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Inverse Relation between MxA Gene Expression and Age in Multiple Sclerosis Patients Reveals a Gender Difference in Response to Interferon Therapy

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory, multifocal, immune-mediated disease of the central nervous system that women are at a higher risk to acquire than men. Myxovirus resistance protein A (MxA) is used as a predictive marker of bioactivity of interferon-beta (IFN-β) therapy in MS patients. This study was undertaken in west of Iran to investigate gender differences in the expression level of MxA in relapsing-remitting MS (RRMS) patients receiving IFN-β therapy, compared with untreated normal individuals.

The expression level of the MxA gene in RRMS samples were compared to untreated normal individuals using the extracted RNA from whole blood of 50 RRMS patients (31 females and 19 males) and 50 normal controls (29 females and 21 males). All patients were HLA-DRB1*15 negative and responded to IFN-β with a normal vitamin D level. The level of MxA gene expression was measured by quantitative RT-PCR.

The levels of gene expression were decreased in RRMS patients compared with normal counterparts (p=0.025). This decrease was significant in females (p=0.009) compared to males (p>0.05). The level of expression varied across different female age-groups with no significant difference in women younger than 30 years, but a significant decrease in expression in women between 30 to 40 years or above 40 years of age was seen.

There was neither linear correlation between the MxA expression level and risk of expanded disability status scale of Kurtzke (EDSS); nor were there any significant correlation between expression status of MxA and duration of the disease. In conclusion, the decrease in the level of MxA expression in MS patients treated with IFN-β when compared to normal individuals was significantly lower in females than males. This demonstrated a gender bias in the response to IFN-β therapy that will need to be confirmed and further investigated in more detail.

Keywords: Gene expression; Interferon-beta; Multiple sclerosis; Mx1

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease that can harm the central nervous system and result in mental and physical disabilities.[1,2] This disease mainly affects middle-aged adults, but women may be 9 to 13 times at greater risk to acquire MS than men.[3] There is no clear evidence as yet on immuno-pathogenic mechanisms involved in the development of the disease although, genetically predisposed people are more likely to contract MS.[4-6] Environmental factors may intensify the disease and there are no known factors that suppress disease activity. Unfortunately, the number of people with MS is reportedly increasing in different countries,[7] particularly in Iran.[8] The inflammatory damages display the autoimmune trait of MS, in this regard association analysis and expression assessment for some of these susceptibility genes, especially HLA and cytokine genes, in Iranian patients were previously investigated.[9-11]

The most common immune-modulatory therapy used for MS is interferon beta (IFN-β).[12] However, the effectiveness of IFN-β therapy is difficult to monitor[13] and the clinical response to IFN-β therapy may exhibit considerable variability in the number and types of MS relapsed cases.[14] Moreover, the effectiveness of IFN-β therapy might vary between men and women because of the marked physiological differences between the genders in their immune response.[15] For example, IFN treatment is sensitive to the regulation of the female hormone, estrogen,[16] .Estrogen affects all the major cells of the immune system including T and B cells, macrophages, dendritic cells and natural killer cells.[15-19]

Recently, a number of studies have shown that the measurement of baseline levels of myxovirus resistance protein A (MxA) mRNA is a potential biomarker of IFN-β bioactivity[20-22] that can be used to predict the response of MS to IFN-β treatment.[23,24] MxA is a 75-kDa cytoplasmic protein member of the dynamin superfamily that is induced by type I interferons (IFN-α and IFN-β). MxA can inhibit the replication of single-strand RNA viruses, and is stable in the absence of viral infections.[25] In addition; MxA has a possible role in the signal transduction of the apoptotic pathway.[26]

Given the potential gender differences associated with MS, the aim of this study was to examine the differences in the gene expression levels of MxA in relapsing-remitting MS (RRMS) female and male patients receiving IFN-β therapy, compared with untreated normal individuals.

