Turk J Gastroenterol 2005; 16 (4): 188-193

Immunomodulatory effects of HBsAg vaccine and levamisole in chronic hepatitis B and hepatitis B carrier children

Kronik hepatit B ve hepatit B taşıyıcı çocuklarda HBsAg aşısı ve levamisolun immünmodulatör etkileri

Fikret DEMİRCİ[1], Ziya BAYRAKTAROĞLU[2], Murat KARAOĞLAN[3], Yavuz COŞKUN[2], İlkay KARAOĞLAN[4], Vahap OKAN[5]

[1]Division of Gastroenterology, [2]Department of Pediatrics, [3]Pediatrics State Hospital of Gaziantep, [4]Department of Infectious Diseases, [5]Department of Internal Medicine, School and Hospital of Medicine, Gaziantep University, Gaziantep

Background/aims: *Pathogenesis of chronic hepatitis B and hepatitis B carrier status is related to deficiencies in the immune system. Thus, treatments regulating the immune system are under discussion. The aim of this study was to investigate the effects of HBsAg vaccine and levamisole on lymphocyte subgroups and immunoglobulins in children with chronic hepatitis B and hepatitis B carriers.* ***Methods:*** *A total of 93 naive children (43 chronic hepatitis B carriers, 50 chronic hepatitis B patients) were treated in three groups with HBsAg vaccine, levamisole or levamisole plus HBsAg vaccine. Levamisole (ketrax®) was delivered as 2.5 mg/kg/day per os, three times per week for three months; the vaccine (Gen HevacB®) was administered subcutaneously as 20, 30, 40 µg at one-month intervals. Both medications were delivered at same dosages in the combined group. The examinations were performed at pre-treatment and at the end of the third month when the treatment concluded.* ***Results:*** *After treatments, CD3, CD4 and CD4/CD8 significantly increased and CD8 significantly decreased in chronic hepatitis B patient groups, except in the levamisole treated group. IgG and IgA were significantly decreased in all groups of chronic hepatitis B patients.* ***Conclusions:*** *It was found that HBsAg vaccine induced cellular immunostimulation in children with chronic hepatitis B; however, levamisole did not. The immune cells of hepatitis B carriers did not manifest a significant change in any treatment group. Although there was no change in B-cell, significant decreases were determined in immunoglobulins (IgG, IgA), especially in chronic hepatitis B patients.*

Key words: Children, chronic hepatitis B, hepatitis B carrier, HBsAg vaccine, levamisole, lymphocyte subsets, immunoglobulins

Amaç: *Kronik hepatit B ve hepatit B taşıyıcılığının patogenezi immün sistemdeki yetersizlikle ilgilidir. Bu nedenle immün sistem düzenleyici tedaviler tartışılmaktadır. Bu çalışmada kronik hepatit B ve asemptomatik hepatit B taşıyıcı çocuklarda, HBsAg aşısı ve levamisolün lenfosit subgrupları ve immünglobülinler üzerine etkileri araştırıldı.* ***Yöntem:*** *50 kronik hepatit B, 43 kronik hepatit B taşıyıcı, toplam 93 naive çocuk hasta HBsAg aşısı, levamisole ve levamisole ilave aşı şeklinde üç grupta tedavi edildi. Levamisole (ketrax®) 2.5 mg/kg/gün, oral olarak, haftada üç kez, 3 ay süreyle, HBsAg aşısı (Gen Hevac B®) 20, 30, 40 µg dozunda bir ay aralıklarla uygulandı. Her iki tedavi aynı dozlarda kombine gruba verildi. Kontroller tedaviden önce ve tedavinin bittiği üçüncü ayın sonunda yapıldı.* ***Bulgular:*** *Tedavilerden sonra, levamisol haricinde kronik hepatit B li hasta gruplarının hepsinde CD3, CD4 ve CD4/CD8 arttı ve CD8 düştü. Kronik hepatit B li tüm hasta gruplarında IgG ve IgA önemli derecede düştü.* ***Sonuç:*** *HBsAg aşısının kronik hepatit B li çocuklarda hücresel immünstümülasyon yaptığı, buna karşın levamisolun etkili olmadığı bulundu. Hepatit B taşıyıcılarının immün hücreleri hiçbir tedavi grubunda önemli değişiklik göstermedi. Her ne kadar B hücrelerinde önemli değişiklik olmadıysa da, özellikle kronik hepatit B hastalarında immünglobülinlerde (IgG, IgA) önemli düşmeler saptandı.*

