

**Detection of Epstein-Barr virus in synovial fluid of rheumatoid arthritis patients**

Mostafa Mahabadi<sup>1</sup>, Ebrahim Faghiloo<sup>2</sup>, Gholam Hossein Alishiri<sup>3</sup>, Mohamad Hossein Ataee<sup>4</sup>, Ramezan Ali Ataee<sup>5</sup>

<sup>1</sup> Ph.D. of Medical Virology, Assistant Professor, Department of Virology and Bacteriology, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

<sup>2</sup> Ph.D. of Medical Virology, Assistant Professor, Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>3</sup> M.D. Rheumatology, Clinical Development Medical Center of Baqiyatallah Hospital, and Department of Rheumatology, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

<sup>4</sup> M.Sc. Molecular and Cellular Biology, Researcher, Research Center of Applied Microbiology, Baqiyatallah University of Medical Sciences, Tehran, Iran

<sup>5</sup> Ph.D. of Medical Microbiology, Professor, Department of Virology and Bacteriology, and Clinical Development Medical Center of Baqiyatallah Hospital, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

**Type of article:** Original

**Abstract**

**Introduction:** Rheumatoid arthritis (RA) is one of the most common chronic inflammatory disorders. Genes and environmental factors contribute to RA. Epstein-Barr Virus (EBV) has been considered as one of the RA pathogenesis. The aim of this study was to detect the EBV genome in patients with RA.

**Methods:** In this cross-sectional study, 50 samples of synovial fluid were obtained from patients with RA from 2010–2012. Using a standard of the EBV genome and EBNA-1-specific primers, the method of PCR was set up. Then, all of the samples of synovial fluids separately were subjected to DNA extraction and Polymerase Chain Reaction (PCR) amplification. Data were analyzed using SPSS version 18.0. The statistical analysis was performed by the t-test.

**Results:** The demographic and laboratory characteristic assay revealed that the mean age of patients was 49, and the patients were 60% males and 40% females. In addition, in all cases, the mean rheumatoid factor (RF) levels of the patients were below the normal level. The results of this study showed that the PCR was able to detect EBV DNA in > 60% of the cases.

**Conclusion:** The results of this study indicated that EBV was frequently detected in the synovial fluid of RA patients. Thus, EBV may be a strong candidate that can act at several levels of the pathophysiology of RA. However, these findings also indicated that EBV may play a role in the pathogenesis of RA. However, the possible relationship between RA and EBV must be determined by further research.

**Keywords:** rheumatoid arthritis, EBV, EBNA-1, PCR

**1. Introduction**

Epstein-Barr virus is one of the human herpes viruses with a 184 kbp-sized, double-stranded DNA genome. It is categorized in the gamma 1 or lymphocryptovirus (LCV) genus, gamma herpes virus subfamily, and herpesviridae family. Epstein-Barr virus is a ubiquitous virus that infects 95% of the world's population (1). In 1964, EBV was the first virus detected from human neoplastic cells (2). EBV is known to have a relationship with Burkitt lymphoma, nasopharyngeal carcinoma (NPC), Hodgkin's lymphoma, and gastric carcinoma. In addition, the association

**Corresponding author:**

Professor Dr. Ramezan Ali Ataee, Department of Virology and Bacteriology, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Tel: +98.2182483427, Fax: +98.2188620843, Email: [Ataee@bmsu.ac.ir](mailto:Ataee@bmsu.ac.ir), and [Ataee216@gmail.com](mailto:Ataee216@gmail.com)

Received: October 16, 2015, Accepted: February 23, 2016, Published: March 2016

iThenticate screening: February 21, 2016, English editing: March 01, 2016, Quality control: March 06, 2016

