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Role of Nasal AQP5 And TREK1 Expression in Biomolecular Background of Pregnancy Rhinitis

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Abstract **Research Article**

Pregnancy rhinitis may lead predisposition to Obstructive Sleep Apnea Syndrome which is associated with critical comorbidities effecting both mother (preeclampsia, hypertension) and newborn (intrauterine growth retardation, low apgar score). We aim to unveil if nasal TREK-1 and AQP5 expression changes during pregnancy. We proposed that these two proteins may take part in the biomolecular background of Pregnancy Rhinitis. Twenty adult Wister albino female rats were enrolled into the study. Two groups constituted as 10 control (group A) and 10 pregnant (group B) rats. We sacrificed the rats at 21th day of gestation by intraperitoneal injection of 400mg/kg Na-pentobarbitone. Intracardiac blood sample was taken before the pulse died out. Serum estradiol (E2) and progesterone (PG) levels were determined by ELISA. Nasal septum with its mucoperichondrium was resected and sent for real time polymerase chain reaction testing. Relative TREK-1 (p=0.001) expression of group B was found to be significantly higher than group A. Relative AQP5 (p=0.003) expression was also found to be significantly higher in group B than group A. E2 was found to upregulate AQP5 expression (p=0.032) while downregulate TREK1 expression (p=-0.011). On the other hand, PG was also found to upregulate nasal AQP5 expression (p=0.024) but not TREK1 expression (p=-0.071). TREK1 and AQP5 take part in pregnancy related biomolecular changes of nasal mucosa. Namely, expression of TREK1 decreases while expression of AQP5 increases during pregnancy. These findings support the researchers asserting triggered allergy as the etiology of PR.

INTRODUCTION

Pregnancy-related nasal symptoms have been known for a long time, but the actual definition of pregnancy rhinitis (PR) was first made by Ellegard and Karlsson in 1999 [1]. They described PR as ‘‘nasal congestion present during the last 6 or more weeks of gestation without other signs of respiratory tract infection and with no known allergic cause, resolving totally within 2 weeks after birth”. Its incidence has been reported as between 9% - 40% [2-5]. Despite its relatively high incidence, the level of public awareness is quite low. However, presence of PR paves the way for Obstructive Sleep Apnea Syndrome (OSAS) which is associated with serious maternal (hypertension, preeclampsia) and fetal comorbidities (low apgar score, intrauterine growth retardation) [2,6-8].

Pathophysiology of PR has not been completely revealed. Increased serum levels of progesterone (PG), estradiol (E2), placental growth hormone and human chorionic gonadotropin have been suggested as the main factor [9-11]. On the other hand, some researchers asserted the activation of subclinical nasal allergy as the triggering factor [2,12]. But a common biomolecular pathway for allergy and PR cannot be revealed yet. In addition, the effect of gonadocorticoids on the nasal mucosa has been studied in the context of various gynecological disorders [13-16]. However, there is very limited data about biomolecular changes regarding PR [17,18].

TWIK-related potassium channel-1 (TREK-1) is a mechano-gated two-pore-domain K^+ channel. Although it was shown to be mainly expressed in central nervous system, it was also detected in tissues like myocytes and endothelial cells[19]. In addition, latest studies unveiled its critical function in airway epithelial barrier integrity [20,21]. It is well known that disruption of epithelial integrity plays a key role in the pathogenesis of chronic rhinosinusitis, allergic rhinitis and asthma [22]. On the other hand, aquaporins are biomolecules in the form of membrane channels which regulates osmotic fluid flux across the cell membrane [23]. Aquaporin 5 (AQP5) was shown to be expressed in mouse and sheep respiratory system and glandular

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cells (24,25). AQP5 was also shown to be expressed in tongue base, salivary glands and gastrointestinal tract [26]. Namely, AQP5 regulates fluid flow through the cytoplasmic membrane while TREK-1 serves for liquid hemostasis through the epithelium by maintaining the epithelial integrity. To the best of our knowledge, the expression of these two proteins has not been studied in terms of pregnancy, either in human or rat nasal mucosa before.

