Reproductive dysfunctions in viral hepatitis

A. M. Kurmanova1, G. M. Kurmanova2, and V. N. Lokshin3

1Scientific Center of Obstetric, Gynecology and Perinatology, Almaty, Kazakhstan; 2Kazakh National Medical University, Almaty, Kazakhstan; and 3Reproduction Medicine Institute, Almaty, Kazakhstan

Abstract
The viral hepatitis abnormalities in the female reproductive system are due to hepatic and extrahepatic damage. On the background of HBV- and HCV-infections, menstrual disorders prevail in the structure of reproductive system pathology; disorders of reproductive function in the form of pregnancy loss and infertility are detected in each second case. Depression of T-cell immunity in the immune status is observed in the patients with intact reproductive function. When miscarriage was in past medical history then divergent changes subpopulations of lymphocytes are found. When patients have infertility, signify depression of T-cell immunity is observed with a decrease in total T-cells, T-helper cells and active lymphocytes.

Introduction
Viral hepatitis is a common infection worldwide. According to various estimates, number of people infected with HBV- and HCV-infections reaches 1 to 2 billion people [1,2]. Kazakhstan is considered to be a region with medium and high viral hepatitis B endemicity. The average carrier state corresponds to 10% [3]. In Kazakhstan, a huge number of symptom-free carriers are first and foremost of epidemiological importance [4].

HBV- and HCV-infections are seen as a mechanism, triggering a cascade of immunological and immunopathological responses that provide the basis for the development of a variety of reproductive functions disorders [5–7].

Abnormalities of female reproductive system in viral hepatitis are due to hepatic and extrahepatic injuries. The former, due to deficiency in the hemostatic system cause the development of hemorrhagic complications, while the latter represent dishormonal manifestations. However, issues of reproductive health of women with chronic viral hepatitis are understudied [8]. Consequently, studying reproductive health and features of immune response in women with chronic viral hepatitis is of interest.

Goal of the study was to study pathology structure of the reproductive system and immune status of women with chronic viral hepatitis.

Material and methods
A complete clinical and laboratory examination of 47 females with pathology of female reproductive system in the setting of chronic viral hepatitis in the middle age of 30.3 ± 6.5 years was conducted. Diagnosis of HBV-infection (in 38 women) was confirmed by HBsAg, antiHBs, antiHBeIgG markers detected in EIA and DNA PCR, and HCV-infection (in nine women) by anti-HCV and RNA, respectively. Depending on severity of reproductive function disorders, all women were divided into 3 groups: Group 1 (27 women) with intact reproductive function, Group 2 (15) with a history of miscarriage, Group 3 (5) with infertility. Control group consisted of 30 healthy women. In 10 women, the levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and lactogenic hormone were measured in blood plasma. Subpopulation analysis of peripheral blood lymphocytes was performed by flow cytometry on “FacsCalibur” device (Becton Dickenson Company, Franklin Lakes, NJ) using monoclonal antibodies to surface antigens of lymphocytes: mature T cells (CD3+), helper T cells (CD4+), cytotoxic lymphocytes (CD8+), natural killer cells (CD16+ and CD56+), B lymphocytes (CD20+), adhesion molecules (CD11b+), early CD25+ and late activation markers (CD95+ and HLA-DR+).

Results and discussion
Among clinical implications of chronic viral hepatitis, asthenic syndrome (60.0%) was prevalent, where patients complained of weakness, irritability, dizziness, fatigue and drowsiness. Next in frequency was dyspeptic syndrome (44.4%), which is manifested by nausea, vomiting and decreased appetite. Migraine-like and hemorrhagic (nasal, gingival bleeding) syndromes occurred in every third patient (33.3%).

Extrahepatic signs of HBV- and HCV-infection included endocrinopathy (46.5%), encephalopathy (33.3%), ophthalmopathy (17.8%), polyarthralgia and Raynaud’s syndrome (6.7%). Other comorbidities were observed with the following frequency, biliary dyskinesia (95.6%), pyelonephritis (44.4%), anemia (35.6%), intestinal dysbacteriosis (24.4%) and myocardiodystrophy (22.2%).

Menstrual irregularities were observed in the overwhelming (73.3%) majority of women examined (Table 1). Beginning of HBV- and HCV-infection development was characterized by prolonged, heavy menstruations that were gradually replaced by infrequent and short ones. In every fifth case patients complained of short and infrequent menstruation...
(hypomenstrual syndrome) and in each fourth case of absence of menstruation, including those associated with the onset of early menopause in 6.7% of patients and the development of a climacteric syndrome in 6.7% women. Algienorhea occurred in 11.1% of women.

