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The Relationship Between TLR 2 and 4 with Microbiota of Mouth and Nose in Hypersensitivity Type 1 Iraqi Patients

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Abstract

Hundreds of commensal bacteria are existed in the mouth and nose, and the interactions between these microbiotas and the Toll-like receptors (TLRs) in different parts of the upper respiratory tract, gastrointestinal tract, and immune cells may assist to maintain the homeostasis of the immune system. Thus, it is important to study the relationship between type one hypersensitivity and normal flora in the mouth and nose. Blood and saliva or sputum samples of seventy-one allergic patients were collected randomly in Baghdad/ Al- Zahraa center for asthma and allergies. Those patients were suffering from different types of hypersensitivity type1 such as skin and respiratory tract allergy (e.g, asthma and Rhinitis). The results revealed that the allergic females percentage were more than males. *Staphylococcus* spp., *Lactobacillus* and Fungi spp. isolated from the mouth and nose were more prevalent than other microorganisms among different age groups. In addition, most age groups were given significant variation in TLR2 level, while TLR4 recorded variation in female more than male patients.

Keywords: TLR2, TLR4, Hypersensitivity and normal flora.

التحقق من العلاقة بين TLR 2 و 4 ونوع البكتيريا في الفم والأنف لدى حالات من العراقيين المصابين بفرط الحساسية النوع الأول

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الخلاصة

توجد المئات من البكتيريا المتعايشة في الفم والأنف ، والتداخلات بين هذه الميكروبات ومستقبلات Toll-like (TLRs) على أجزاء مختلفة من الجهاز التنفسي العلوي والجهاز الهضمي والخلايا المناعية ممكن ان تساعد في الحفاظ على التوازن في الجهاز المناعي. لذا من المهم دراسة العلاقة بين فرط الحساسية النوع الأول والنبات الطبيعي في الفم والأنف. لذلك تم جمع واحد وسبعون حالة من فرط الحساسية (عينات الدم، اللعاب أو البلغم) بشكل عشوائي في مركز الزهراء / بغداد لعلاج الربو والحساسية ، الذين كانوا يعانون من أنواع مختلفة من فرط الحساسية النوع الأول مثل حساسية الجلد والجهاز التنفسي (مثل الربو والتهاب الأنف والجهاز التنفسي). وكشفت النتائج أن نسبة الحساسية لدى الإناث كانت أكثر من الذكور. وأن المكورات العنقودية الذهبية ، عصيات الحليب والفطريات المعزولة من الفم والأنف كانت أكثر انتشارا من الكائنات الحية الدقيقة الأخرى بين الفئات العمرية المختلفة. وكان مستوى TLR2 متباين في معظم الفئات العمرية ، بينما سجل TLR4 تباين واضح في حالات الإناث منها في الذكور.

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Introduction

Microbes are all over the place. They crowd the air, the water, the soil, and have even changed intimate relationships with plants and animals. Starved of microbes, life on earth would cease. This is due mainly to the needed roles microbes play in the systems that provision life on earth, such as nutrient cycling and photosynthesis. Normal flora can be originated in many sites of the human body including the skin and mucous membrane [1]. Hypersensitivity type 1 (Immediate type) is an allergic reaction triggered by re-exposure to an exact type of antigen denoted to as an allergen [2]. In type 1 hypersensitivity, B-cells are stimulated to produce IgE antibodies specific to an antigen, that made a variance between a normal infective immune response and type 1 hypersensitivity. Mast cells and blood basophils play important role in this type of hypersensitivity [2]. Later exposure to the similar allergen cross-links the bound IgE on sensitized cells, resulting in anaphylactic degranulation, which is the fast and explosive release of pharmacologically active pre-formed mediators from storing granules and simultaneous synthesis of inflammatory lipid mediators from arachidonic acid [3]. This release of pre-formed mediators represented by histamine allows not only rapid anaphylactic reactions and allergic responses but also initiates recruitment of leukocytes to sites of pathogen attack, stimulation of innate immune processes, and inflammatory responses [4].

