Aydogan et al., 2007). To the best of our knowledge, this is the first tonsil-sample-based study in world and Iraq that aimed to investigate the effects of tonsillitis on GHR-gene expression and to explore any variations in this expression in males and females of under and older than 10 years of old. In the case of children of less than 10 years of old, females showed higher expression of the GHR gene than that in males, and this agrees with (Aydogan et al., 2007; Egeli and Inalkaç, 1997). Levels of transcribed GHR gene are affected by psychological stress, and GH activity is related to body energy and body weight. However, GH needs GHR because GH is not a steroid hormone, and its main function is related to the signaling that is generated at the GHR point. Therefore, deficient in the GHR could affect the GH function to reduce GHR-based signaling. It may also show a decrease in the pituitary functions that cause a high risk of metabolic syndrome via insufficiency in the GH/GHR-based functions (Yang, 2016).

In the case of children of older than 10 years of old, males showed higher expression of the GHR gene than that in females, and this agrees with. The current study results agree with (Cohen et al., 2008; Fourage et al., 2013; Nachalon et al., 2014; Yang, 2016) who found decreases in the growth of females more than that in males by measuring the heights of those children. Moreover, Nachalon et al., (2014) incorporated infants and toddlers in their study to evaluate them before and after tonsillectomy for height, weight, circulating highly sensitive C reactive protein and found that surgery not only decreased inflammation but also stimulated growth in those patients (Richard, 2001). In addition, (Fourage et al., 2013; Koycu et al., 2016; Vontetsianos et al., 2005) found significantly low levels of GHR-gene mRNA in different tissues in obesity which resulted in decreased GHR availability. However, Aydogan et al., (2007) revealed different results when measured height and weight to compare between groups that suffered from recurrent tonsillitis. They found that recurrent tonsillitis had no effect on delaying growth in children.

The current study results of the histopathological changes in tonsillitis showed necrosis, hyperplasia; enlargement of the lobes, and there is pus mixed with debris cells and collagenous fibers. These results agree with (Arora et al., 2008; Mogoantă et al., 2008) who found hyperplasia and hypertrophy of the lymphoid fol-

licles in the tonsils. In the current study, microhemorrhages and hematic extravasations were also noticed inside the follicles, and collagenous-fiber enrichment was shown in the conjunctive stroma. Previously, chronic tonsillitis, follicular tonsillitis, chronic suppurative tonsillitis, lymphoid hyperplasia, and lymphoma were observed (Al-malaak, 2014). Collagenous fibers and hypertrophied-germinative clear-center in the tonsilar follicle were seen. Moreover, Tonsillar crypt had dead-cell remains and lymphocytes in the lumen (Dakhil, 2017). These literature agree with the current study results. Hypertrophy of tonsil and adenoid may be caused by recurrent tonsillitis and upper airway obstruction in children. A reduced dietary intake and failure to gain weight are frequently reported by parents of children with a history of recurrent acute tonsillitis and adenotonsillar hypertrophy (Adoga et al., 2011).

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