Anahtar kelimeler: Çocuk, kronik hepatit B, hepatit B taşıyıcı, HBsAg aşısı, levamisole, lenfosit subgrupları, immunoglobulinler

INTRODUCTION

Hepatitis B infection is a serious threat worldwide and becomes chronic at a rate of 5% in regions of high risk. It is estimated that 350 million people in the world suffer from hepatitis B and that 1 million people die from cirrhosis or hepatocellular cancer related to chronic hepatitis B (CHB) every year (1, 2).

Address for correspondence: Fikret DEMİRCİ
Flat No: 21, Block B, University Building, 27310, Şahinbey, Gaziantep, Turkey
Phone: +90 342 360 46 26 • Fax: +90 342 360 30 02
E-mail: demirci@gantep.edu.tr

Manuscript received: 28.09.2005 **Accepted:** 10.11.2005

Many studies indicate that hepatitis B virus (HBV) is not cytopathic. The absolute eradication of an HBV that has entered into the human body or its continuation as a chronic disease is dependent upon the condition of the immune system. Both humoral and cellular immunity play a significant role in the pathogenesis of hepatitis B. Although TH1 antibody reaction against viral envelope antigens clears the virus from blood, the cytotoxic T-lymphocytes (CTL), stimulated by T-helper lymphocytes, kill the hepatocytes infected with the virus and clear the virus from the liver. Moreover, CTLs inhibit the HBV gene expression by secreting antiviral cytokines (3, 4). Polyclonal and multispecific cytotoxic T-cell and T-helper responses against viral antigens were observed in totally healed acute hepatitis B patients (4, 5). Strong multispecific T-cell responses were also observed after treatment with interferon and lamivudine in improving patients who showed a decrease in viral load (6, 7).

Hepatitis B virus persistence is thought to be related to poor HBV-specific T-cell responses. It is known that, in CHB patients and carriers, neither the antigen presenting to the immune system nor the responses of the immune system to the antigen are sufficient in themselves to combat the disease (1). In patients with viral replication, only one-third of the viral load decreases as a result of interferon alpha therapy, and nucleoside analogs, such as lamivudine and adevofir dipivoxil, inhibit the replication of HBV and improve the liver histology (8-10). Breakthroughs often occur, however, due to relapses in short-term treatment and development of viral variants in long-term treatment (11-13). These potentially useful treatments are nonetheless only virostatic, the ultimate goal being to eradicate viral replication and eliminate residually infected hepatocytes. The aim of the treatment of CHB patients is to enable the responses of specific T-cells to HBV to attain the same level as that seen in cured patients (4).

The immunogenicity of selected HBV envelope- or capsid-based vaccine formulations for the induction or expansion of T-cell and B-cell responses, of which HBV chronic carriers exhibit a deficiency, is currently being researched in animal models and in clinical trials (14-17). As a result of the addition of dendritic cells or granulocyte-macrophage colony-stimulating factor (GM-CSF) to the vaccine, antigen presentation can be increased, producing an improved immune response (18, 19).

Levamisole, a phenylimidothiazole derivative, has long been used as an anti-helmintic. It began to be used in many diseases after 1972, when it was discovered to have an immunomodulatory effect (20-22). Levamisole stimulates macrophages and T-lymphocytes and improves cellular immunity by increasing the secretion, chemotaxis, and proliferation of these cells (23). For this reason, in CHB patients, levamisole was applied solely or in combination with interferon (24-26).

In our study, we investigated the effects of HBsAg vaccine and levamisole therapy on the lymphocyte subsets and immunoglobulins of the cellular and humoral immune system in children with chronic hepatitis B and those who were inactive hepatitis B carriers.