Trillions of commensal bacteria (microbiota) exist in the gastrointestinal tract from mouth to anus, and the interactions between gut microbiota and the toll-like receptors (TLRs) on different site of epithelial cells and immune cells assist to maintain the homeostasis of the immune system [5].

In healthy human gut, a constant homeostasis is kept by the perfect regulation of microbial load and the immune response made against it. Failure of this balance may effect on various pathological conditions [5, 6]. The role of TLR2 in epithelial cells is to preserve tolerance to ubiquitous commensal lipoproteins. Whereas TLR4 is involved in both defense against pathogens and preserving tolerance to commensal bacteria. Nonstop recognition of selective commensals by TLR4 under steady-state conditions is essential in mucosal defense against exogenous injury [7]. This family of receptors recognizes a wide range of microbial agents with different TLR signaling the existence of different microbial components [8].

Latest researches are suggested that numerous mutually useful interactions occur between a human and their microbiome, together with those that are essential for good health. These discoveries reveal that the micro biomes of the respiratory tract and gut contribute to the pathogenesis of asthma and allergy [9]. Thus, this study was aimed to investigate the relationship between mouth and nose normal flora and TLR2, TLR4 as well as their roles in inducing hypersensitivity type 1.

Material and Methods:

Samples collection: Seventy-one patients of hypersensitivity type 1 and 64 normal subjects, included 40 female and 24 male, acts as control were collected randomly. Blood and saliva or sputum, as well as nose swabs samples, were collected from outpatients of Al- Zahraa center /Baghdad for asthma and allergies, suffering from different types of hypersensitivity type1 represented in the skin (Eczema, pruritus and /or respiratory tract(asthma, Rhinitis..etc.). This study was carried out during the period of February/2018 to May/2018. All the samples were grouped according to age into (10-30), (31-50) and (51-71) years.

Isolation of bacterial isolates: Each sample of the saliva, sputum and nasal swab was cultured immediately on blood agar, chocolate agar, and MacConkey agar plates, all these media are supplemented by Himedia/India.

Identification of bacterial isolates: In order to verify the bacteria, coexist of the bacterial characterization method, several examinations were carried out. Bacterial phenotype was determined using diagnostic microbiology guide [10]. Moreover, other suspected bacterial isolates were re-characterized using VITEK 2bioMérieux/ France.

Determination of the TLR 2 and 4 concentrations: According to the company instruction, TLR 2 and 4 kits / MyBioSource/USA were used to measure the concentration of both receptors in the collected samples of blood.

Statistical analysis: The Statistical Analysis System- SAS (2012) program was used to investigate the effect of different factors in study parameters. T-Test or Least Significant Difference-LSD was used to compare between means in this study.

Result and discussion

Samples (saliva, sputum, blood) were randomly collected from patients suffering from skin or respiratory tract hypersensitivity. The result showed that female patients were significantly more than male patients ($P < 0.001$), Table-1, with a percentage of 70.4 % for female and 29.5% for male. This result was agreed with that reviewed by Chen et al., [11] who reported that this variation may due to hormonal differences between the sex. The experiment on rodents estrogens revealed that the effect was on mast cell activation and allergic sensitization, while progesterone is shown to suppress histamine release however, potentiate IgE was induced. Dehydroepiandrosterone may antagonize the production of Th2 cytokines but the effect of testosterone and the other androgens remains less defined [12].