MATERIALS AND METHODS

Patients and Controls

Blood sample was collected from 50 RRMS patients (31 females and 19 males, mean age: 37.4±5.2, age of onset: 31.36±2.4, duration of disease: 6.1±3.1 years, expanded disability status scale EDSS: 2.72±2.3) during interferon treatment and also samples from 50 healthy controls (29 females and 21 males, mean age: 36.9±4.6) receiving no treatment. Clinical profiles of MS patients and healthy individuals are shown in Table 1. RRMS in patients was identified with magnetic resonance imaging (MRI), based on McDonald criteria.[27,28] All of the patients were HLA-DRB1*15 negative,[29] they were clinically stable, and received daily injections of IFN-β (CinnoVex, Cinagene Company, Iran) during period of blood collection .

Blood Sampling

A 5 mL peripheral blood sample was obtained from each participant–control and patients groups. The local Ethics Committee of Shahid Beheshti University of Medical Sciences approved (No. 3275) the collection of blood samples at Iran's MS Society Clinic and Imam Hossein Hospital in Tehran.

Table 1. Demographic and clinical profiles of patients with relapsing-remitting multiple sclerosis and healthy controls

Variables	MS patient	Control
Female/Male [no. (%)]	31 (62%)/19(38%)	29 (58%)/21 (42%)
Age (mean ± SD, Years)	37.4 ± 5.2	36.9 ± 4.6
Age range (Years)	17-69	19-63
Age of onset (mean ± SD, Years)	31.36 ± 2.4	-
Duration (mean ± SD, Years)	6.1 ± 3.1	-
EDSS[a](mean ± SD)	2.72 ± 2.3	-

a; Expanded disability status scale of Kurtzke.

Table 2. The sequences of probes and primers for expression analysis of MX dynamin like GTPase 1(MXA) gene and hypoxanthine phosphoribosyltransferase 1 (HPRT1) as reference gene

Property	HPRT1	MxA
NCBI accession number	BC000578	M30817
Forward primer	AGCCTAAGATGAGAGTTC	CAGCACCTGATGGCCTATCAC
Amount of use	10 picomol	10 picomol
Reverse primer	CACAGAACTAGAACATTGATA	GAGCATGAAGAACTGGATGATCAA
Amount of use	10 picomol	10 picomol
Probe	Fam-CATCTGGAGTCCTATTGACATCGC-Tamra	Fam-AGCAAGCGCATCTCCAGCCACATC -Tamra
Amount of use	7.5 picomol	7.5 picomol
Amplicon length	101bp	81bp
Optimized annealing temperature	60°C	60°C

Quantitative Real Time-PCR

The RNA was extracted using Geneall Hybrid-R blood RNA extraction kit (cat No.305-101, Korea) according to the manufacturer's instruction. The cDNA was synthesized with Biosystems High-Capacity cDNA Reverse Transcription Kit (PN: 4375575, USA). Allele ID 7 (Premier Biosoft, Palo Alto, USA) was applied to design the sequence of the specific probes and primers shown in Table 2. The HPRT1 housekeeping gene was used as a positive control and internal reference in the expression studies. The Applied Biosystems TaqMan Universal PCR Master Mix (PN: 4304449, USA) was used to run the Real-time quantitative PCR in a Corbett Rotor Gene 6000 machine (Corbett Life Science, USA). The no template control (NTC) was used as a negative control in each run for each primer. The expression analysis for each sample was performed in triplicates.

Statistical Methods

Independent T-test was adopted in order to compare data obtained from participants. The one-way ANOVA test was also used to complete statistical computations. Mann–Whitney test was applied to test for differences in each group with small size, due to the skewed distributions of data. The *p* value for the level of significance was set at $p \leq 0.05$. The analyses were performed using the SPSS version 18 windows statistical package (Chicago, IL, USA).

RESULTS

MxA Expression Level and Risk of RRMS

The expression level of MxA gene in RRMS patients was compared with normal individuals (Table 3). The data for the total number of participants (i.e.,

Table 3. MxA expression level in relapsing-remitting multiple sclerosis patients, compared with control group, based on age and sex of the participants

MxA expression		Control no.	RRMS patient no.	*p* value	Expression ratio	Std. Error	95% CI
Total		50	50	**0.025**	0.478	0.498	0.123-2.536
Male		21	19	0.27	0.817	0.747	0.676-2.327
Female		29	31	**0.009**	0.358	0.501	0.455-2.447
<30	Male	9	5	0.75	0.91	1.813	0.312-4.495
	Female	13	11	0.11	0.612	0.663	0.284-2.41
30-40	Male	7	6	0.71	0.8	1.615	0.879-4.101
	Female	9	9	0.03	0.301	1.144	1.4-6.107
>40	Male	5	8	0.24	0.69	1.174	0.668-4.871
	Female	7	11	0.01	0.212	0.792	0.456-1.773

RRMS, relapsing-remitting multiple sclerosis;

age and sex combined) revealed a statistically significant decrease in MS patients, compared to normal controls (p=0.025, expression ratio=0.478, 95% CI=0.123-2.536).