Expression of both TREK1 and AQP5 were shown to be upregulated by Estradiol (E2) and progesterone (PG) [27-30]. It is well-known fact that these two gonadocorticoids exhibit a gradual increase during gestation [3]. In addition, effect of gonadocorticoids on nasal mucosa has been revealed in various aspects [31-36]. Considering these findings, we hypothesized that nasal TREK1 and AQP5 expression would be up regulated by increased serum levels of E2 and PG during pregnancy. Uncovering such a relationship can partially clarify the pathophysiology of PR.

In this study, the physiological and gestational levels of TREK-1 and AQP5 in rat nasal mucosa were evaluated. We also investigated the effect of E2 and PG on these proteins. We hereby will provide preliminary data concerning the relationship between nasal expression of these 2 proteins and pregnancy.

MATERIALS and METHODS

Ethical approval

This animal experimental research was approved by the Laboratory Animals Local Ethics Committee of Manisa Celal Bayar University (28.04.2015/77.637.435-29) .

Animals

Institutional Laboratory Animals Local Ethics Committee was approved this study. In addition, it was done in institutional Experimental Animals Research and Application Center.

Twenty Wister albino female rats (12-week-old) in the were enrolled in the study. They were housed at room temperature (22 ± 2 °C) on a 12 hours light-dark cycle. Female rats were kept together with male rats in the ratio of 4/1 for overnight. The male rats were fended off in the next day. Pregnant rats were determined by detecting sperm in vaginal smears as described previously [37-39]. Control group (group A) was established from rats having negative smears. The rats having sperm positive smears assigned as the pregnant group (group B). Average pregnancy period of Wister albino rat was reported as 22 (21-26) days [38,40]. For this reason, they were sacrificed at 20th day of pregnancy by intraperitoneal injection of sodium-pentobarbitone (400mg/kg) solution as described previously [39]. We waited for the loss of righting reflex and consciousness. Approximately 15 ml of blood sample was taken by a 23 G needle from the hearth before the pulse disappears. Samples were utilized for detection of serum E2 and PG levels by ELISA. Then we shaved the nasal dorsum. We separate the nasal bones from the maxilla in an upward manner and revealed the nasal cavity macroscopically (Figure 1). Cartilaginous part of the septum (Cartilago septi nasi) with its mucoperichonrium was resected and reserved for real time polymerase chain reaction testing (PCR).

Figure 1. External view of rat's rhinarium (dorsal hair shaved). Nasal roof (Os nazale) are mobilized in upward direction and internal macroscopic view of both nasal cavities is seen. The septum (Septum nasi osseum) and middle nasal chonca (Concha nasalis media) are identified. *: Septum nasi osseum.

Detection of E2 and PG levels by ELISA

Blood samples were mixed with sodium citrate, heparin and EDTA. Centrifugation of the admixture was done for 10 minutes at 3000 rpm and stored at -80 °C. We used Rat E2 (estradiol) ELISA Kit and General Progesterone (PG) ELISA Kit for quantitative measurement of serum E2 and PG levels, respectively (MyBioSource, Inc.,CA, USA)(39).

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR) Analyses

We used PureLink® RNA Mini Kit (Thermo Fisher Scientific, 12183555) along with TRIzol® Reagent TRIzol® Reagent for extraction of total RNA from the VCM. Forward AQP5F1

primer 5'-GACTTTCCAGCTAGCCCTCTG-3', reverse AQP5R1 primer 5'-GATGGCCCAGTGTGACAGAC-3', forward TREKF1 5'-ACATCTCCCCACGAACTGAAG-3', reverse TREKR1 5'-ATGGTTCCAAGCTGATCCCC-3' and QuantiFast SYBR Green qRT-PCR Kit (Qiagen, 204154) were used for quantification of qRT-PCR analysis of AQP5 and TREK1 RNA expression levels.

