CLINICAL STUDY

Toll-like receptor 9 polymorphism in patients with erythema multiforme, Stevens-Johnson syndrome and Stevens-Johnson syndrome/toxic epidermal necrolysis overlap syndrome

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Abstract: Background: “Toll like receptor” (TLR) 9 functions in stepping in of native immune system against different viral and bacterial pathogens and induction of adaptive immune response effectively. TLR 9 gene polymorphism makes host predisposed to microbial pathogens by affecting the functional capabilities of the receptor.

Objective: We aimed to determine if TLR 9 gene polymorphism makes a predisposition to “erythema multiforme” (EM), “Stevens-Johnson syndrome” (SJS) and “Stevens-Johnson syndrome/toxic epidermal necrolysis overlap syndrome” (SJS/TEN).

Methods: Forty-two patients clinically and/or histopathologically diagnosed as EM, SJS, and SJS/TEN overlap syndrome and 50 healthy control subjects were enrolled in our study. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied for TLR 9 gene 1237 thymine/cytosine (T/C) polymorphism. Genotypes were determined according to bands occurring on agarose gel electrophoresis.

Results: In patients group, the frequencies of TT and TC genotypes were 73.8 % and 26.2 % while CC genotype wasn’t detected. In control group, the frequencies of TT, TC and CC genotypes were 74 %, 24 %, and 2 %. There wasn’t a statistically significant difference for TT, TC and CC genotypes between patients and controls. The frequencies of T and G alleles were 84.5 % and 15.5 % in patients and 86 % and 14 % in controls, respectively.

Conclusion: Our results showed that there isn’t any association between TLR gene polymorphism and EM, SJS, SJS/TEN overlap syndrome (Tab. 1, Fig. 1, Ref. 30). Full Text in free PDF www.bmj.sk.

Key words: erythema multiforme, Stevens-Johnson syndrome, Stevens-Johnson syndrome/toxic epidermal necrolysis overlap syndrome, toll like receptor 9, polymorphism.

Erythema multiforme (EM), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are progressive mucocutaneous diseases that cause serious morbidity and mortality. Considering the histopathological similarities and overlapping characteristics in the patterns of these three diseases, they are accepted as variants of the same process with only differences in involvement (1). The etiology of EM, SJS and TEN includes medical drugs and infections. The most frequent cause of EM is infection with Herpes simplex virus (HSV) type 1 and 2 (2). Other identified agents are mainly Mycoplasma pneumoniae and fungal infections (3). Medical drugs are considered to be the etiologic agent in SJS and TEN, still infectious agents are found to be responsible for about 50 % of SJS cases, and 5 % of TEN cases (4).

Human skin is the first line of immune defense against environmental pathogens like viruses, bacteria and fungi. Receptors, which are located on immune cells and recognize the pathogens, were first discovered on Drosophila flies in 1991 and were given the name “toll”(5). The first human homologue of the “toll” receptor family known as “Toll-like receptor (TLR) 4” was identified in 1997 (6). Following this discovery, 10 different TLRs and their ligands were identified up till now. It has been illustrated that the dendritic cells, monocytes, natural killer cells, lymphocytes and epithelial cells of the human innate immune system variably express TLR (7).

TLR 9 is located in the cytoplasm and its specific ligand is cytidine-guanine (CpG) DNA. As with bacterial DNAs, HSV type 1 DNA also contains high levels of CpG dinucleotide strands that have not undergone methylation and these strands are recognized by TLR 9 (8–10). TLR 9 plays a role in antibacterial and antiviral immune defense by enabling interferon β (IFN-β), IFN-γ, IFN-γ interferleukin 6 (IL–6), and IL-12 induction (11).

In the past century, individual variations and genetic susceptibilities were defined for various infectious diseases. The most familiar example to this is Malaria caused by Plasmodium species. While Hemoglobin S homozygote individuals suffer from sickle cell anemia; hemoglobin S heterozygote individuals develop resistance against Plasmodium falciparum infection.
The genetic variation referred to in this example reduces susceptibility towards infection; however, genetic variations that facilitate the occurrence of infection have also been identified. It is well known that the occurrence of various infections like tuberculosis and leprosy is higher in monozygotic twins, when compared to dizygotic twins. TLR mutations as well as TLR gene polymorphisms can make the host more susceptible to various infections or inflammatory diseases and studies are being conducted to explain the variations in such susceptibilities by genetic polymorphism in TLR genes (12).

Currently it has been demonstrated that polymorphisms in TLR9 gene is related to asthma, Crohn disease and atopy with an increasing risk (13–15). On the other hand, in their studies conducted with patients suffering from SJS/TEN, Ueta et al (16) have identified that TLR 3 gene polymorphism may lead to susceptibility for SJS/TEN.

EM and SJS can be recurrently triggered by both viral and bacterial factors. Since TLR 9 has the capability to recognize bacterial and viral (especially HSV) DNA that contains unmethylated CpG DNA dinucleotide strands, we aimed to investigate the role of TLR 9 polymorphism in this group of diseases.

Materials and method

Patients

Forty-two patients referred to our department between January 2000 and June 2008 were included in the study. The patients were diagnosed as clinically and/or histo-pathologically EM, SJS and SJS/TEN overlap syndrome disease and were classified according to the classification system proposed by Bastuji-Garin et al (1). Fifty healthy volunteers without any known or previously diagnosed dermatologic, allergic or systemic disease were taken as the control group.

The study was approved by the local ethics committee (Ethics Committee Certification no: 2008–5/20) and informed consent form was taken from the patients and control group.