Table 1-the percentage of patients according to gender

Total No. (%)	Male (%) No.	Female (%) No.
71	(29.5 %) 21	(70.5 %) 50
Chi- square	11.637 **	

** ($P < 0.01$).

In order to fix this problem ; the patients and control subjects were grouped according to the age, the results in Table-2 revealed that all groups were showed significant variation ($P \leq 0.01$) between female and male patients in a percentage 76.7 % , 57.1% and 84.6% for female groups respectively. Whereas, the male recorded 23.3% ,42.9% and 15.4 % respectively. These results agreed with Yao *et al.*, [13], who reported an increase of hypersensitivity with increasing age due to total immunoglobulin E levels increased with age until 14-15 years, and declined thereafter. In addition, elevated of the Body Mass Index (BMI) was associated with greater prevalence of wheezing and eczema. Laboratory studies further suggested a dynamic change of the gender-specific distribution in atopic diseases. The overall sensitization rate and sensitization to mites, grass and tree pollens were significantly higher in the female gender, especially after 8 years of age[13]. In another study, specific IgE antibodies to aero-allergens and food were significantly higher in women than men above the age of 21 years [14, 15].

The elevation of hypersensitivity in elderly group (Table-2), was confirmed with Victoria and her coworkers [16] who elucidated that, the mechanisms of allergic diseases in the elderly may due to immunosenescence and specific organ changes. While *Untersmayr et al.*, [12] explained that the increase of hypersensitivity in female than male may due to a frequently, hypersensitivity reactions affect more than one sex hormone such as estrogen and progesterone. Since the menstrual cycle dependent symptoms range from skin afflictions, gynecological problems to non-specific reactions, different pathophysiological mechanisms seem possible.

Table 2-The number and percentage of patient's gender according to the age group

Age group (year)	Total No. (%)	Male No. (%)	Female No. (%)	Chi- square
10-30 Year	30	7 (23.3%)	23 (76.7%)	12.62 **
31-50 year	28	12 (42.9%)	16 (57.1%)	6.02 **
51-71Year	13	2 (15.4%)	11 (84.6%)	13.49 **

** ($P < 0.01$).

The oral and nasal cavity is a dynamic ecosystem that varies over time in ways that influence spatial patterns of microbial community assembly. Table-(3A) illustrated the results of 10 – 30 age group, which showed a significant variation in most of the normal flora between male and female, especially *Lactobacillus spp.* that showed highly significant variation ($P < 0.01$), followed by *S. epidermidis* , *Streptococcus spp.* and *S. aureus* with a percentage in female 88.2%,83.3%,80% and 72.2% respectively than male percentages and compared with control Table-(3 B). In spite of the bacterial isolates that were found in patients represented as mouth and nose normal flora, these isolates in addition to fungi may be formed as a good antigen especially in case of anaphylaxis which is act as inflammation with its mediators. This result was explained by Kniker who mentioned that the clinical presentation of anaphylaxis can also be produced by intravascular antigen-antibody reactions that activate the complement system. In this case, the antibodies may be of the IgG or IgM class. Peptides that are split from activated complement components act on mast cells and basophils to induce the release of the same mediators [17].

Furthermore, the group of age 31 – 50 results revealed a significant variation ($P \leq 0.01$) for *S. epidermidis* between male and female, with a percentage in female 68.8% than male 31.2%, compared with control Tables-(4 A and B). Nonetheless, the male in this group showed significant variation ($P \leq 0.01$) for *S. saprophyticus* and fungi with a percentage of 100 and 62.5% respectively compared with female. In this group *Lactobacillus* spp. showed significant variation ($P \leq 0.05$) in female 55.6% compared with male 44.4%. This variation may due to the mouth surfaces are varied with respect to their proximity to the nearest salivary gland, a major source of environmental disturbance in the mouth. The minor salivary glands form a dense and expansive network that punctuates the labial, palatal, and buccal mucosa, releasing viscous, highly proteinaceous secretions with poor buffering capacity [18]. These secretions bathe the surfaces from which they emanate, as well as opposing surfaces, creating heterogeneity that likely explains, in concert with other factors [18, 19]. The three major salivary glands are differing in their secretory rates and salivary composition, giving rise to gradients in salivary film velocity, oral clearance, and intra-plaque pH across the teeth. Moreover, the salivary glands also give rise to spatial variation in patterns of wetness and dryness across different geographic regions of the mucosa, suggesting that microbial communities inhabiting soft tissues may vary along with moisture or pH gradient [20] Therefore, the types of individual microbiota are differed from person to person depending on several parameters such as the type of nutrition, general health condition, mouth health care and type of mouth hygiene (acidic or alkaline). All these parameters play an important role in the determination of bacterium inhabitation.