Separately prepared mixture of QuantiFast SYBR Green, TREKF1, TREKR1, AQP5F1 and AQP5R1 was analyzed in the Rotor-Gene Q (Qiagen, Hilden, Germany). We used β-microtubulin (B2M) (B2MF1 5'-TCTCTCTTTCTGGCCTGGA-3', B2MR1 5'-TGTCGGATGGATGAAACCC-3') and hypoxanthine phosphoribosyl transferase (HPRT1) (HPRT1F1 5'-CGTCTTGCTCGAGATGTGAT-3', HPRT1R1 5'-TTCAGTGCTTTGATGTAATCCAG-3') as a housekeeping gene to normalize the expressional changes. The related forward and reverse primers were synthesized by Metabion company (Germany). Program of the qRT-PCR cycling conditions started with the reverse transcription step of 50°C (10 min), followed by PCR step comprised of an initial activation/denaturation stage of 95°C (10 min), followed by 40 cycles of denaturation 95°C (15 s), combined annealing/extension 60°C (45 s). For calculation of the relative changes in gene expression determined from the Real-Time PCR analysis, we used 2-ΔΔCT method [41].

Statistical analyses

Data distribution was assessed by Shapiro-Wilk test. We compared levels of TREK-1 and AQP5 expression of group A and B by independent samples t-test or Mann-Whitney U Test according to the results of Shapiro-Wilk test. The effect of serum E2 and PG levels on TREK-1 and AQP5 was analyzed by Pearson correlation test. Statistical significance was defined as $p<0.05$. Results were presented as mean ± standard deviation (SD). The Statistical Package for the Social Sciences (SPSS) Version 21.0 (IBM Corp.; Armonk, NY, USA) was used for statistical calculations.

RESULTS

Twenty Wister albino female rats (10 controls, 10 pregnant) in total were enrolled into the study. The mean values of TREK-1 in group A and B was 0.679±0.203 and 0.0347±0.018, respectively. The mean values of AQP5 in group A and B was 1.346±0.609 and 2.327±0.683, respectively. The mean serum E2 levels of group A and B was 19.254±5.287 pg/ml and 74.179±4.324 pg/ml, respectively. The mean PG levels of group A and B was 14.335±1.456 ng/ml and 32.589±4.195 ng/ml, respectively. Distribution of whole data except AQP5 ($p=0.487$) were found to be abnormally distributed ($p<0.05$). For this reason, Mann-Whitney U Test was used for comparison of TREK1 and serum sex hormone (E2, PG) levels between groups. But for comparison of APQ5 between groups independent samples t-test was used.

Relative TREK-1 ($p=0.001$) expression was found to be significantly low in group B. Group B exhibited higher expression of AQP5 ($p=0.003$) than group A (Figure 2). E2 and PG levels of group B were also found significantly higher than group A ($p<0.001$) (Table 1). We found positive correlation between E2 and AQP5 expression ($p=0.032$) (Figure 3) while negative correlation between E2 and TREK1 expression ($p=-0.011$) (Figure 4). Similarly, a positive correlation between serum PG levels and AQP5 ($p=0.024$) (Figure 5) was found, but we failed to show any correlation between PG levels and TREK1 expression. (TREK-1 ($p=-0.071$)

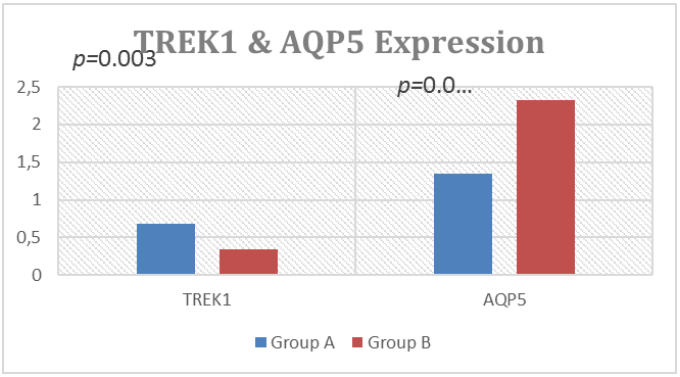

Figure 2. Graphic showing change in relative expression of TREK1 and AQP5 expression during pregnancy.