Comparing sex-linked subgroups of patients with normal individuals, the female patients showed a statistically significant decrease (p value=0.009, expression ratio = 0.358, 95% CI=0.455-2.447), whereas the male patients only showed a slight non-significant decrease in MXA expression (p=0.27).

The level of expression varied across different female age-groups, with no significant difference in women younger than 30 years (p=0.11), but a significant decrease in expression in women between 30 to 40 years (p=0.03) or above 40 years of age (p=0.01).

The Taqman Q-RT-PCR results are demonstrated as fold changes in the graphs (Figure 1).

MxA mRNA Expression Level and EDSS

The correlation between EDSS and MxA relative quantitation was measured in the RRMS patients group and no significant linear correlation (R^2=0.1876) was found between EDSS and MxA expression (Figure 2).

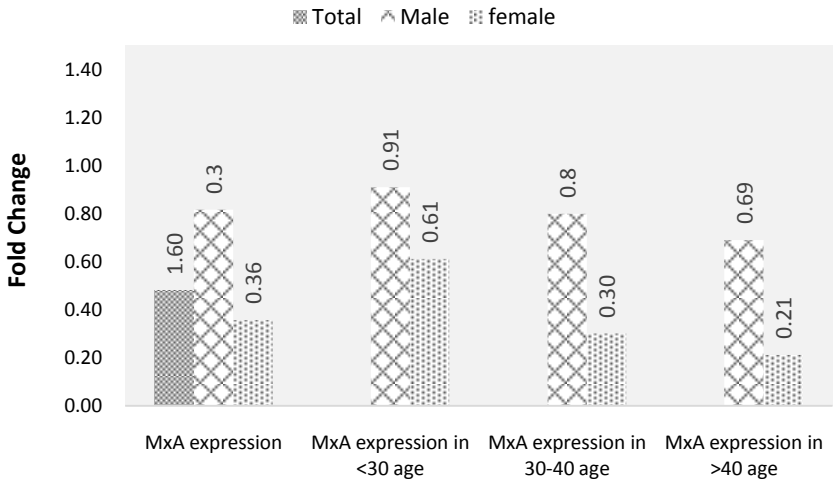

Figure 1. Fold change of MxA gene expression by Real time-PCR comparing multiple sclerosis patients of different ages

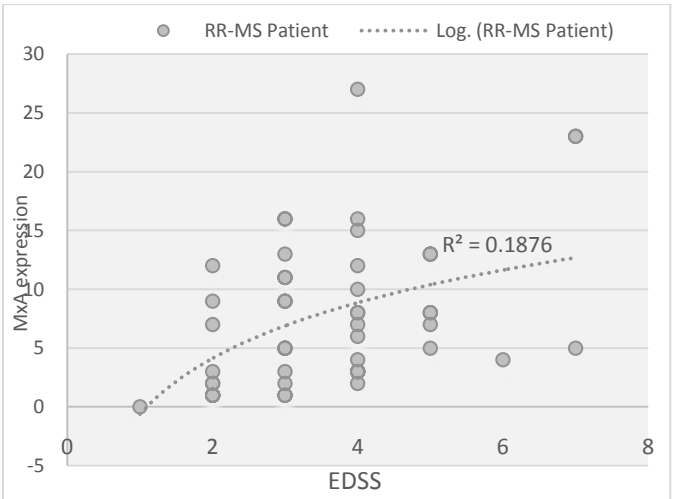

Figure 2. Correlation between MX dynamin like GTPase 1 MxA relative quantitation and disease severity (expanded disability status scale, EDSS scores) in multiple sclerosis patients