Table-5 A revealed that, the age group 51 - 71 showed the presence of *Streptococcus* spp., *S. saprophyticus*, *Lactobacillus* spp. and fungi. All these microorganisms were recorded high significant variation ($P < 0.01$) between female and male with a 100% percentage in female. Moreover, *S. aureus*, *S. epidermidis* were 85.7% and 83.3% respectively, compared with the male and control Table-(5 B). Based on results, an important implication can be established: all age groups of control have fungi with mouth and nose microbiota. Several studies elucidated the mechanisms governing community state type transitions in nasal and mouth communities and now being defined. One mechanism is the elaboration of proteins or small molecules by one organism to antagonize or induce the growth of another [21]. This observation may define the role of fungi and *Lactobacillus* spp. in counteracting another microbiota growth. On the other hand, *S. epidermidis* recorded prevalence in the all age groups of allergic patients. This result was in disagreement with Diana et al., [22] who reported that *S. epidermidis* is represented as less common microorganism in mouth and nose. Meanwhile, the results of all allergic age groups showed a relationship between *Staphylococcus* spp. and *Lactobacillus* spp. presence, this result agreed with [23] who mentioned that the ability of some microbiota to secrete molecules may encourage others to grow.

Table 3 A-The number and percentage of normal flora for (10 – 30) age group patients

Bacteria	Total No.	Number and percentage for female	Number and percentage for male	Chi square
<i>Streptococcus SPP.</i>	10	8(80%)	2(20%)	13.25 **
<i>S. aureus</i>	18	13(72.2%)	5(27.8%)	12.28 **
<i>S. epidermidis</i>	12	10(83.3%)	2(16.7%)	13.42 **
<i>S. saprophyticus</i>	0	0 (0.00%)	0 (0.00%)	0.00 NS
Fungi	11	8(72.7%)	3(27.7%)	12.28 **
<i>Lactobacillus SPP.</i>	17	15(88.2%)	2(11.8%)	13.92 **

**($P < 0.01$).

Table 3 B- The number and percentage of normal flora for 10 – 30 age group control

Bacteria	Total No.	Number and percentage for male	Number and percentage for female	Chi-Square
<i>Streptococcus SPP</i>	2	1(50%)	1(50%)	0.00 NS
<i>S. aureus</i>	4	2(50%)	2 (50%)	0.00 NS
<i>S. epidermidis</i>	5	3(60%)	2(40%)	7.25 **

<i>S. saprophyticus</i>	0	0 (0.00%)	0 (0.00%)	0.00 NS
Fungi	5	2(40%)	3(60%)	7.25 **
<i>Lactobacillus SPP.</i>	4	2(50%)	2 (50%)	0.00 NS

** (P<0.01).

Table 4 A-The number and percentage of normal flora in 31 – 50 age group

Bacteria	Total No.	Number and percentage for female	Number and percentage for male	Chi- square
<i>Streptococcus SPP</i>	8	4(50%)	4(50%)	0.00 NS
<i>S. aureus</i>	13	6(46.2%)	7(53.8%)	2.51 NS
<i>S. epidermidis</i>	16	11(68.8%)	5(31.2%)	9.96 **
<i>S. saprophyticus</i>	1	0(0%)	1(100%)	15.00 **
Fungi	8	3(37.5%)	5(62.5%)	9.26 **
<i>Lactobacillus SPP</i>	18	10(55.6%)	8(44.4%)	4.53 *

* (P<0.05), ** (P<0.01).

Table 4 B-The number and percentage of normal flora for (31 – 50) age group of control.

