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Original Article

The Prevalence of Hepatitis B Surface Antigen and Anti-Hepatitis B Core Antibody in Iran: A Population-Based Study

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Background: **Hepatitis B virus infection is a very common cause of chronic liver disease worldwide. It is estimated that 3% of Iranians are chronically infected with hepatitis B virus. Current population-based studies on both rural and urban prevalence of hepatitis B virus infection in Iran are sparse with results that do not always agree. We performed this study to find the prevalence of hepatitis B surface antigen, anti-hepatitis B core antibody, and associated factors in the general population of three provinces of Iran.**

Methods: **We randomly selected 6,583 subjects from three provinces in Iran, namely Tehran, Golestan, and Hormozgan. The subjects were aged between 18 and 65 years. Serum samples were tested for hepatitis B surface antigen and anti-hepatitis B core antibody. Various risk factors were recorded and multivariate analysis was performed.**

Results: **The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran was 2.6% and 16.4%, respectively. Predictors of hepatitis B surface antigen or anti-hepatitis B core antibody in multivariate analysis included older age, not having high-school diploma, living in a rural area, and liver disease in a family member. We did not find any significant differences between males and females.**

Conclusion: **In spite of nationwide vaccination of newborns against hepatitis B virus since 1992, hepatitis B virus infection remains a very common cause of chronic liver disease in Iran which should be dealt with for at least the next 30 – 50 years.**

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Introduction

Hepatitis B virus (HBV) infection is a serious global health problem with over two billion people infected worldwide and 350 million suffering from chronic infection. It is the 10th leading cause of death worldwide and results in 500,000 to 1.2 million deaths per year due to cirrhosis and hepatocellular carcinoma (HCC).[1] HBV infection is highly prevalent in the Western Pacific and South-East Asia where rates as high as 35% have been reported.[2] However, in the Middle-East, different rates are reported. Some limited studies indicate that HBV infection is the cause of about 75% of cirrhosis and HCC in Iran.[3–5] But, nationwide population-based studies about the prevalence of HBV infection and its risk factors are sparse in Iran.[5]

Iran has been known as a medium-prevalence area. The first estimates of the prevalence of HBV

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infection in Iran, about 30 years ago, indicated that approximately 35% of Iranians were exposed to HBV and 3% were chronic carriers.[6,7] These studies, carried out on blood donors during the early years of the foundation of the Blood Transfusion Organization in Iran, may have been a good estimate of chronic HBV infection but cannot be generally assumed to be representative of the general population. In another study performed during 1990 – 1991 on 39,841 subjects, randomly selected from all the country, a mean prevalence of 1.7% was observed for hepatitis B surface antigen (HBsAg) positivity.[8] This study included subjects as young as two years old.

During the last 30 years, many researchers have studied the prevalence of HBV infection in special populations in Iran. These populations include subjects with hemophilia, major thalassemia, sexually transmitted diseases, Behcet's disease, patients on chronic hemodialysis, pregnant women, prisoners, drug abusers, truck drivers, etc.[9–16]

Vaccination against HBV has been routinely performed for newborns and high-risk groups since 1992 and adulthood vaccination has been done since 2007. It is believed to change the epidemiologic pattern of HBV infection especially in children and adolescents. The effects are now discernible in younger Iranians.[17] Continuous surveillance of this infection and its risk factors is essential for better health planning.

New population-based studies are rather sparse. From current literature, it is obvious that the prevalence is significantly varied in different provinces of Iran. Nevertheless, serial studies on the prevalence of HBV infection in various provinces are essential for health planning and monitoring.

In this study, we assessed the prevalence and determinants of exposure to HBV infection in three provinces of Iran.

Materials and Methods

Design and settings

This cross-sectional study was performed during 2006 in three provinces of Iran.

Study population

The general population of three provinces in Iran were studied. The provinces included Golestan, North-East of Iran; Tehran, North-Center; and Hormozgan, south of Iran. Both urban and rural populations were included in Tehran and Golestan. In Hormozgan, due to practical considerations, only the urban population was studied. The demography of Iran and the three study provinces is given in Table 1.