© 2016 The Authors. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

between EBV and some autoimmune diseases, such as systemic lupus erythematosus (SLE) and RA, have been documented (2). The association between EBV and rheumatoid arthritis was first reported by Alspaugh and Tan (3). This study showed that sera from RA patients contain antibodies that precipitate antigens associated with EBV-infected B cell lines (3). The antigen was named RA nuclear antigen (RANA), and it is identical to Epstein-Barr nuclear antigen 1 (EBNA-1) (4). Many other studies have shown that patients who have rheumatoid arthritis have higher levels of serum antibodies against early antigen (EA), Epstein-Barr nuclear antigen (EBNA), and viral capsid antigen (VCA) than normal people (5-11). There is a similarity in the sequence and the cross reaction between Epstein-Barr virus proteins and joint proteins (12). However, several studies have shown that Epstein-Barr virus was present in the synovial tissue obtained from RA patients (13-19). Scotet et al. showed that synovial tissues from RA patients contain cytotoxic T lymphocytes specific for EBV-encoded proteins (20). The synovial tissue of an RA patient has a fibroblast-like cell line that contains EBV (21). However, some studies have failed to detect EBV DNA in synovial tissue from RA patients (22), but the expression of the latent membrane protein 1 (LMP1) and the small EBV-encoded RNAs (EBERs) of EBV have been reported in synovial cells from eight out of 34 RA patients (17). This study was designed to evaluate the presence of the EBV genome in the synovial tissue of RA patients.

## **2. Material and Methods**

### *2.1. Patient Population*

This cross sectional study was performed between 2010 and 2012 on 50 patients. The samples of synovial fluids that were used for this study were taken from 50 patients with rheumatoid arthritis who were admitted to Baqiyatallah Hospital in Tehran, Iran. For each case, information of age, gender, and laboratory parameters was collected. These samples were related to patients with confirmed rheumatoid arthritis from 2010–2012, and the mean RF factor of all patients was below the normal level. This study was approved by the Research Ethics Committee at Baqiyatallah University of Medical Sciences, Tehran, Iran.

### *2.2. Extraction of DNA*

DNA was isolated from samples of the synovial fluids using QIAamp DNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Briefly, a 500- $\mu$ l aliquot of synovial fluids was added to 6  $\mu$ l of mutanolysin, and the mixture was incubated at 37 °C for 30 min. Then, 50  $\mu$ l of Proteinase K with a concentration of 20 mg/ml and 500  $\mu$ l of AL buffer were added, and the sample was incubated at 56 °C for 30 min. After this, 500  $\mu$ l of ethanol were added, and the DNA was purified by using the columns provided in the kit (Qiagen, Valencia, CA).

### *2.3. Polymerase chain reaction (PCR)*

The PCR was carried out with forward primer (5'-GGGCCAAGACATAGAGATGGTGTCC-3') and reverse primer (5'-GCGGTGGAGACCCGGATGATGATGA-3') (23). The PCR for the amplification of the EBV EBNA-1 was performed by adding 10  $\mu$ L of the extracted DNA to 40  $\mu$ L of the reaction mixture that contained 26  $\mu$ L distilled water, 5  $\mu$ L 10X PCR buffer, 4  $\mu$ L dNTP, 2.5  $\mu$ L MgCl<sub>2</sub>, 1  $\mu$ L forward primer, 1  $\mu$ L reverse primer, and 0.5  $\mu$ L of Taq DNA polymerase. Amplification conditions consisted of 5 min at 95 °C, 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min with a final extension at 72 °C for 10 min. All PCRs were run with positive and negative controls. The PCR amplicons were 226 bp. The PCR products were analyzed by electrophoresis on a 1.5% agarose gel and viewed under UV light.

### *2.4. Statistical methods*

The data were analyzed using SPSS statistical package version 18.0 (SPSS, Inc, Chicago, IL, USA). The statistical analysis was performed by the chi-squared test and Fisher's exact test. The t-test was performed to compare the means of the continuous variables. All P values were two-sided and considered significant at  $p < 0.05$ .