MATERIALS AND METHODS

Between April 2000 and May 2001, we studied 93 child patients in total: 50 CHB and 43 hepatitis B carriers, aged 6-14 years, with an average age of 10.4±4.4 and with a 1.4±0.6 year disease history. The patients, 58 of whom were male (62%) and 35 female (38%), had not been previously treated with drugs for hepatitis. The characteristics of patient groups before therapy are shown in Table 1. All subjects and their parents agreed to participate in the study after full explanation of the nature and purpose of the investigation was made to them. This study was reviewed and accepted by the academic board of the medical faculty. The patients were diagnosed as suffering from CHB or as hepatitis B carriers using clinical, biochemical and serologic tools. Patients who had ALT values of 1.5 times the normal and HBV-DNA positivity for more than six months were defined as suffering from CHB. Patients who were HBsAg positive more

Tablo 1. Characteristics of patient groups before therapy

GROUPS	L (N=32)	V (N=28)	LV (N=33)
Age (year)	10.15±4.84	10.93±4.19	10.20±4.38
Gender (M/F)	17/15	18/10	23/10
Duration of illness (year)	1.56±0.98	1.66±1.28	1.26±1.02
CHB/HBVC	17/15	15/13	18/15
Liver biopsy	17/6	15/7	18/9
Knodell (average)	6.2±1.64	4.57±2.44	6.70±4.8
ALT (IU/L)			
<45	15	13	15
>45	17	15	18
HBV-DNA positivity	22/32	17/28	24/33
HBV-DNA (pg/ml)	621±196.52	727±219.21	675±194.85

L: Levamisole, V: Vaccine, LV: Levamisole plus vaccine, CHB: Chronic hepatitis B, HBVC: Hepatitis B virus carrier

than six months but had no clinical signs of liver disease and no elevated ALT values were defined as chronic HBV carriers. A liver biopsy was performed on 22 of the 50 patients with CHB. Of the 22 biopsy patients, 17 had mild hepatocellular damage, three had moderate damage, and two had advanced hepatocellular damage. The mean Knodell score was found to be 5.77±1.23.

Other hepatitis indications -- anti-HCV, anti-Delta, anti-HAV IgM and anti-HIV -- were negative in all of the patients accepted into the study. The patients had not used any anti-hepatitis medication or medication for any other important disease previously.

The patients were divided into three similar treatment groups according to such attributes as age, gender and disease period, as follows: I-Levamisole group (L: 32 patients), II- Vaccine group (V: 28 patients), III-Levamisole + vaccine group (LV: 33 patients). Levamisole (Ketrax®) was delivered as 2.5 mg/kg/day (p.o), three times per week for three months to the L group; HBsAg vaccine (Gen Hevac B®) was administered three times subcutaneously in doses of 20, 30, 40 µg at one-month intervals to the vaccine group. Both medications were delivered at the same dosages to the LV group. Although some patients reported nausea and weakness during the treatments, there were no important complications. Examinations were performed prior to treatment and at the end of the third month after treatment had ended.

antibodies were CD3/CD4 FITC/PE (for T-helper lymphocytes), CD3/CD8 FITC/PE (for cytotoxic T-lymphocytes), CD3/CD16+56 FITC/PE [for natural killer (NK) cells], CD3/CD19 (for total B- and T-cells), CD45/CD14 leucogate and immunofluorescent staining techniques were used. Serum immunglobulins were studied by the nephelometric method using the Behring Nephelometer 100 Analyzer device with its own kits. HBV-DNA was analyzed with microcolon DNA probe RIA (Abbot) and Quantiplex branched DNA; Bayer Diagnostics, Emeryville, California. The results are given as pg/ml.

Statistical Methods

In order to evaluate the parameters, Wilcoxon matched-pairs test was used for the dependent groups 190and Mann-Whitney U test for independent groups. Analyses were evaluated using the Windows SPSS 6.0 program.