Salpingo-oophoritis was detected in 31.1% of women, ovarian cysts in 20.2%, hysteromyoma in 15.6% and secondary infertility in 13.3%. With the same frequency (11.1%) adhesions process in the lower pelvis, diffuse mastopathy and endometriosis occurred. Cervical erosion was detected in 6.7% of patients.

Miscarriage occurred in 31.9% of cases, as a spontaneous abortion and non-developing pregnancy. In addition to pregnancy loss in women with chronic viral hepatitis, pregnancy was complicated with threatened miscarriage (33.3%), gestosis (17.8%), including preeclampsia and polydramnios (8.9%). There was poor uterine contraction strength registered during labor (35.6%) and premature detachment of normally situated placenta (13.5%). Postpartum hemorrhage was observed in 48.8% of patients.

It should be noted that reproductive function disorders in HBV- and HCV-infection are caused by generalized vascular disease. Immune complex vasculitis cause degenerative changes in hypothalamic-pituitary region with subsequent development of hormonal homeostasis dysfunction. Women with polycystic ovaria type dysmenorrhea in phase 1 of menstrual cycle exhibited increased concentration of FSH (90.7 IU/ml) in normal LH and lactogenic hormone concentrations. When hypomenstrual syndrome, there was a decrease in FSH level 1 of the menstrual cycle phase (5.8 mIU/l), the trend of increasing the concentration of LH and prolactin. Hyperprolactinemia was registered in 40% of women with amenorrhea (600 mIU/ml), while LH and FSH content were normal.

The data characterizing features of population structure of peripheral blood lymphocytes in different conditions of women’s reproductive functions are presented in Tables 2 and 3.

There was relative lymphocytosis (43.1–45.9%) was observed in all study groups. It was found that the first group of women with reproductive function disorders, relative content of CD3+, CD4+ lymphocytes were reduced significantly (p <0.05). However, their absolute content was not significantly different from control group. Relative and absolute contents of natural killer cells (CD16+ and CD56+) and lymphocytes expressing adhesion receptors (CD11b+) did not differ from controls either.

Analysis of the expression levels of activation markers has shown that relative and absolute contents of CD25+ lymphocytes and HLA-DR+ lymphocytes were significantly increased in patients with chronic viral hepatitis, pregnancy was associated with HBV- and HCV-infection (M ± m).

| Measure % | Control n = 30 | Undisturbed RF, n = 27 | Miscarriage n = 15 | Infertility n = 5 |
|-----------|---------------|------------------------|-------------------|------------------|
| Lymphocytes | 37.0 ± 7.3 | 45.9 ± 9.6 | 43.1 ± 6.0 | 44.8 ± 5.4 |
| CD3+ | 54.8 ± 3.2 | 25.9 ± 6.9* | 33.3 ± 11.7 | 29.2 ± 3.4* |
| CD4+ | 39.9 ± 3.3 | 22.1 ± 5.0* | 28.4 ± 7.9 | 23.2 ± 3.4* |
| CD8+ | 23.9 ± 2.8 | 19.7 ± 6.8 | 23.3 ± 6.4 | 25.0 ± 9.6 |
| CD16+ | 10.9 ± 2.6 | 15.2 ± 6.4 | 17.2 ± 7.0 | 17.0 ± 4.0 |
| CD20+ | 10.1 ± 3.0 | 14.2 ± 4.9 | 14.5 ± 7.4 | 12.0 ± 2.8 |
| CD11b+ | 10.8 ± 2.2 | 13.1 ± 3.4 | 15.0 ± 3.3 | 10.3 ± 3.6 |
| CD95+ | 6.8 ± 1.1 | 11.1 ± 3.6 | 13.5 ± 4.6 | 8.4 ± 4.3 |
| CD25+ | 1.3 ± 0.1 | 10.3 ± 3.3* | 11.0 ± 4.6* | 5.5 ± 5.0 |
| CD56+ | 16.5 ± 1.0 | 19.9 ± 7.7 | 22.5 ± 9.8 | 16.0 ± 15.0 |
| HLA-DR+ | 5.3 ± 0.6 | 13 ± 3.4* | 14.6 ± 4.7 | 14.5 ± 5.5 |

*Disparities with control are reliable with p ≤ 0.05.