## **3. Results**

Out of the synovial fluid samples from the 50 patients with confirmed rheumatoid arthritis, 30 cases (60%) had EBV DNA in their synovial fluids. This represented a significant difference with the control group ( $p \leq 0.04$ ). Among the 50 patients, 30 samples were from men (60%) and 20 samples were from women (40%). The statistical analysis of the patients with rheumatoid arthritis and the mean changes of the laboratory parameters, including rheumatoid factor, Alanine Transaminase, Alanine Aminotransferase, White Blood Cell Count in Blood, Platelets, C Reactive Protein, Erythrocyte Sedimentation Rate, Alkaline Phosphatase, anti-cyclic citrullinated peptide antibody, White Blood Cell Count in Synovial fluid, and Polymorphonuclear leukocytes in the synovial fluid, are shown in Table 1. The mean age for all patients was 49. The mean levels of the laboratory parameters RF, ALT, AST, and WBC in

blood; PLT, CRP, ESR, ALP, Anti-CCP, and WBC in the synovial fluid; and PMN in the synovial fluid were as 1.9167, 28.9048, 21.4286, 707.2222, 247.7600, 1.5217, 31.2000, 174.1250, 138.22424, 336.9767, and 68.9512 per ml, respectively. The mean duration of disease (years) and duration of treatment (years) for all patients were 5.8068 and 3.6667, respectively.

**Table 1.** Statistical analysis of patients and laboratory parameters

Variable	n	Minimum	Maximum	Mean	SD
Age	60	16.00	70.00	49.0000	13.24249
Duration of disease (year)	44	1.00	30.00	5.8068	4.75592
Number of Injection	22	0.00	10.00	3.4091	2.71958
Duration of treatment (year)	3	2.00	6.00	3.6667	2.08167
Cumulative drug Dosage	23	0.20	2.00	.6609	0.52374
WBC in Blood	60	1020.00	13500.00	7072.2222	2237.98092
PLT	60	126.00	451.00	247.7600	86.42881
CRP	60	1.00	4.00	1.5217	0.89796
ESR	60	4.00	96.00	31.2000	21.28628
RF	60	1.00	3.00	1.9167	0.66856
AST	21	10.00	72.00	21.4286	13.61092
ALT	21	6.00	145.00	28.9048	29.77399
ALP	8	88.00	258.00	174.1250	61.48969
ANTICCP	19	0.00	500.00	138.2242	142.21142
WBC in Synovial fluid	43	1000.00	10000.00	4336.9767	2731.18306
PMN in Synovial fluid	41	25.00	95.00	68.9512	16.87891

#### 4. Discussion

Rheumatoid arthritis (RA) as an inflammatory disorder that involves synovial destruction. The etiology of RA still is unknown, but genetic and environmental factors may contribute to the disease (24). Among environmental factors, viruses play important roles for triggering this disease, and, among them, Epstein-Barr virus (EBV) was first suggested as a causative agent (3). This study investigated EBV DNA genomic in RA patients' synovial fluids using PCR methods for the EBV EBNA-1 gene. Our results showed that EBV DNA was detected in  $\geq 60\%$  of rheumatoid arthritis patients' synovial fluids. The high prevalence of EBV that was detected in this study was similar to a previous study. For example, Takeda et al. evaluated the existence of EBV DNA in the synovial tissue of patients with RA using Southern blot hybridization and PCR methods. Epstein-Barr virus DNA was detected by PCR in 15 of the 32 samples from the RA patients (47%), but it was not detected in any of the 30 osteoarthritis patients. This study demonstrated that EBV was detected frequently in the synovial tissue of rheumatoid arthritis patients (25). Synovial tissue from 84 patients with RA and 81 patients with non-RA were investigated for EBV DNA/RNA using PCR method, and the results indicated that the EBV DNA and/or RNA were significantly higher in RA patients than in non-RA patients. Thus, the results suggested the EBV is related to RA pathogenesis (26).

Chiu and et al.'s study showed that all synovial fluid samples from patients with non-resolving inflammation of rheumatoid arthritis showed positive expression of Epstein-Barr virus-encoded small RNA1 (EBER1), whereas none of the synovial samples from patients with osteoarthritis showed expression of EBER1. They suggested that non-resolving rheumatoid arthritis inflammation is related strongly to the presence of EBER1 and Epstein-Barr virus. EBER1 is responsible for synovial fibroblast interleukin-6 production (27). Balandraud and et al.'s study results showed that patients with rheumatoid arthritis have higher EBV load in peripheral blood lymphocytes than healthy controls. This study recommended anti-EBV antibody responses should be considered as one of the chronic autoantibody responses that are most relevant to the development of rheumatoid arthritis (28). One study produced results that showed elevated EBV load in their peripheral blood mononuclear cells (PBMCs), which demonstrated in patients with RA, the Epstein-Barr virus DNA load was increased almost 10-fold. This study showed that patients with rheumatoid arthritis had elevated EBV DNA viral load in their peripheral blood (29). This study showed a high prevalence of EBV DNA detection in synovial fluid samples. So, this study suggested that EBV could play a role in the pathogenesis of rheumatoid arthritis. The EBV viral particles may act as potent superantigens that are involved in triggering inflammatory diseases, such as RA. It should be noted that this is not the only superantigen that contributes to RA disease, since recent research has indicated that several other bacterial superantigens, including

