INFLAMMATORY MEDIATOR SEROTONIN RECEPTOR GENE (5-HTR3A) EXPRESSION CHANGES ON HUMAN PERIPHERAL BLOOD LYMPHOCYTES IN RHEUMATOID ARTHRITIS

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The 5-HT3 receptor is a pentameric ligand-gated cation channel located in the central and peripheral nervous system and on extraneuronal locations like lymphocytes, monocytes and fetal tissue. Serotonin receptor gene expressions and their alterations in RA diseases have not been reported. The aim of this study is to show whether the serotonin receptor gene expresses on peripheral blood lymphocytes and also to characterise the lymphocyte serotonin receptor expression profiles in patients suffering from rheumatoid arthritis (RA). In the present study, using RT-PCR technique, the research team investigated 5-HT3A receptor gene expression in peripheral blood lymphocyte cells (PBMC) of forty healthy individuals compared to forty RA patients. The PBMC was separated from whole blood by Ficoll-hypaque. Total cellular RNA was extracted and then cDNA was synthesized. The research team analyzed quantititavely gene expression profile by Real time-PCR using primer pairs specific for 5-HT3A receptor and for β-actin as internal control. Each PCR product of 5-HT3A receptor was confirmed by DNA sequencer ABI 3700 capillary system (Applied Biosystem, USA). The results showed that the 5-HT3A receptor gene is detected on the lymphocytes of both normal control and RA patients. There was a significant difference between 5-HT3A receptor expression profile in RA and that of healthy individuals. Moreover, no SNP-based change on sequenced fragments was observed. In conclusion, the present study indicated that not only human lymphocytes in normal individuals and patients express 5HT3A receptor, but the expression pattern of 5HT3A receptor gene is different between normal controls and RA patients. Moreover, after sequencing no changes in either controls or patients were observed. The above-mentioned changes can contribute to new information related to the pathogenesis of RA disease.

Autoimmune diseases in general are complex genetic diseases where genes and environment interact in unknown ways (1-2). Rheumatoid arthritis (RA) is one of the common autoimmune disorders usually considered as a chronic, inflammatory, multisystem and destructive arthropathy that cannot be cured and has substantial personal, social, and economic costs (3-4). Although the cause of rheumatoid arthritis is a very active area of worldwide research (5-6), the etiology of rheumatoid arthritis (RA) is obscure (7).

Key words: rheumatoid arthritis (RA), 5-hydroxytryptamine (5-HT), serotonin receptor, gene expression, peripheral blood lymphocytes, Real time-PCR

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Lymphocytes apparently carry active receptors and transport systems for the neurotransmitters such as serotonin and dopamine (8). 5-HT also presents in a variety of peripheral tissues including in constituents of the immune system. In recent years, it has been possible to study new important aspects of 5-HT in the immune system (9). The immune and nervous systems have a bilateral relationship through a wide variety of common mediators including neurotransmitters and cytokines (10). Recently new evidence has come to light elucidating the modulatory role of neurotransmitters in immune functions (11). A recent survey revealed that serotonergic system in the central nervous system is implicated in the etiology and pathogenesis of affective disorders. Serotonergic system also plays several roles in the immune system through the expression of a number of its receptor subtypes in the immune cells (12). The expression of the serotonin receptor is characterized in the brain but little work has been done to examine its expression in other organs. Moreover, in certain diseases of the immune systems, alterations in serotonin receptor gene expression in different cells and tissues have not been reported (13). We conducted this study to examine whether the 5HT3A receptor gene expression changes and mutates at RNA levels which encode receptors to be involved in the pathophysiology of RA.

MATERIALS AND METHODS

In the present study, patients with established RA attending the outpatient clinic of the Rheumatology department, Taleghani Hospital, Tehran, Iran, were enrolled between 2006 and 2008. All participants gave informed consent. All data used for this study were registered at enrolment. All subjects were Iranians, who complied with at least four criteria of the American College of Rheumatology (ACR), especially one definite radiographic bone erosion in hands or feet (14).