| Measure ×10^3/l | Control n = 30 | Undisturbed RF, n = 27 | Miscarriage n = 15 | Infertility n = 5 |
|-----------------|---------------|------------------------|-------------------|------------------|
| Lymphocytes | 2500 ± 900 | 2800 ± 900 | 3000 ± 900 | 2500 ± 600 |
| CD3+ | 1359 ± 45.5 | 771.2 ± 372.5 | 919.0 ± 397.5 | 743.4 ± 214.0* |
| CD4+ | 1005 ± 39.9 | 630.3 ± 242.3 | 776.9 ± 273.1 | 578.0 ± 125.7* |
| CD8+ | 607.2 ± 27.6 | 559.8 ± 243.7 | 630.0 ± 200.1 | 617.0 ± 260.9 |
| CD16+ | 274.0 ± 27.4 | 448.2 ± 221.7 | 477.5 ± 227.2 | 416.6 ± 102.4 |
| CD20+ | 256.1 ± 23.4 | 426.7 ± 216.3 | 402.9 ± 215.7 | 279.2 ± 32.6 |
| CD11b+ | 266.7 ± 55.6 | 402.2 ± 175.3 | 397.9 ± 115.6 | 261.8 ± 67.9 |
| CD95+ | 166.7 ± 27.8 | 319.9 ± 139.6 | 364.3 ± 159.0 | 238.1 ± 23.8* |
| CD25+ | 3.4 ± 0.2 | 271.0 ± 99.7* | 292.7 ± 144.3* | 130.9 ± 68.9 |
| CD56+ | 425.0 ± 9.3 | 559.4 ± 261.6 | 622.0 ± 325.2 | 263.3 ± 235.8 |
| HLA-DR+ | 107.0 ± 5.7 | 333.1 ± 83.7* | 407.8 ± 162.2 | 284.6 ± 37.4* |

*Disparities with control are reliable with p ≤ 0.05.

(p <0.05) as compared with that of control group. In this group of patients an increased receptor expression to IL-2 (CD25) indicates an adequate immune response to lymphocytes activation with Th1 profile cytokines. In the study of co-relation between indicators of blood lymphocytes population structure it was found that in an intact reproductive function there was a direct strong relationship (r>0.7) observed between CD3+ and CD4+, CD16+ and CD25+, CD95+ and CD25+ (Figure 1).

In the second group (with a history of miscarriage), relative and absolute content of mature T lymphocytes, T helper cells and cytotoxic CD8+ cells were not significantly different from the control group. The activation markers analysis has found a significant (p <0.05) increase in the expression to IL-2 (CD25+). The content of other activation markers CD95+ and HLA-DR+ was not statistically different from the control group, due to the wide scatter of values. In the content of natural killer cells both high and low values were observed. CD16+ and CD56+ lymphocytes levels were high in half of the patients while the other half had low levels, and CD16+ level was much lower in 1/5 of patients.

In women with a history of miscarriage direct strong correlations were observed between the values of CD3+ and CD4+, CD3+ and CD8+, CD4+ and CD8+, CD4+ and CD8+, CD16+ and CD95+, CD16+ and CD25+, CD95+ and CD25+. Emergence of new strong correlations in the group of women with impaired reproductive function indicates the stress of the immune system with the inclusion of a great number of structural components (Figure 2).

Third group (infertile) exhibited significant reduction in the relative and absolute number of mature T-lymphocytes and
detected, particularly CD56 + CD16 + natural killer cells. This is associated with HBV- and HCV-infection in peripheral blood in correlations were only observed between CD95 + and absolute content of lymphocytes expressing late activation from the control group. In this group there was an increase of cellular cytotoxicity (ADCC), were not significantly different receptor expressing for Fc-IgG, i.e. capable of antibody dependent T-helper cells. The content of natural killer cells, both general and infertile female patients.

Figure 2. Correlation between lymphocytes subpopulation patterns in fertile female patients.

Figure 2. Correlation between lymphocytes subpopulation patterns in patients with miscarriage.

Figure 3. Correlation between lymphocyte subpopulation patterns in infertile female patients.

T-helper cells. The content of natural killer cells, both general and receptor expressing for Fc-IgG, i.e. capable of antibody dependent cellular cytoxicity (ADCC), were not significantly different from the control group. In this group there was an increase of absolute content of lymphocytes expressing late activation markers (CD95+, HLA-DR+). With infertility direct strong correlations were only observed between CD95 + and CD25 + lymphocytes (Figure 3).