Bacteria	Total No.	Number and percentage for male	Number and percentage for female	Chi-Square
<i>Streptococcus SPP.</i>	2	1(50%)	1(50%)	0.00 NS
<i>S. aureus</i>	3	1(33.3%)	2(66.7%)	9.31 **
<i>S. epidermidis</i>	2	1(50%)	1(50%)	0.00 NS
<i>S. saprophyticus</i>	2	1(50%)	1(50%)	0.00 NS
Fungi	5	3(60%)	2(40%)	7.25 **
<i>Lactobacillus SPP.</i>	5	2(40%)	3(60%)	7.25 **

** (P<0.01).

Table 5 A-The number and percentage of normal flora of 51 - 71 age group patients

Bacteria	Total No.	Number and percentage for female	Number and percentage for male	Chi square
<i>Streptococcus SPP.</i>	4	4(100%)	0(0%)	15.00 **
<i>S. aureus</i>	7	6(85.7%)	1(14.3%)	13.56 **
<i>S. epidermidis</i>	4	5(83.3%)	1(16.7%)	13.44 **
<i>S. saprophyticus</i>	2	2(100%)	0(0%)	15.00 **
Fungi	5	5(100%)	0(0%)	15.00 **
<i>Lactobacillus SPP.</i>	6	6(100%)	0(0%)	15.00 **

** (P<0.01).

Table 5 B-The number and percentage of normal flora of 51 - 71 age group control

Bacteria	Total No.	Number and percentage for male	Number and percentage for female	Chi-Square
<i>Streptococcus SPP.</i>	2	1 (50.00%)	1 (50.00%)	0.00 NS
<i>S. aureus</i>	4	2 (50.00%)	2 (50.00%)	0.00 NS
<i>S. epidermidis</i>	6	3 (50.00%)	3 (50.00%)	0.00 NS
<i>S. saprophyticus</i>	0	0 (0.00%)	0 (0.00%)	0.00 NS
Fungi	5	3 (60.00%)	2 (40.00%)	7.25 **
<i>Lactobacillus spp.</i>	4	2 (50.00%)	2 (50.00%)	0.00 NS

** (P<0.01).

TLRs are expressed on antigen presenting cells, such as macrophages and dendritic cells (DCs). TLR engagement leads to nuclear factor (NF)- κ B and/or interferon regulatory factor (IRF)3/7-induced production of inflammatory mediators, which attempt to control infection, as well as anti-inflammatory molecules [24, 25].based on results presented in Table-(6A) , the male showed a

significant variation among different age groups for TLR2, with the bias toward the high percentage compared with control. Table-6B showed a normal percentage more than other variation. In addition, a significant variation has been recorded for the same receptor in the all age groups of a female with the bias toward high-level compared with the control that revealed toward normal values Table-(7A and 7B). Meanwhile, TLR4 results revealed no change and recorded significant result toward the normal level in a male group Table-(6A, 6B). Furthermore, in the females, the results showed a significant variation toward normal values. Allergic asthma is immune-mediated diseases. Pattern recognition receptors are proteins expressed by cells in the immune system to identify microbial pathogens and endogenous ligands. Toll-like receptors (TLRs) is a member of this family and could represent a molecular link between microbial infections and immune-mediated diseases. These findings were explained by Bjørnvold and coworkers [26] who mentioned that allergic asthma was significantly associated with the TLR2 rs3804100 T allele and further associated with the haplotype including this SNP, possibly representing a susceptibility locus common for this disease. Moreover, Oh et al., [27] demonstrated that, TLR2 polymorphisms have been associated with deficits in immune regulation such as allergic asthma, and atopic disease. This is consistent with an earlier observation that sublingual TLR2 agonist therapy concurrent with allergen exposure can abrogate airway hyper responsiveness in mice [28]. A multitude of studies demonstrate that TLR2 stimulation with systemic or mucosal administration of synthetic agonists can prevent antigen presenting cells from eliciting a T_H2-polarized response, thereby reducing IgE antibodies and allergenicity in murine asthma models [29]. Noteworthy [30] reported that, the impacts of TLR2 activation on the processing and tolerance to foods have to be characterized. Interestingly, many common foods such as processed meats, chocolate, yoghurt, and cheese contain TLR2 activators. Concomitantly Alison et al., [31, 32] improved the complex roles for TLR2, TLR4, and MyD88 in promoting the development and induced allergic airway disease. Thus, TLR signaling is likely required for both the development of asthma and the suppression of asthma by *S. pneumonia*.