Table 1. Expression of TREK-1 and AQP5 in nasal mucosa, and serum E2 and PG levels based on groups.

Biomolecules & Sex Hormones	Control (Group A)	Pregnant (Group B)	P value
Biomolecules			
TREK-1 (REV)[a]	0.679±0.203	0.0347±0.018	p=0.001[b]
AQP5 (REV)[a]	1.346±0.609	2.327±0.683	P=0.003[c]
Serum Sex Hormone Levels			
Estradiol (pg/ml)	19.254±5.287	74.179±4.324 pg/ml	p<0.001[b]
Progesterone (ng/ml)	14.335±1.456	32.589±4.195	p<0.001[b]

[a] Denotes for Relative Expression Value.
[b] P values obtained by Mann-Whitney U Test.
[c] p values obtained by Independent Samples t- test.

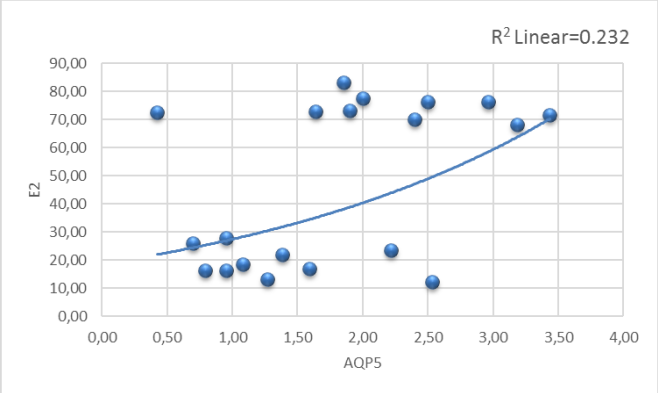

Figure 3. Scatter dot graphic showing correlation between E2 and AQP5.

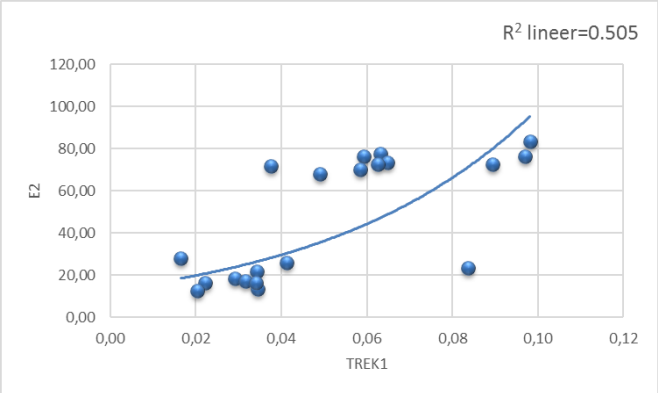

Figure 4. Scatter dot graphic showing correlation between E2 and TREK1.

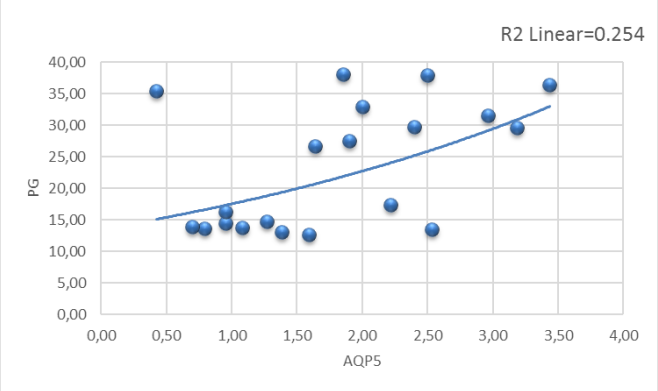

Figure 5. Scatter dot graphic showing correlation between PG and AQP5.