The results of our study indicate that in miscarriages associated with HBV- and HCV-infection in peripheral blood in some patients, there were high levels of cytotoxic lymphocytes detected, particularly CD56 + CD16 + natural killer cells. This is consistent with the studies of Coulam C.B. (1997), Fukui A. (1999), De Maria A. (2011); Mardanian F. (2015) on the increase of CD56 + CD16 + cells fraction in peripheral blood in miscarriage [9–12].

At the same time in another group of patients with miscarriages there were sharply reduced levels of CD56 + and CD16 + lymphocytes registered, this downward trend remained in patients with infertility. Reduced levels of natural killer cells in peripheral blood were also noted and Baczkowski (2007), indicating that the percentage of total NK cells (CD56 + CD16+, CD56 + CD16− and CD56− CD16+) peripheral blood in infertile women was lower compared to control group while percentage of CD56 bright CD16− cells was higher in control group compared with the group of women with infertility [13].

Such differences in the cytotoxic lymphocytes content are obviously due to peculiarities of viruses or the presence of a number of endocrine dysfunctions [14]. As was demonstrated above, in the reproductive system pathology pattern associated with HBV- and HCV-infections, there are various menstrual disorders, ovarian cysts, hyperprolactinemia and endocrinopathies present. Therefore, further research is needed to study the immune status in the context of clinical manifestations.

Conclusion

Thus, against the background of HBV- and HCV-infection irregular menstrual domination structure of reproductive system in female patients; every other patient exhibits reproductive disorders in the form of pregnancy loss or infertility. There is T-cell immunity depression observed in the immune status of patients with intact reproductive function; with a history of miscarriage there is a picture of divergent changes in lymphocytes subpopulation patterns; while with infertility there is a severe depression of T-cell component, both with a decrease in total T-cells and T-helper cells and active lymphocytes.

Declaration of interest

Authors report no conflict of interests.

References

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepatol. 2004;11:97–107.
2. Morel P, Aufrere AL. Hepatite B: une nouvelle. Eurobiologiste 1992:26:109–16.
3. Shuratov IC, Kuatbaeva AM, Sermbaeva MK, et al. Characteristics of the epidemic process of viral hepatitis B in the city of Almaty. Hyg Epid Immunobiol 2001;1:2:85–9.
4. Shachgildyan IV, Onishenko GG, Chuchlovitch PA. The results of the study and the outstanding issues of epidemiology and prevention of parentral viral hepatitis in Russia. J Microbiol 1994;5:26–32.
5. Dionne-Odom J, Tita AT, Silverman NS. Hepatitis B in pregnancy screening, treatment, and prevention of vertical transmission. Am J Obstet Gynecol 2016;214:6–14.
6. Huang QT, Huang Q, Zhong M, et al. Chronic hepatitis C virus infection is associated with increased risk of preterm birth: a meta-analysis of observational studies. J Viral Hepat 2015;22:1033–42.
7. Giakoumelou S, Wheelhouse N, Cuschieri K, et al. The role of infection in miscarriage. Hum Reprod Update 2016;22:116–33.
8. Adambekov S, Kaiyrylyzyz A, Igisinnov N, Linkov F. Health challenges in Kazakhstan and Central Asia. J Epidemiol Community Health 2016;70:104–8.
9. Coulam CB, Clark DA, Beer AE, et al. Current clinical options for diagnosis and treatment of recurrent spontaneous abortion. Clinical guidelines recommendation committee for diagnosis and treatment of recurrent spontaneous abortion. Am J Reprod Immunol 1997;38:57–74.
10. Fukui A, Fujii S, Yamaguchi E, et al. Natural killer cell subpopulations and cytotoxicity for infertile patients undergoing in vitro fertilization. Am J Reprod Immunol 1999;41:413–22.

11. De Maria A, Bozzano F, Cantoni C, Moretta L. Revisiting human natural killer cell subset function revealed cytoltyic CD56 (dim) CD16+ NK cells as rapid producers of abundant IFN-gamma on activation. Proc Natl Acad Sci USA 2011;108:728–32.

12. Mardanian F, Kazerounizadeh M, Rashidi B. Evaluation of CD56(dim) and CD56(bright) natural killer cells in peripheral blood of women with IVF failures. Iran J Reprod Med 2015;13: 577–82.

13. Baczkowski T, Kurzawa R. Immunophenotypic profiles of peripheral blood lymphocytes on the day of embryo transfer in women undergoing in vitro fertilization. Folia Histochem Cytobiol 2007;45: 73–7.

14. Kaur R, Gupta K. Endocrine dysfunction and recurrent spontaneous abortion: an overview. Int J Appl Basic Med Res 2016;6: 79–83.