In each province, subjects between 18 and 65 years of age were included. Other inclusion criteria were Iranian nationality, being a permanent inhabitant of the household, and willing to participate.

Sample size calculation

The prevalence of HBsAg positivity in Iran has been previously reported to be approximately 3%.[7] Even though hepatitis B vaccination has been included in the national vaccination program since 1992, this prevalence was used to calculate the sample size, because vaccination may not have significantly changed the prevalence in adults.

We calculated our sample size for the detection of a prevalence of 3% with a confidence level (CI) of 95% and error of 0.75% for each province. This yielded a sample size of 2000 subjects for each province. In Tehran, 2500 subjects were selected.

Sample selection

Clustered random sampling was used in each province.

We estimated that each research team could collect data of 20 subjects per day in Hormozgan and Golestan and 25 subjects in Tehran (due to the higher population density). So, a cluster size of 20 or 25 was chosen respectively which resulted in 100 clusters in each province to provide the calculated sample size.

In Tehran and Hormozgan, we used the list of postal codes (which are updated annually) for randomly selecting 100 clusters (families). In

Table 1. Census information on Iran and the three provinces studied.*

	Area (km^2)	Population	Percent urban	Percent male	Literacy
Iran	1,648,195	70,495,782	68.5%	50.9%	84.0%
Tehran	18,814	13,422,366	91.3%	51.4%	91.3%
Hormozgan	70,697	1,403,674	47.1%	51.7%	82.4%
Golestan	20,367	1,617,087	49.2%	49.7%	82.1%

*Source: Iran national population and housing census, 2006, http://www.sci.org.ir/portal/faces/public/sci_en

Golestan, family registry of health department, which was the most updated population profile, was used to select clusters. In each cluster, the first 20 (or 25 in Tehran) eligible cases were recruited. If an eligible subject was not available on the first visit, a second appointment was scheduled for data collection. Subjects were excluded if they refused to participate or contact was not possible after two attempts and they were replaced with the next subject in the cluster.

Data collection

Data collection was performed during 2006. Questionnaires were completed by a trained interviewer for each subject. The questionnaire included demographic and anthropometric data and risk factors of HBV infection. The number of missing and filled teeth was also recorded as an indicator of oral health.

Laboratory tests

A blood sample was collected from each participant and then transferred to the regional laboratory. Serum was separated and stored at -20°C. Frozen sera were transferred to the Iran Blood Transfusion Organization (IBTO) Research Center for HBsAg and anti-hepatitis B core antibody (anti-HBc) tests. Serum levels of HBsAg and anti-HBc were evaluated by Enzygnost HBsAg 5.0 kit (Dade Behring, Germany) and Hepanostica anti-HBc Uni-Form kit (Biomerieux, France), respectively.

Statistical analysis

In each province, to overcome different response rates, weighted frequencies of HBsAg and anti-HBc were calculated with weights equal to male and female population. For univariate analysis, table of frequencies was used to calculate the prevalence of HBsAg and anti-HBc by different potential risk factors. Weighted logistic regression was applied to calculate the odds ratio and 95%CI for each potential risk factor. Then, two different multivariate weighted logistic regressions were developed to calculate the independent effects of predictors of HBsAg and anti-HBc. In each model, the variables with $P<0.2$ in univariate analysis were entered and weights were equal to population of provinces. Due to high missing values of risk factor data in Golestan, multivariate analysis was restricted to Tehran and Hormozgan. Data were analyzed by Stata 10.0 (StatCorp, College Station, TX).

Ethics

The study was approved by the Institutional Review Board of the Digestive Disease Research Center of Tehran University of Medical Sciences. Written or informed consents were obtained from all participants before data collection.

Results

A total of 6,583 subjects were interviewed in the three provinces. After reviewing the data, we noticed that the age criterion was not strictly observed in Hormozgan and Golestan Provinces and 381 subjects were younger than 18 or older than 65 who were excluded. Five hundred and twenty-four subjects refused to give blood samples or their samples were unsuitable. So, a total of 5,678 samples were analyzed. Demographic characteristics of the study population are shown in Table 2.