Staphylococcal (30-32) and Mycoplasma superantigens (33, 34), were reported in the synovial fluids of RA patients. In addition, the existence of Staphylococcal superantigens in CSF of meningitides patients were reported (35). However, the similarity point of the individual multiple superantigens in pathophysiology of the disease is unknown, and more research will be required to clarify their roles.

### **5. Conclusions**

The main findings of this study indicate that the EBV DNA gene were amplified in 30 cases (60%) of synovial fluids of rheumatoid arthritis patients. However, the results of this study suggested a possible role for the EBV in the pathophysiology of RA as an autoimmune disease and specific antiviral treatment may be recommended for RA patients. More research is needed to define the pathophysiology of RA disease.

### **Acknowledgments:**

The authors thank the staff of the Deputy of the Clinical Development Medical Center of Baqiyatallah Hospital for their assistance. In addition, the authors thank the staff of the Department of Virology, Rheumatology, and Microbiology for their cooperation.

### **Conflict of Interest:**

There is no conflict of interest to be declared.

### **Authors' contributions:**

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

### **References**

- 1) Epstein MA, Barr YM, Achong BG. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet*. 1964; 1(7335): 702–3. doi: 10.1016/S0140-6736(64)91524-7, PMID: 14107961.
- 2) Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer*. 2004; 4(10): 757–68. PMID: 15510157.
- 3) Alspaugh MA, Jensen FC, Rabin H, Tan EM. Lymphocytes transformed by Epstein-Barr virus: induction of nuclear antigen reactive with antibody in rheumatoid arthritis. *J Exp Med*. 1978; 147(4): 1018–27. PMID: 206643.
- 4) Baboonian C, Halliday D, Venables PJ, Pawlowski T, Millman G, Maini RN. Antibodies in rheumatoid arthritis react specifically with the glycine alanine repeat sequence of Epstein-Barr virus nuclear antigen-1. *Rheumatol Int*. 1989; 9: 161–6. PMID: 2481875.
- 5) Kosaka S. Detection of antibody to a new antigen induced by Epstein-Barr virus in rheumatoid arthritis. *Tohoku J Exp Med*. 1979; 127: 157–60. PMID: 216133.
- 6) Catalano MA, Carson DA, Slovin SF, Richman DD, Vaughan JH. Antibodies to Epstein-Barr virus-determined antigens in normal subjects and in patients with seropositive rheumatoid arthritis. *Proc Natl Acad Sci U S A*. 1979; 76: 5825–8. PMID: 230491.
- 7) Alspaugh MA, Henle G, Lennette ET, Henle W. Elevated levels of antibodies to Epstein-Barr virus antigens in sera and synovial fluids of patients with rheumatoid arthritis. *J Clin Invest*. 1981; 67: 1134–40. PMID: 6259211.
- 8) Ferrell PB, Aitcheson CT, Pearson GR, Tan EM. Seroepidemiological study of relationships between Epstein-Barr virus and rheumatoid arthritis. *J Clin Invest*. 1981; 67: 681–7. PMID: 6259207.
- 9) Sculley TB, Walker PJ, Moss DJ, Pope JH. Identification of multiple Epstein-Barr virus-induced nuclear antigens with sera from patients with rheumatoid arthritis. *J Virol*. 1984; 52: 88–93. PMID: 6090712.
- 10) Hazelton RA, Sculley TB, Pope JH. The prevalence of antibodies to an Epstein-Barr virus-induced polypeptide (EBNA-2) in the sera of rheumatoid arthritis families. *Br J Rheumatol*. 1987; 26: 193–6. PMID: 3034370.
- 11) Shimizu N, Yamaki M, Sakuma S, Ono Y, Takada K. Three Epstein-Barr virus (EBV)-determined nuclear antigens induced by the BamHI E region of EBV DNA. *Int J Cancer*. 1988; 41: 744–51. PMID: 2835324.
- 12) Fox R, Sportsman R, Rhodes G, Luka J, Pearson G, Vaughan J. Rheumatoid arthritis synovial membrane contains a 62,000-molecular-weight protein that shares an antigenic epitope with the Epstein-Barr virus-encoded associated nuclear antigen. *J Clin Invest*. 1986; 77: 1539–47. PMID: 2422209.