Eighty individuals, forty healthy controls and forty patients (age 25-55 years), took part in this study. Peripheral blood samples (4 ml) were obtained from the cubital vein and collected in cell preparation tubes containing an anticoagulant. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density centrifugation (Lymphoprep ™, Oslo, Norway), the density of which is 1.077 ± 0.001 g/ml (20°C) and the osmolity is 290 ± 15 mOsm. The buffy coat was collected in tubes. Then buffy coat was washed three times in Phosphate buffer saline (PBS) (15). The total mRNA was isolated from PBMC by High pure RNA isolation Kit (Roche, Germany), in compliance with the manufacturer's instructions. The amount and purity of RNA were determined by spectrophotometery (16). Total RNA was reversely transcribed into first-strand cDNA by using M-MuLV RT (recombinant moloney murine leukemia virus reverse transcriptase) according to the manufacturer's protocols (Revert Aid ™ First Strand cDNA Synthesis Kit #K1622, Fermentas, EU). Samples were reverse transcribed with oligodT primers (17). For the amplification, reactions were run for 55 cycles, 10 second (s) at 95°C, 10 s at 55°C, 18 s at 72°C, and 5 min at 72°C. PCR was carried out in a Real Time-PCR (Roach, Germany) with a Cyber green fluorogenic nucleotide to monitor cDNA amplification by the increase in fluorescence intensity and by using primer pairs specific for 5HT3A mRNAs and β-actin as internal control (Table I). All prepared reagents were from Fermentas, UE.

The specificities of the obtained PCR products for the respective serotonin receptor fragments were confirmed by sequenced analysis capillary system (Applied Biosystem, USA) (18). The PCR products visualized by gel electrophoresis on a 2% agarose gel to assure known concentration of cDNA for each sample.

Statistical analysis

We used SPSS 16.0 (SPSS, Inc, Chicago, IL) and *t* **test** to analyse the data of RA patients in comparison with healthy individuals. Relative mRNA expression was calculated by the δδCt-method (19). The significant value in this study was less than 0.05 ($P \leq 0.05$).

RESULTS

We detected mRNA expressions of serotonin receptors in PBMC using highly sensitive methods. We focused on one subtype of serotonin receptors (5HTR3A) on the basis of the latest research, whereby only this receptor is functionally related to some diseases. We divided nine exones into four segments and then expression of the different serotonin receptor gene segments were studied by analyzing total RNA extracted from the samples. The results revealed that all segments of serotonin receptor gene were expressed on PBMC of healthy and patient individuals. In order to detect serotonin gene receptor expression at the RNA level, Real time PCR was used for the regions of different serotonin receptor segments. The specificities of the obtained PCR products for the respective serotonin receptor

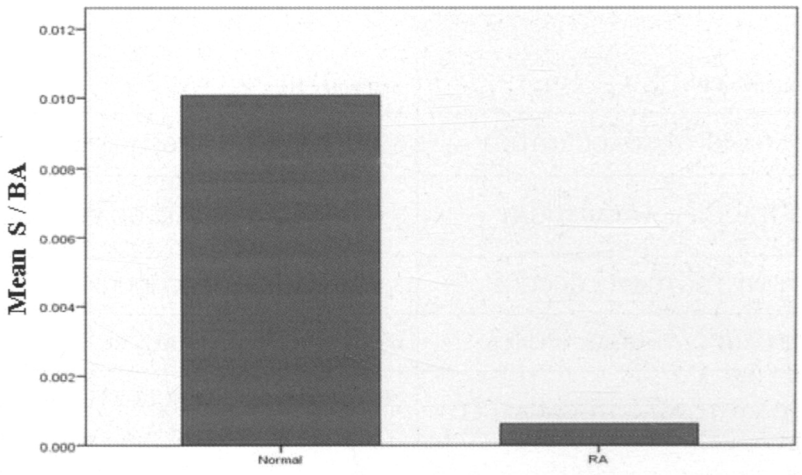

Fig. 1. *Significant quantitative decrease of 5HT3A serotonin receptor gene expression in RA patients compared to normal control. P value of this expression change was 0.001 (P>0/05)*