Methods

5 ml blood with EDTA was taken from both the patients and healthy volunteers. Then using the commercial Dr. Zeydani DNA isolation kit (Turkey), genomic DNA was obtained from leukocytes as proposed by the manufacturer of the kit. In our study, 1237 T/C polymorphism of TLR 9 gene was studied. The gene section was replicated with polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method by using primers specified for this polymorphism, which has a singular nucleotide variation for 154 bp (15). To identify products replicated with PCR, 2% agarose gel electrophoresis was used. The obtained PCR products were displayed by using gel display system. Alleles were identified using BstN I (Genemark, Russia) enzyme that recognizes the studied single nucleotide strand. PCR product of 10 μL was digested by 5 U BstN I restriction enzyme for 12 h at 60 °C. Migration was performed on 4% agarose gel stained with ethidium bromide. At the end of slicing process, the gene sections with C allele were sliced from two points, while the gene sections with T allele were sliced from a single point. Genotyping was done as T/T if there were two bands on the sample aligned with 129 bp and 25 bp; as T/C if there were four bands aligned with 129 bp, 81 bp, 48 bp and 25 bp; and as C/C if there were three bands aligned with 81 bp, 48 bp, and 25 bp (Fig. 1).

Statistical analysis

The variables with categorical values (gender, genotype) were entered with cross charts and the variations between the two groups were analyzed by using the Pearson’s chi-square and the Fisher’s chi-square tests. For the age variable, which obtains a continuous value, the normality test was conducted using the Shapior–Wilks test. The age variable was compared between the two groups using a non-parametric test, Mann-Whitney U test. The level of significance was accepted as p=0.05.

Results

The patient group included 17 males (40.5 %) and 25 females (59.5 %). Their ages varied between 8–73 years with an average of 38.3±17.3. The control group was consisted of 26 males (52 %) and 24 females (48 %). Their ages varied between 9–72 years with an average of 33.9±13.5. In the patient group; 14 (8 females, 6 males) were diagnosed as EM, 17 (9 females, 8 males) as SJS and 11 (8 females, 3 males) as SJS/TEN overlap syndrome. There was no significant difference in age and gender between the groups, (p>0.05).

Among the patients in the study group, TT genotype was identified in 31 patients (73.8 %); TC genotype in 11 patients (26.2 %); but the CC genotype was not identified. In the control group, TT genotype was identified in 37 people (74 %); TC genotype in 12 (24 %), and CC genotype in 1 (2 %). When the TT, TC and CC genotypes were compared, no significant difference was observed between the patient and control groups (p>0.05).

The relation between the frequency of the T and C alleles in the
Based on the possible potential effects of HSV and streptococcal antigens in triggering the Behcet’s disease, Ito et al. (26) studied the TLR 9 polymorphism in a group of 200 Japanese patients suffering from Behcet’s disease and 102 healthy volunteers. The most frequent 9 single nucleotides seen in the Japanese population have been studied and the study concluded that there was no significant relation between the TLR 9 gene polymorphism and Behcet’s disease. Based on the susceptibility of patients with atopic dermatitis to viral and bacterial diseases and effect of these infectious pathogens on the course of the disease, Novak et al. (27) studied the TLR-9 1237T/C polymorphism in a group consisting of 483 children with parents suffering from atopic dermatitis, 274 atopic dermatitis patients and 252 healthy controls. The study concluded that especially the intrinsic sub-group of atopic dermatitis cases was related to TLR-9 polymorphism. Again, Ng et al. (28) studied the relation between systemic lupus erythematosus (SLE) and TLR 9 gene polymorphism in a group consisting of 467 SLE patients and 799 healthy volunteers. As a conclusion of the study, they did not identify any relation between TLR 9 polymorphism and the disease occurrence. In a similar study, where 350 SLE patients and 330 controls were studied, it has been found that TLR 9 gene polymorphism does not lead to susceptibility to SLE (29).

It has been indicated that opportunistic infectious agents such as Staphylococcus aureus and Staphylococcus epidermidis can be found on ocular surfaces of patients with SJS/TEN (30). This fact leads us to consider that there is possibly a relation between defective innate immune system and SJS and TEN. There is only one research in the literature that studied the TLR gene polymorphism on SJS and TEN. In this referred study, Ueta et al. (16) studied TLR 3 gene polymorphism. The study included 57 Japanese patients suffering from SJS/TEN with ocular complication and 160 healthy Japanese volunteers. Using the database, they have identified 7 single nucleotide polymorphism of TLR 3 gene declared previously in the Japanese community and all these polymorphisms were investigated. They concluded that 299698T/G, 293248A/A and 299698T/T genotype patterns have a strong relation with SJS/TEN. In our study, where we studied TLR 9 1237T/C polymorphism, we have concluded that there is not a significant difference between the patients and the control group, in terms of the genotype patterns. Also in our study, no significant difference was detected in frequency of the allele groups of the patients and the control group.

Our study is the one that investigates the relation between EM, SJS and SJS/TEN overlap syndrome and TLR 9. To conclude, we could not show a significant relation between TLR 9 gene polymorphism and EM, SJS, SJS/TEN overlap syndrome. However, when our study is compared to other polymorphism studies, our study is limited in terms of the number of cases studied. Considering this fact, the conclusion we have reached needs to be supported by studies including higher number of cases to reveal a relation between other single nucleotide polymorphisms of TLR 9 gene and EM, SJS, SJS/TEN overlap syndrome.
References


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