- 13) Alspaugh MA, Shoji H, Nonoyama M. A search for rheumatoid arthritis-associated nuclear antigen and Epstein-Barr virus specific antigens or genomes in tissues and cells from patients with rheumatoid arthritis. *Arthritis Rheum.* 1983; 26: 712–20. PMID: 6190485.
- 14) Fox RI, Chilton T, Rhodes G, Vaughan JH. Lack of reactivity of rheumatoid arthritis synovial membrane DNA with cloned Epstein Barr virus DNA probes. *J Immunol.* 1986; 137: 498–501. PMID: 3013992.
- 15) Brousset P, Caulier M, Cantagrel A, Dromer C, Mazieres B, Delsol G. Absence of Epstein-Barr virus carrying cells in synovial membranes and subcutaneous nodules of patients with rheumatoid arthritis. *Ann Rheum Dis.* 1993; 52: 608–9. PMID: 8215625.
- 16) Zhang L, Nikkari S, Skurnik M, Ziegler T, Luukkainen R, Mo'ttonen T, et al. Detection of herpesviruses by polymerase chain reaction in lymphocytes from patients with rheumatoid arthritis. *Arthritis Rheum.* 1993; 36: 1080–6. doi: 10.1002/art.1780360808, PMID: 8343184.
- 17) Takei M, Mitamura K, Fujiwara S, Horie T, Ryu J, Osaka S, et al. Detection of Epstein-Barr virus-encoded small RNA 1 and latent membrane protein 1 in synovial lining cells from rheumatoid arthritis patients. *Int Immunol.* 1997; 9: 739–43. PMID: 9184919.
- 18) Mousavi Jazi M, Bostrom L, Lovmark C, Linde A, Brytting M, Sundqvist VA. Infrequent detection of cytomegalovirus and Epstein-Barr virus DNA in synovial membrane of patients with rheumatoid arthritis. *J Rheumatol.* 1998; 25: 623–8. PMID: 9558160.
- 19) Saal JG, Krimmel M, Steidle M, Gerneth F, Wagner S, Fritz P, et al. Synovial Epstein-Barr virus infection increases the risk of rheumatoid arthritis in individuals with the shared HLA-DR4 epitope. *Arthritis Rheum.* 1999; 42: 1485–96. PMID: 10403278.
- 20) Scotet E, David-Ameline J, Peyrat MA, Moreau-Aubry A, Pinczon D, Lim A, et al. T cell response to Epstein-Barr virus transactivators in chronic rheumatoid arthritis. *J Exp Med.* 1996; 184: 1791–800. PMID: 8920867, PMCID: PMC2192863.
- 21) Koide J, Takada K, Sugiura M, Sekine H, Ito T, Saito K, et al. Spontaneous establishment of an Epstein-Barr virus-infected fibroblast line from the synovial tissue of a rheumatoid arthritis patient. *J Virol.* 1997; 71: 2478–81. PMID: 9032386.
- 22) Alspaugh MA, Shoji H, Nonoyama M. A search for rheumatoid arthritis-associated nuclear antigen and Epstein-Barr virus specific antigens or genomes in tissues and cells from patients with rheumatoid arthritis. *Arthritis Rheum.* 1983; 26: 712–20. PMID: 6190485.
- 23) Wang W, Chien YC, Jan JS, Chueh CM, Lin JC. Consistent Sequence Variation of Epstein-Barr Virus Nuclear Antigen 1 in Primary Tumor and Peripheral Blood Cells of Patients with Nasopharyngeal Carcinoma. *Clin Cancer Res.* 2002; 8: 2586-90. PMID: 12171888.