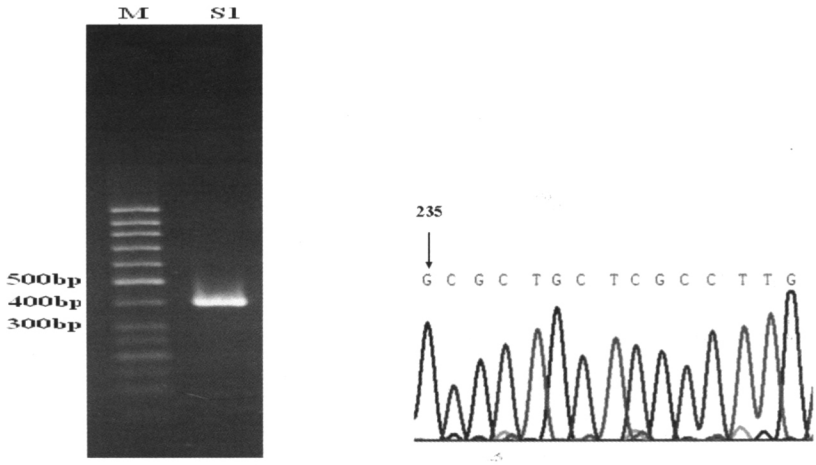

Fig. 2. *Agarose gel electrophoresis of PCR products of serotonin receptor gene (**left**) and sequencing of RA patient fragment which adapted with its sequence in NCBI (**right**).*

fragments were confirmed by capillary sequence analysis (ABI 3700).

However, firstly, receptor gene fragment expression of RA patients and matched healthy controls were analyzed by Real time PCR and normalized to the housekeeping gene β-actin for each sample using the δδCt method (19). The measure of mRNA concentration of each sample was then calculated and also Mean, Standard deviation and P Value for 5HTR3A gene expression was determined (Tables II-IV). The concentration mean for RA patients was 6.2418, whereas concentration mean for the control group was 20.1200 (Table II).

As a result, there is a quantitatively substantial difference of expression (Mean 20, 1200 – 6.2418) at RNA level following comparison healthy and patient individuals (Fig. 1). P value of this expression change was 0.001 (P>0/05). Using ABI 3700 capillary system we sequenced PCR products of 5-HT3A receptor and confirmed by a DNA sequencer. After sequencing, no changes in control and patient group were observed. As an example, Fig. 2 shows agarose gel electrophoresis of 5HTRA gene and related exon sequence compared with its sequence

Table I. *Primer sequences of serotonin receptor fragments (5HT3A) and β-actin.*

Gene	Sequence FP	Sequence RP	Amplicon Size
Fragment 1	5'CAGAAGGTGTGAGCAGTGG3'	5'ACATCTGGTACCGGCAGTAC3'	398 bp
Fragment 2	5'GGTACCGGCAGTACTGGA3'	5'GGTACCGGCAGTACTGGA3'	472 bp
Fragment 3	5'CTATGTGGTCATCCGCCGG3'	5'CTATGTGGTCATCCGCCG3'	457 bp
Fragment 4	5'CTCAGCCATGGGAAACCAC3'	CTCAGCCATGGGAAACCAC3'	392 bp
β-actin	5'TGAAGTGTCACGTGGACATCCG3'	5'GCTGTCACCTTCACCGTTCCAG3'	146 bp

Table II. *Mean, standard error and standard deviation for concentration in normal and RA samples.*

Concentration 5HT3A

Samples	Mean	Std. Error Mean	Std. Deviation
Normal	20.1200	0.728761	2.726775
RA	6.2418	0.115905	0.742155

Table III. *Mean, standard error and standard deviation for cross in normal and RA samples.*

Cross 5HT3A

Samples	Mean	Std. Error Mean	Std. Deviation
Normal	27.0500	0.30327	1.35627
RA	29.9149	0.56304	3.60522

in NCBI, nor were changes observed in other exons.