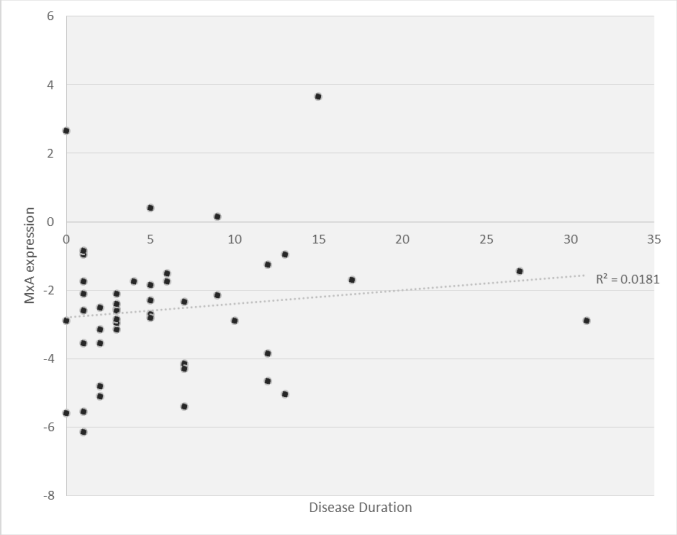

Figure 3. Correlation between MX dynamin like GTPase 1 MxA relative quantitation and disease duration in multiple sclerosis patients

MxA Expression and Disease Duration

The correlation between MxA relative quantitation and disease duration in RR-MS patient group is shown in Figure3. No significant correlation (R^2=0.0181) was found between expression status of MxA and duration of the disease.

DISCUSSION

MS is known as an immune-mediated demyelinating disease of the central nervous system, which is treated mostly by IFN-β.[12] However, some patients do not respond to this treatment as effectively as expected.[12] Since IFN induces MxA proteins in brain tissue of MS patients,[30] the MxA mRNA level has been used to predict relapses in patients diagnosed with MS.[31] single-nucleotide polymorphism (SNPs) on the MxA promoter region appear to have an important role in the pathophysiology of MS patients,[32] and increased levels of MxA and IFN in MS patients not treated with IFN have been attributed to an increase in viral infections.[33] Thus, the reliability of MxA as a biological marker for monitoring IFN-β therapy has been questioned in a number of studies.[23,24]

In this study we compared the expression levels of the MxA gene in RRMS patients with those of normal controls. The results showed a significant association between the age and sex of the patients, but not with the severity of their disease. That is, the MxA levels significantly decreased in women with RRMS, who were over 30 years of age. The male RRMS patients; however, did not show a similar pattern and there was no statistically significant decrease in their level of MxA. In contrast to the women, the male MxA expression level did not fluctuate across different age groups. These results are consistent with the known gender differences for MS as women are 9 to 11 times more likely to acquire MS than men.[3] The female and male sex hormones are known to enhance IFN action.[19,34] For example, estrogen was found to double the effect of IFN-γ and also to have a smaller additive effect for IFN-α in mouse cells.[16] Therefore, it is likely that the decrease in the MxA expression levels in women over 30 years of age was due to a reduced effect of IFN therapy in menopausal women.[35] This increase in IFN resistance during menopause may result directly or indirectly from the drop in the production of female sex hormones or from changes associated in the production and regulation of cytokines and other host factors.[15] In addition, the sex differences is reported to make women more susceptible for developing autoimmune diseases than men because of the role of sex chromosome complement in female bias and the increased cellular and hormonal immune responses in women. Therefore, it should be considered to explain the observed gender differences for MS in this study and for[36] future analyses of IFN resistance. Moreover, more studies are needed to elucidate the role

of sex hormones and other gender factors that might affect IFN treatment for MS or other autoimmune diseases[16] or viral infections.[37,38]

However, the important question still remains that if IFN induces MxA, then why did MxA decrease in responder patients who were taking IFN? The answer could be that MS patients without IFN injections had significantly lower levels of MxA expression compared with those who had been treated with IFN. Therefore, further studies are needed not only on patients being treated with IFN but also on patients who are not taking the same medications. Another question that will need to be resolved is whether sex hormone therapy in conjunction with IFN treatment in menopausal women might help restore the effectiveness of IFN treatment and raise the MxA gene levels. All together, the present study revealed that the decrease in the levels of MxA expression in MS patients treated with IFN-β when compared to untreated normal individuals was significantly lower in females than males. This demonstrated a likely gender bias in the response to IFN-β therapy.