RESULTS

CD3 (total T-lymphocyte) increased in all CHB patient groups at the end of the treatment. The increase in the V and LV groups was statistically significant ($p<0.01$). CD3 was also increased at the end of the treatment in inactive B carriers; the increase in the LV group was significant ($p<0.01$). CD19 (total B lymphocyte) did not manifest a significant change in any treatment group. CD4 (T-helper) increased in every CHB patient group

Tablo 2. Lymphocyte subsets and immunoglobulins in inactive hepatitis B carrier groups before and after treatment

	Before treatment			After treatment					
	L	V	LV	L		V		LV	
CD3	1449.9	1587.5	1471.3	1851.2	P<0.01	1752.6	P>0.05	1811.3	P<0.01
CD19	237.5	343.3	310.4	305.0	P>0.05	360.4	P>0.05	368.2	P>0.05
CD4	923.2	948.6	892.5	1049.2	P>0.05	978.6	P>0.05	899.0	P>0.05
CD8	727.8	692.3	1039.0	654.4	P>0.05	609.4	P>0.05	661.9	P>0.05
CD4/CD8	1.54	1.77	1.21	1.82	P>0.05	1.65	P>0.05	1.43	P>0.05
CD16/56	262.5	299.6	335.1	323.3	P>0.05	365.2	P>0.05	385.0	P>0.05
IgG	1577.4	1812.6	1493.1	1304.9	P<0.05	1280.2	P<0.05	1247.4	P<0.05
IgA	166.9	198.5	178.2	129.1	P<0.05	150.1	P>0.05	148.8	P>0.05
IgM	168.1	142.8	156.6	114.3	P<0.05	114.1	P>0.05	120.1	P>0.05

Lymphocyte subsets: mm^3, Immunoglobulins: mg/dl

The total lymphocyte count was analyzed by an automatic MS 9 blood-counting device (Melet Schloesing Laboratories). In order to determine the lymphocyte subgroups, an FAC Sort Flowcytometer device (Becton Dickinson Immunocytometry Systems, USA) was used. The monoclonal

after the treatment, with the increase in the V and LV groups showing statistical significance ($p<0.01$). Although CD4 values were higher in all of the inactive carrier groups at the end of the treatment, the difference was not statistically significant. CD8 (cytotoxic/suppressor T-lymphocytes)

Tablo 3. Lymphocyte subsets and immunoglobulins in chronic hepatitis patient groups before and after treatment

	Before treatment			After treatment					
	L	V	LV	L		V		LV	
CD3	1680.0	1440.7	1450.4	1890.0	**P**>0.05	1970.6	**P**<0.01	1995.0	**P**<0.01
CD19	405.0	472.7	423.4	436.2	**P**>0.05	378.6	**P**>0.05	452.5	**P**>0.05
CD4	956.3	835.8	991.3	1057.9	**P**>0.05	1069.4	**P**<0.01	1166.4	**P**<0.01
CD8	883.1	1051.1	927.3	690.0	**P**>0.05	705.5	**P**<0.01	663.3	**P**<0.01
CD4/CD8	1.36	1.01	1.38	1.67	**P**>0.05	1.77	**P**<0.01	1.89	**P**<0.05
CD16/56	289.2	404.3	331.9	328.8	**P**>0.05	398.7	**P**>0.05	429.6	**P**>0.05
IgG	1730.7	1659.8	1688.0	1403.3	**P**<0.05	1146.2	**P**<0.01	1256.4	**P**<0.01
IgA	176.5	160.1	178.7	132.5	**P**<0.05	123.2	**P**<0.05	121.0	**P**<0.05
IgM	173.0	131.1	184.0	132.5	**P**>0.05	104.3	**P**>0.05	112.1	**P**<0.05

showed a decrease in all carrier and chronic hepatitis groups. The decrease in the V and LV groups of CHB sufferers was significant ($p<0.01$). The CD4 and CD8 ratio was found to have increased in chronic hepatitis patients. This increase was significant in the V and LV groups ($p<0.01$). Although there was an increase in the carrier groups, it was not significant. Post-treatment changes in CD16/56 (NK) were not significant in any of the patient groups.

Before treatment, immunoglobulins were at a normal level in relation to age. After treatment, IgG dropped significantly in all of the CHB and carrier groups ($p<0.01$, $p<0.05$ respectively). IgA showed a significant decrease in all chronic hepatitis patient groups ($p<0.05$), but only in the L-hepatitis B carrier group did it manifest a significant decrease ($p<0.05$). The decrease in IgM was significant only in group L of chronic carriers ($p<0.05$). In Tables 2 and 3, the pre-treatment and post-treatment values are listed comparatively in CHB and hepatitis B carrier groups.