DISCUSSION

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic monoamine with different functions in many physiological and pathophysiological processes such as neuropsychiatric disorders such as depression, schizophrenia, anxiety, diet, sexual behavior, asthma, etc. Several 5-HT receptor types/subtypes have been reported on human immunocytes, such as lymphocytes, monocytes and dendritic cells (20). In the brain, serotonin modulates neuronal activity and is actively involved in mediating many cognitive functions and behaviors. In the periphery, serotonin is involved in vasoconstriction, inflammation, and cell growth, among other processes. The serotonergic system plays several roles in the immune system through the expression of a number of its receptor subtypes in the immune cells. It is well established that various biological effects of 5-HT are mediated through different serotonin receptors and their signal transduction pathways. Initially, the idea that neurotransmitters could act as immunomodulators emerged with the discovery that their release and diffusion from nervous tissue could lead to signaling through lymphocyte cell-surface receptors and the modulation of immune function. It is now evident that neurotransmitters can also be released from leukocytes and act as autocrine or paracrine modulators (21).

Although in certain diseases of the nervous system and in some immune system diseases, alterations in neurotransmitter receptor gene expressions in different cells have been reported, there is no evidence of investigation of serotonin receptor

Table IV. *Comparison of the expression pattern of human serotonin receptor gene in RA patients and normal individuals.*

Serotonin Receptor (5HT3A)	Normal (Mean ± SD)	RA (Mean ± SD)	P value* P≤ 0.05
Cross	27.0500±1.35627	29.9149±3.60522	0.000
Concentration	20.1200±2.726775	6.2418±0.742155	0.001

**All value of serotonin receptor genes concentration and cross is relative to beta actin*

gene expression in peripheral blood lymphocytes in RA patients (22). In this study, we looked at the expression profiles of 5-HT_3 receptor and by one-step reverse transcription polymerase chain reaction (RT-PCR) we examined the presence of mRNAs for 5-HT3A receptor subtype in the human PBMCs of rheumatoid arthritis patients in comparison to that of healthy individuals. As expected, 5-HT3A receptor gene was consistently detected and also mRNA concentration for 5-HT3A receptor exons in the human PBMCs measured. Our study indicates that there are significant differences in receptor gene expression between the patient and the control groups ($P < 0.01$).These observed differences may be helpful for primary diagnosis and treatment of RA. On the other hand, there is evidence that 5-HT3 receptor antagonists provide considerable benefit in some rheumatic diseases such as rheumatoid arthritis. Thus, understanding of alteration of 5HT3A receptor expression can help to access an effective drug for treatment of RA diseases. However, further research is necessary to give conclusive evidence on the possible function of this receptor in lymphocytes.

The aim of the present study is to show that the 5HT3A receptor expresses on human peripheral blood lymphocytes, similarly to dopamine which reflects to some extent brain status (23), and also the PBMC can be considered as one of the subsets of serotonergic models in the neuroimmune system. Our hypothesis is that lymphocyte serotonin receptor expression profiles are changed in rheumatoid arthritis disease and this alteration results in dysfunction of the serotonergic system and as in the hypothesis of dopamine, a persisting dysfunction of the serotonergic system could contribute to a biological cause the chronic character of autoimmune system diseases such as rheumatoid arthritis (24).

The present study demonstrates that serotonin receptor genes are expressed by HPBL, both in normal control and RA patients, where it is localized on mononuclear cells. The results clearly show that leukocytes in a human RA patient group express serotonin receptor (5HT3A) with significant difference in comparison with healthy individuals. It is suggested that possibly significant changes in serotonergic system including 5HT3A receptor gene profile can lead to RA.

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