- 24) Sewell KL, Trentham DE. Pathogenesis of rheumatoid arthritis. *Lancet.* 1993; 1: 283–6. PMID: 8093927.
- 25) Takeda T, Mizugaki Y, Matsubara L, Imai S, Koike T, Takada K. Lytic Epstein-Barr virus infection in the synovial tissue of patients with rheumatoid arthritis. *Arthritis Rheum.* 2000; 43: 1218–25. doi: 10.1002/1529-0131(200006)43:6<1218::AID-ANR4>3.0.CO;2-2, PMID: 10857780.
- 26) Saal JG, Krimmel M, Steidle M, Gerneth F, Wagner S, Fritz P, et al. Synovial Epstein-Barr virus infection increases the risk of rheumatoid arthritis in individuals with the shared HLA-DR4 epitope. *Arthritis Rheum.* 1999; 42: 1485–96. doi: 10.1002/1529-0131(199907)42:7<1485::AID-ANR24>3.0.CO;2-7, PMID: 10403278.
- 27) Chiu WC, Chen CM, Cheng TT, Huey-Ling You HL, Yu SF, Weng LH, et al. EBV-encoded small RNA1 and nonresolving inflammation in rheumatoid arthritis. *Kaohsiung J of Med Sci.* 2013; 29: 606-10. doi: 10.1016/j.kjms.2013.04.002, PMID: 24183354.
- 28) Balandraud N, Roudier J, Roudier C. Epstein-Barr virus and rheumatoid arthritis. *Autoimmunity Reviews.* 2004; 3: 362–7. doi: 10.1016/j.autrev.2004.02.002, PMID: 15288002.
- 29) Balandraud N, Meynard JB, Auger I, Sovran H, Mugnier B, Revirion D, et al. Epstein-Barr Virus Load in the Peripheral Blood of Patients With Rheumatoid Arthritis. *Arthritis Rheum.* 2003; 48: 1223–8. doi: 10.1002/art.10933, PMID: 12746895.
- 30) Ataee RA, Karami A, Izadi M, Aghania A, Ataee MH. Molecular screening of staphylococcal enterotoxin B gene in clinical isolates. *Cell J.* 2011; 13: 187-92. PMID: 23508641. PMCID: PMC3584468.
- 31) Ataee RA, Ataee MH, Alishiri GH, Esmaeili D. Staphylococcal enterotoxin C in synovial fluid of patients with rheumatoid arthritis. *Iran Red Crescent Med J.* 2014; 16: e16075. doi: 10.5812/ircmj.16075.
- 32) Zahiri YS, Ataee RA, Alishiri GH, Movahedi M. Bacteriological and molecular assessment of staphylococcal enterotoxin e in the blood of patients with rheumatoid arthritis. *Jundishapur J Microbiol.* 2015; 8: e16621. doi: 10.5812/jjm.16621.

- 33) Golmohammadi R, Ataee RA, Alishiri GH, Mirnejad R, Mehrabi TA, Esmaili D. Design of PCR-based method for detection of a gene-encoding *Mycoplasma arthritidis* mitogen superantigen in synovial fluid of rheumatoid arthritis patients. *Iran J Microbiol.* 2014; 6: 415-20. PMID: 25926960. PMCID: PMC4411428.
- 34) Ataee RA, Golmohammadi R, Alishiri GH, Mirnejad R, Najafi A, Esmaili D et al. Simultaneous Detection of *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Mycoplasma arthritidis* in Synovial Fluid of Patients with Rheumatoid Arthritis by Multiplex PCR. *Arch Iran Med.* 2015; 18: 345-50. doi: 015186/AIM.004.
- 35) Ataee RA, Mehrabi-Tavana A, Izadi M, Hosseini SM, Ataee MH. Bacterial meningitis: a new risk factor. *J Res Med Sci.* 2011; 16: 207-10. PMID: 22091233, PMCID: PMC3214305.