Table 6A-Number and percentage of TLR2 and TLR4 in male patients groups

Age group	Total No.	TLR2 normal	TLR2 low	TLR2 high	Chi square	TLR4 normal	TLR4 low	TLR4 high	Chi square
10-30 Year	7	(28.6%)2	(28.6%)2	(42.8%)3	4.92 *	(100%) 7	(0%) 0	(0%) 0	15.00 **
31-50 year	12	(16.7%)2	(33.3%)4	(50%)6	9.43 **	(100%) 12	(0%) 0	(0%) 0	15.00 **
51-71 Year	2	(0%)0	(0%)0	(100%)2	15.00	(100%) 2	(0%) 0	(0%) 0	15.00 **
Total No.	21	4	6	11	--	21	0	0	---

* (P<0.05), ** (P<0.01).

Table 6 B-Number and percentage of TLR2 and TLR4 in male control group

Age group	Total No.	TLR2 normal	TLR2 low	TLR2 high	Chi square	TLR4 normal	TLR4 low	TLR4 high	Chi square
10-30 Year	9	(55.6%)5	(22.2%)2	(22.2%)2	9.21 **	(55.6%) 5	(22.2%) 2	(22.2%) 2	9.21 *
31-50 year	11	(36.4%)4	(36.4%)4	(27.2%)3	4.28 *	(36.4%) 4	(36.4%) 4	(27.2%) 3	4.28 *
51-71 Year	4	(50%)2	(25%)1	(25%)1	8.33 **	(50%) 2	(25%) 1	(25%) 1	8.33 **
Total No.	24	11	7	6	--	11	7	6	

* (P<0.05), ** (P<0.01).

Table 7A-Number and percentage of TLR2 and TLR4 in female patients groups

Age group	Total No.	TLR2 normal	TLR2 low	TLR2 high	Chi square	TLR4 normal	TLR4 low	TLR4 high	Chi square
10-30 Year	23	(21.7%) 5	(30.4%)7	(47.9%)11	8.36 **	(65.2%) 15	(8.7%) 2	(26.1%) 6	10.89 **
31-50 year	16	(6.3%)1	(25%)4	(68.7%) 11	10.57 **	(62.5%) 10	(6.2%) 1	(31.3%) 5	9.51 **
51-71 Year	11	(18.18%)2	(36.4%)4	(45.5%)5	8.91 **	(100%) 11	(0%)0	(0%) 0	15.00 **
Total No.	50	8	15	27	---	36	3	11	---

** (P<0.01)

Table 7 B-Number and percentage of TLR2 and TLR4 in female control groups

Age group	Total No.	TLR2 normal	TLR2 low	TLR2 high	Chi square	TLR4 normal	TLR4 low	TLR4 high	Chi square
10-30 Year	18	(44.4%)8	(27.8%)5	(27.8%)5	5.28 *	(44.4%) 8	(27.8%) 5	(27.8%) 5	6.14 **
31-50 year	14	(35.8%)5	(35.8%)5	(28.4%)4	2.48 NS	(42.8%)6	(28.6%)4	(28.6%)4	4.63 *
51-71 Year	8	(50%)4	(25%)2	(25%)2	8.33 **	(50%)4	(25%)2	(25%)2	8.33 **
Total No.	40	17	12	11	---	18	11	11	---

* (P<0.05), ** (P<0.01).

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