DISCUSSION

We investigated the effects of HBsAg vaccine and levamisole therapy on lymphocyte subsets and serum immunoglobulin levels in CHB patients and chronic HBsAg carriers.

At the end of the treatment, the number of total T-lymphocytes (CD3) manifested an increase in both groups (patients and carriers), and with the exception of the levamisole group of CHB and the vaccine group of CHB carriers, the increase was significant ($p<0.01$). The total T-lymphocyte increase in our cases was thought to be caused by the increase in CD4. According to some studies, the increase in CD4 cells is reported to have been due to HBV vaccination in CHB patients, like in our study (27, 28). Levamisole was reported to increase the phagocytic activity, activation of lymphocytes, and lymphokine release and to increase the absolute lymphocyte count, especially of T-lymphocytes, in various diseases (29, 30). In our study, however, CD4 and CD3 cells did not increase in CHB patients treated with levamisole. This may be related to the different immunologic behavior of CHB patients.

The CD4 cells manifested a significant increase in patients with CHB treated with vaccination and levamisole plus vaccination ($p<0.01$). The core mechanism in the treatment with a HBV vaccine, the CD4 increase, has been demonstrated in many studies. In a study conducted on CHB patients, HBsAg vaccine was reported to induce and proliferate CD4 T-cells (31). It was shown that as a result of the stimulation of the CD4 T-cells by means of interferon alpha and gamma, the existing TH2 dominance shifted to TH1 in CHB (32). In our cases, however, no significant change in CD4 cells was determined in the chronic HBsAg carriers in all treatment groups. This finding may imply that chronic HBsAg carriers do not respond properly at least on cellular levels of immunity to the various immunostimulant therapies.

In the post-treatment period, CD8 T-lymphocytes manifested a significant reduction in CHB groups, except in the levamisole group ($p<0.01$). No significant change was observed in the carrier groups. In one study, the HBV vaccine was reported not to induce CD8 T-lymphocytes (28). In a study performed on mice, cytotoxic T-lymphocytes (CTL) were reported to have accumulated in the spleen during a hepatic infection caused by a HBV genome producing adenovirus (Ad-HBV). After vaccination, an accumulation in the liver and a decrease in the CTL count in the peripheral blood were recorded (33). The inhibition of CD8 T-lymphocytes by the adjuvant substance (aluminium hydroxide) used with the vaccine was also determined (34). In our study, CD4/CD8 was significantly high in the

chronic hepatitis groups ($p<0.01$), except in the levamisole group, and no significant change was observed in any of the carrier groups.

CD19 (total B-lymphocytes) did not manifest a significant change in carrier or chronic hepatitis groups. Although an increase in the number of B-lymphocytes was found after levamisole treatment in patients suffering from chronic pneumonia and chronic bronchitis (23), it was reported that no significant change in the B-lymphocyte count occurred in CHB (35, 36). This finding may imply that CHB patients and chronic HBsAg carriers do not respond properly at least on humoral cell levels of immunity to the various immunostimulant therapies.

The natural killer (NK-CD16/56) T-lymphocyte did not manifest a significant change in any patient group by the end of the treatment. There is little evidence about the role of NK cells in chronic HBV infection. Although an increase in NK was determined in the early stage of acute HBV infection, normal values were reported during the healing period (37). In another study, levamisole treatment was reported to be ineffective on NK T-cell and interferon production in both normal subjects and cancer patients (38).

The eradication of the virus in hepatitis B infection can be achieved only with cellular and humoral immunity treatments together. Although in our study, no significant change in the B-lymphocyte count was observed in any treatment group, the immunoglobulin levels were found to be low after the treatment. IgG manifested a significant decrease in all of the chronic hepatitis groups and in the carrier groups, with the exception of the levamisole plus vaccine group. IgA showed a significant decrease in all the chronic hepatitis groups and in the levamisole treatment group of carriers. IgM manifested a significant decrease only in the levamisole group of carriers. Although no change in immunoglobulin levels was reported after the administration of the hepatitis B vaccine in a previously concluded study, in our study there was a significant decrease in IgG and IgA after treatment especially in CHB patients (39).