DISCUSSION

PR is a quite common gonadocorticoid related rhinological disorder [9-11] which may indirectly lead predisposition to comorbidities like maternal hypertension, preeclampsia, low APGAR score and fetal growth retardation [2,6-8]. Although some epidemiological and physiological studies have been done on PR [3,42], histopathological and biomolecular background has not been studied thoroughly. In addition, there are very few studies concerning the treatment of PR [43,44]. In this study we unveiled some biomolecular changes of nasal mucosa in pregnant rats. These findings may assist in understanding the physiopathology of PR and may create opportunity for new treatment modalities.

In the current study, expression of AQP5 in nasal mucosa was found to be significantly increased in pregnant rats (Figure 2). Similarly, increased nasal AQP5 expression was also shown in allergic rhinitis by Lei et al. [45]. Thus, this finding supports that PR and allergic rhinitis may share a common biomolecular pathway. On the other hand, AQP5 was shown to facilitate fluid secretion in submucosal glands and be involved in inflammatory processes [46,47]. This finding is compatible with the nasal obstruction experienced by PR patients. In addition, we found upregulatory effect of E2 and PG on nasal AQP5 expression (Figure 3,5) which is also concordant with previous studies [28,30]. This relationship may also shed light on the pathophysiology of other gonadocorticoid related nasal disorders like vicarious menstruation [48, 49].

We revealed that nasal TREK1 expression decreases with pregnancy. We also revealed downregulatory effect of E2 on nasal TREK1 expression (Figure 4). At first glance this finding seems to contradict with some previous studies concerning the effect of gonadocorticoids on TREK1 [27, 29]. This negative correlation also partially contradicts with our beginning hypothesis but there are some studies supporting our data in different pathological states of nose. For instance, Wang et al. revealed decreased expression of nasal TREK1 in allergic rhinitis patients [50]. Similarly, Kim et al. asserted downregulation of TREK1 in rhinosinusitis patients ending up with disrupted epithelial barrier function [51]. As expected, the disruption of epithelial barrier would end up with oedema and inflammation of nasal mucosa leading nasal obstruction. On the other hand, we failed to show any effect of PG on nasal TREK1 expression.

Exacerbation of subclinical allergy has been suggested as the etiology of PR and some studies support this phenomenon [2,11,14]. Namely, Ellegard et al. revealed increased level of IgE against house dust mite in PR patients [12] and Toppozada et al. showed some changes like AR in specimens of PR patients by electron microscopy [14]. Our study also supports these previous data in the context of AQP5 and TREK1. Namely, upregulation of AQP5 and downregulation of TREK1 is concordant with the studies concerning allergic rhinitis [45, 50]. Thus, according to the current study it can be asserted that allergic rhinitis and PR may share same biomolecular pathway. Intranasal steroids constitute the main

treatment modality in allergic rhinitis patient so according to the current study they may also be used in the treatment of PR.

It's a well-known fact that nasal obstruction is one of the etiological factors of OSAS [52]. On the other hand, OSAS is the main risk factor for the abovementioned maternal and fetal comorbidities leading serious public health issues [53]. In fact, pregnancy itself is a risk factor for OSAS [54]. Thus, diagnosis and treatment of PR is very important regarding maternal and fetal health. The current study partly unveils the physiopathology of PR in biomolecular level which may create opportunity for new treatment modalities. Future clinical trials with nasal steroids may be promising for this insidious gestational rhinological disorder.

CONCLUSION

According to the current study TREK1 and AQP5 take role in pregnancy related biomolecular changes of nasal mucosa. Namely, expression of TREK1 decreases while expression of AQP5 increases during pregnancy. These findings support the hypothesis that PR is caused by the activation of subclinical allergy that is present before pregnancy. Thus, basing this relevancy we may prescribe topical nasal steroids in pregnancy rhinitis, particularly in the last trimester.

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Conflict of interest

The authors declare that they have no conflict of interest.

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