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REFERENCES

1. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis results of an international survey. Neurology 1996; 46(4):907-11.
2. Berer K, Krishnamoorthy G. Microbial view of central nervous system autoimmunity. FEBS lett 2014; 588(22):4207-13.
3. Beeson PB. Age and sex associations of 40 autoimmune diseases. Am J Med 1994; 96(5):457-62.
4. Oksenberg JR, Barcellos LF. The complex genetic aetiology of multiple sclerosis. J Neurovirol 2000; 6(2):S10-4.
5. Milo R, Kahana E. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmun Rev 31; 9(5):A387-94.
6. Taheri M, Nemati S, Movafagh A, Eftekharian MM, Mirfakhraie R, Saberi M, et al. TRAIL gene expression analysis in multiple sclerosis patients. Hum Antibodies 2016; 24(1-2):338.
7. Eftekharian MM, Ghannad MS, Taheri M, Roshanaei G, Mazdeh M, Musavi M, et al. Frequency of viral infections and environmental factors in multiple sclerosis. Hum Antibodies 2015; 24(1-2):17-23.
8. Izadi S, Nikseresht A, Sharifian M, Sahraian MA, Jahromi AH, Aghighi M, et al. Significant increase in the prevalence of multiple sclerosis in iran in 2011. Iran J Med Sci 2014; 39(2):152-3.
9. Eftekharian, Mohammad Mahdi, et al. "RAR-related orphan receptor A (RORA): A new susceptibility gene for multiple sclerosis. Journal of the Neurological Sciences 2016; 369:259-62.
10. Eftekharian MM, Sayad A, Omrani MD, Ghannad MS, Noroozi R, Mazdeh M, Mirfakhraie R, Movafagh A, Roshanaei G, Azimi T, Inoko H. Single nucleotide polymorphisms in the FOXP3 gene are associated with increased risk of relapsing-remitting multiple sclerosis. Human Antibodies. 2016 21(Preprint):1-6.
11. Nazdik MK, Taheri M, Omrani MD, Sajjadi E, Arsang-Jang S, Koohpar ZK, Inoko H, Sayad A. Increased expression ratio of matrix metalloproteinase-9 (MMP9) and tissue inhibitor of matrix metalloproteinase (TIMP-1) RNA levels in Iranian multiple sclerosis patients. Human Antibodiesh 2016 22(Preprint):1-5.
12. Bertolotto A, Granieri L, Marnetto F, Valentino P, Sala A, Capobianco M, et al. Biological monitoring of IFN-β therapy in Multiple Sclerosis. Cytokine Growth Factor Rev 2015; 26(2):241-8.
13. Stone LA, Frank JA, Albert PS, Bash CN, Calabresi PA, Maloni H, McFarland HF. Characterization of MRI response to treatment with interferon beta-1b: contrast-enhancing MRI lesion frequency as a primary outcome measure. Neurology 1997; 49(3):862-9.
14. Arababadi MK, Mosavi R, Khorramdelazad H, Yaghini N, Zarandi ER, Araste M, et al. Cytokine patterns after therapy with Avonex®, Rebif®, Betaferon® and CinnoVex™ in relapsing-remitting multiple sclerosis in Iranian patients. Biomark Med 2010; 4(5):755-9.
15. Mekky RY, Abdelaziz AI. Sex hormones and HCV: an unresolved mystery. Expert Rev Gastroenterol Hepatol 2013; 7(1):69-75.
16. Panchanathan R, Shen H, Zhang X, Ho SM, Choubey D. Mutually positive regulatory feedback loop between interferons and estrogen receptor-α in mice: implications for sex bias in autoimmunity. PLoS One 2010; 5(5):e10868.
17. Ahmed SA, Penhale WJ, Talal N. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex

hormone action. Am J Patho 1985; 121(3):531-51.
18. Cutolo M, Sulli A, Capellino S, Villaggio B, Montagna P, Seriolo B, Straub RH. Sex hormones influence on the immune system: basic and clinical aspects in autoimmunity. Lupus 2004; 13(9):635-8.
19. Nakaya M, Tachibana H, Yamada K. Effect of estrogens on the interferon-γ producing cell population of mouse splenocytes. Biosci Biotechnol Biochem 2006; 70(1):47-53.
20. Cunningham S, Graham C, Hutchinson M, Droogan A, O'Rourke K, Patterson C, et al. Pharmacogenomics of responsiveness to interferon IFN-beta treatment in multiple sclerosis: a genetic screen of 100 type I interferon-inducible genes. Clin Pharmacol Ther 2005; 78(6):635-46.
21. Vallittu AM, Salmi AA, Erälinna JP. MxA protein induction in MS patients treated with intramuscular IFNβ-1a. Neurol Sci 2006; 26(6):438-43.
22. Hesse D, Sellebjerg F, Sorensen PS. Absence of MxA induction by interferon β in patients with MS reflects complete loss of bioactivity. Neurology 2009; 73(5):372-7.
23. Gilli F, Marnetto F, Caldano M, Sala A, Malucchi S, Capobianco M, et al. Biological markers of interferon-beta therapy: comparison among interferon-stimulated genes MxA, TRAIL and XAF-1. Mult Scler 2006; 12(1):47-57.
24. Matas E, Bau L, Martínez-Iniesta M, Romero-Pinel L, Mañé MA, Cobo-Calvo Á, et al. Baseline MxA mRNA expression predicts interferon beta response in multiple sclerosis patients. PloS one 2014; 9(11):e112758.
25. Ronni T, Melen K, Malygin A, Julkunen I. Control of IFN-inducible MxA gene expression in human cells. J Immunol 1993; 150(5):1715-26.
26. Mibayashi M, Nakade K, Nagata K. Promoted cell death of cells expressing human MxA by influenza virus infection. Microbiol Immunol 2002; 46(1):29-36.
27. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001; 50(1):121-7.
28. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011; 69(2):292-302.
29. Mazdeh M, Taheri M, Sayad A, Bahram S, Omrani MD, Movafagh A, et al. HLA genes as modifiers of response to IFN-beta-1a therapy in relapsing-remitting multiple sclerosis. Pharmacogenomics 2016; 17(5):489-98.
30. Al-Masri AN, Heidenreich F, Walter GF. Interferon-induced Mx proteins in brain tissue of multiple sclerosis patients. Eur J Neurol 2009; 16(6):721-6.
31. Van der Voort LF, Vennegoor A, Visser A, Knol DL, Uitdehaag BM, Barkhof F, et al. Spontaneous MxA mRNA level predicts relapses in patients with recently diagnosed MS. Neurology 2010; 75(14):1228-33.
32. Furuyama H, Chiba S, Okabayashi T, Yokota SI, Nonaka M, Imai T, et al. Single nucleotide polymorphisms and functional analysis of MxA promoter region in multiple sclerosis. J Neurol Sci 2006; 249(2):153-7.
33. Chieux V, Chehadeh W, Hautecoeur P, Harvey J, Wattré P, Hober D. Increased levels of antiviral MxA protein in peripheral blood of patients with A chronic disease of unknown etiology*. J Med Virol 2001; 65(2):301-8.
34. Hughes GC, Thomas S, Li C, Kaja MK, Clark EA. Cutting edge: progesterone regulates IFN-α production by plasmacytoid dendritic cells. J Immunol 2008; 180(4):2029-33.
35. Mekky RY, Hamdi N, El-Akel W, Esmat G, Abdelaziz AI. Estrogen-related MxA transcriptional variation in hepatitis C virus-infected patients. Transl Res 2012; 159(3):190-6.
36. Smith-Bouvier DL, Divekar AA, Sasidhar M, Du S, Tiwari-Woodruff SK, King JK, et al. A role for sex chromosome complement in the female bias in autoimmune disease. J Exp Med 2008; 205(5):1099-108.
37. Hayashi J, Kishihara Y, Ueno K, Yamaji K, Kawakami Y, Furusyo N, et al. Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. Arch Intern Med 1998; 158(2):177-81.
38. Villa E, Karampatou A, Cammà C, Di Leo A, Luongo M, Ferrari A, et al. Early menopause is associated with lack of response to antiviral therapy in women with chronic hepatitis C. Gastroenterology 2011; 140(3):818-29.