In summary, after treatment, CD3, CD4 and CD4/CD8 increased and CD8 decreased in the vaccine and levamisole plus vaccine groups in CHB patients. The difference was no more significant in the combination treatment group than in the vaccine group alone. CD3, CD4 and CD8 cells did not significantly differ in CHB patients treated with levamisole. The immune cells of CHB carriers did not respond to any of the immunostimulant therapies used in this study. No significant change was found in total B-cells or in the NK cells. Although there were no significant changes in B-cells, it was found that immunoglobulins decreased especially in CHB patients, and thus the humoral immune system was thought to be affected qualitatively.

In conclusion, it was found that HBsAg vaccine induced cellular immunostimulation in children with chronic hepatitis B; however, levamisole treatment did not. In addition, levamisole plus vaccine therapy was not superior to vaccine treatment alone. Although there was no significant change in B-cell, significant decreases were determined in immunoglobulins (IgG and A) especially in CHB patient groups.

Conflict of Interest

The authors have declared that no conflict of interest exists.

REFERENCES

1. Mahoney FJ. Update on diagnosis, management, and prevention of hepatitis B virus infection. Clin Microbiol Rev 1999; 12(2): 351–6.
2. Pol S, Couillin I, Michel ML, et al. Immunotherapy of hepatitis B by anti HBV vaccine. Acta Gastroenterol Belg 1998; 61(2): 228-33.
3. Chisari FV. Cytotoxic T cells and viral hepatitis. J Clin Investig 1997; 99: 1472-7.
4. Chisari F, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 1995; 13: 29–36.
5. Ferrari C, Penna A, Bertoletti A, et al. Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. J Immunol 1990; 145: 3442–9.
6. Boni C, Bertoletti A, Penna A, et al. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. J Clin Invest 1998; 102: 968 –75.
7. Boni C, Penna A, Ogg GS, et al. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatology 2001; 33(4): 963-71.
8. Hoofnagle JH, di Bisceglie AM. The treatment of chronic viral hepatitis. N Engl J Med 1997; 336: 347–56.
9. Yurdaydin C, Bozkaya H, Sahin T, et al. Lamivudine vs lamivudine and interferon combination treatment of HBeAg (-) chronic hepatitis B. J Viral Hepat 2005; 12(3): 262-8.
10. Marcellin P, Chang TT, Lim SG, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003; 348: 808–16.

11. Pai SB, Bozdayı AM, Pai RB, et al. Emergence of a novel mutation in the FLLA region of hepatitis B virus during lamivudine therapy. Antimicrob Agents Chemoter 2005; 49(7): 2618-24.
12. Bozdayı AM, Uzunalimoglu O, Turkyilmaz AR, et al. YSDD: a novel mutation in HBV-DNA polymerase confers clinical resistance to lamivudine. J Viral Hepat 2003; 10(4): 256-65.
13. Angus P, Vaughan R, Xiong S, et al. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 2003; 125: 292–7.
14. Chow YH, Huang WL, Chı WK, et al. Improvment of hepatitis B virus DNA vaccines by plasmids coexpressing hepatitis B surface antigen and interleukin-2. J Virol 1997; 71: 169-78.
15. Michel ML, Pol S, Brechot C, et al. Immunotherapy of chronic hepatitis B by anti HBV vaccine: from present to future. Vaccine 2001; 19: 2395-9.
16. Hui CK, Lau GK. Advances in immunomodulating therapy of HBV infection. Int J Med Sci. 2005; 2(1): 24-9.
17. Mancini M, Hadchouel M, Tiollais P, et al. Induction of anti-hepatitis B surface antigen (HBsAg) antibodies in HBsAg transgenic mice: a possible way of circumventing 'nonresponse' to HbsAg. J Med Virol 1993; 39: 67–74.
18. Wang J, Zhu Q, Zhang T, et al. A pilot study on the combined therapy of granulocyte-macrophage colony-stimulating factor and hepatitis B vaccine on chronic hepatitis B virus carrier children. Chin Med J 2002; 115 (12): 1824-8.
19. Li YG, Chen M, Zhang DZ, et al. Clinical research on the treatment effect of autologous dendritic cell vaccine on the patients with chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi 2003; 11(4): 206-8.
20. Holcombe RF, Li A, Stewart RM. Levamisole and interleukin-2 for advanced malignancy. Biotherapy 1998; 11(4): 255-8.
21. Tanphaichitra D, Srimuang S. Cellular immunity (T-cell subset using monoclonal antibody) in tuberculosis, melioidosis, pasteurellosis, penicilliosis; and role of levamisole and isoprinosine. Dev Biol Stand 1984; 57: 117-23.
22. Garszon MC. Levamisole treatment in HIV-infected Zambian children. Lancet 1992; 340: 1099-100.
23. Borisova AM, Novikova TA, Glazko AV. Effect of levamisole on cellular immunity indices in chronic bronchitis and chronic pneumonia patients. Ter Arkh 1984; 56(10): 29-31.
24. Fattovich G, Cadrobbi P, Crivellaro C, et al. Virological changes in chronic hepatitis type B treated with levamisole. Digestion 1982; 25(2): 131-7.
25. Bosch O, Moraleda G, Castillo I, et al. Treatment of chronic hepatitis B with recombinant interferon alpha versus recombinant interferon alpha plus levamisole. J Hepatol 1993; 19(3): 437-41.
26. Ruiz-Moreno M, Garcia R, Rua MJ, et al. Levamisole and interferon in children with chronic hepatitis B. Hepatology 1993; 18(2): 264-9.
27. Ren F, Hino K, Yamaguchi Y, et al. Cytokine-dependent anti-viral role of CD4 positive T cells in therapeutic vaccination against chronic hepatitis B viral infection. J Med Virol 2003; 71(3): 376-84.
28. Jung MC, Gruner N, Zachoval R, et al. Immunological monitoring during therapeutic vaccination as a prerequisite for the design of new effective therapies: induction of a vaccine-specific CD4+ T-cell proliferative response in chronic hepatitis B carriers. Vaccine 2002; 20(29-30): 3598-612.
29. Khanna A, Ojha KN, Gupta RM. Effect of levamisole as an immunomodulating agent in trophoblastic lesions. Indian J Pathol Microbiol 1993; 36(1): 32-7.
30. Scherak O, Smolen JS, Menzel AJ, et al. Effect of levamisole on immunological parameters in patients with systemic lupus erthematosus. Scand J Rheumatol. 1980; 9(2): 106-12.
31. Coullin I, Pol S, Mancini M, et al. Specific vaccine therapy in chronic hepatitis B: induction of T cell proliferative responses specific for envelope antigens. J Infect Dis 1999; 180(1): 15-26.
32. Abar SM, Abe SM, Masumato T, et al. Mecanism of action of vaccine therapy in murine hepatitis B virus carriers: vaccine-induced activation of antigen presenting dendritic cell. J Hepatol 1999; 30(5): 755-64.
33. Isogawa M, Kakimi K, Kamamoto H, et al. Differential dynamics of the peripheral and intrahepatic cytotoxic T lymphocyte response to hepatitis B surface antigen. Virology 2005; 333(2): 293-300.
34. Gupta RK, Siber GR. Adjuvant for human vaccines - current status, problems and future prospects. Vaccine 1995; 14: 1263-75.
35. Pozsonyi T, Feher J, Jakab L. Lymphocyte populations in chronic active liver disease: in vitro effect of levamisole on T-lymphocyte populations. Allergol Immunopathol 1981; 9(1): 37-44.
36. Thomas HC, Freni M, Sanchez-Tapias J, et al. Peripheral blood lymphocyte populations in chronic liver disease. Clin Exp Immunol 1976; 26(2): 222-7.
37. Chemello L, Mondelli M, Bortolotti F, et al. Natural killer activity in patients with acute viral hepatitis. Clin Exp Imunol 1986; 64: 59-64.
38. Liberati M, Boreden EC, McBain JA, et al. Effect of levamisole on human natural killer and killer cell activity and production of interferons. Immunopharmacology 1982; 5(1): 11-8.
39. Kietduriyakul V, Charuchaimontri C. Serum immunoglobulin levels before and after hepatitis B vaccinations. J Med Assoc Thai 1991; 74(1): 19-23.