The rate of HBsAg and anti-HBc positivity is given in Table 3. The total prevalence was calculated by adjusting for the population of each province in addition to sex ratio.

Data for rural area of Hormozgan was not available; thus, our reports on rural areas are based on data from Golestan and Tehran only.

The prevalence of neither HBsAg ($P=0.2$) nor anti-HBc ($P=0.9$) was different according to gender. The prevalence of HBsAg and anti-HBc increased significantly with age ($P<0.0001$) [Figures 1a and 1b]. In univariate analysis, HBsAg was correlated with history of blood transfusion, history of traditional phlebotomy (hejamat, cupping), history of tattooing, having a family member with liver diseases, living in rural areas,

Table 2. Demographic data of the study population.

	Participants interviewed	Number valid	Samples collected	Percent male	Percent rural	Mean age ± SD (yr)
Tehran	2561	2561	2327	41.6%	5.2%	35.6±13.6
Hormozgan	1987	1745	1455	45.2%	0%	33.4±11.9
Golestan	2035	1896	1896	32.0%	48.2%	38.8±12.9
Total	**6583**	**6202**	**5678**	**39.3%**	**18.6%**	**36.1±13.1**

Table 3. Prevalence of anti-HBc and HBsAg in three provinces of Iran.

		Anti-HBc	HBs Ag
Tehran	Male	15.4%	2.7%
	Female	13.0%	1.8%
	Weighted total	14.2%	2.3%
Hormozgan	Male	14.8%	3.4%
	Female	11.7%	2.0%
	Weighted total	13.3%	2.7%
Golestan	Male	39.8%	5.4%
	Female	34.0%	4.8%
	Weighted total	36.9%	5.1%
Weighted total*	Male	17.7%	3.0%
	Female	15.0%	2.1%
	Total	16.4%	2.6%

* Adjusted for the population of each province.

and less than 12 years of education. Anti-HBc was correlated with history of surgery, history of traditional phlebotomy, having a family member with liver diseases, living in rural areas, and less than 12 years of education (Tables 4). But in multivariate analysis, only age and having a family member with liver disease was correlated with both HBsAg and anti-HBc. Furthermore, living in rural area and less than 12 years of education was associated with HBsAg and anti-HBc in multivariate analysis respectively (Table 5).

Discussion

The sera of 5,678 randomly selected subjects were tested in three provinces of Iran for the presence of HBsAg and anti-HBc. The rate of HBsAg and anti-HBc positivity was 2.6% and 16.4%, respectively.

Other than being population-based, there are some characteristics of our study which should be noted. Very few studies in the literature have commented on the prevalence of HBV infection in rural areas of Iran. We demonstrated that exposure rates were higher in rural vs. urban areas of Iran. Furthermore, our study population was composed of more females than males, whereas many previous studies have reported almost exclusively on males. This is especially true for blood donor studies where the female population has always been much smaller since only around 10% of blood donors are women. Unlike most previous studies, we did not find a significantly higher rate of HBsAg or anti-HBc in men vs. women.

Routine vaccination of newborns against HBV has been implemented in Iran since 1992. We have studied subjects older than 18 years, so none of our subjects had received HBV vaccination as part of the routine nationwide vaccination program. The rate of HBsAg positivity is an indicator of the prevalence of chronic HBV infection whereas anti-HBc positivity can be regarded as an indicator of past exposure to HBV. Anti-HBc positivity underestimates past exposure to HBV since subjects who clear the infection may gradually loose anti-HBc.

In Figure 1, it is seen that the prevalence of HBsAg and anti-HBc increases with age, indicating exposure to HBV in all age groups. There is a small peak at age 40 for both HBsAg and anti-HBc. The significance of this peak is unknown.

Another important characteristic of our study is multivariate analysis of risk factors which has rarely been performed in earlier reports from Iran. Risk factors such as tattooing, cupping, and non-intravenous drug abuse, disappear when controlling for other variables such as education and living in rural areas. This might indicate that when a good level of education is present,

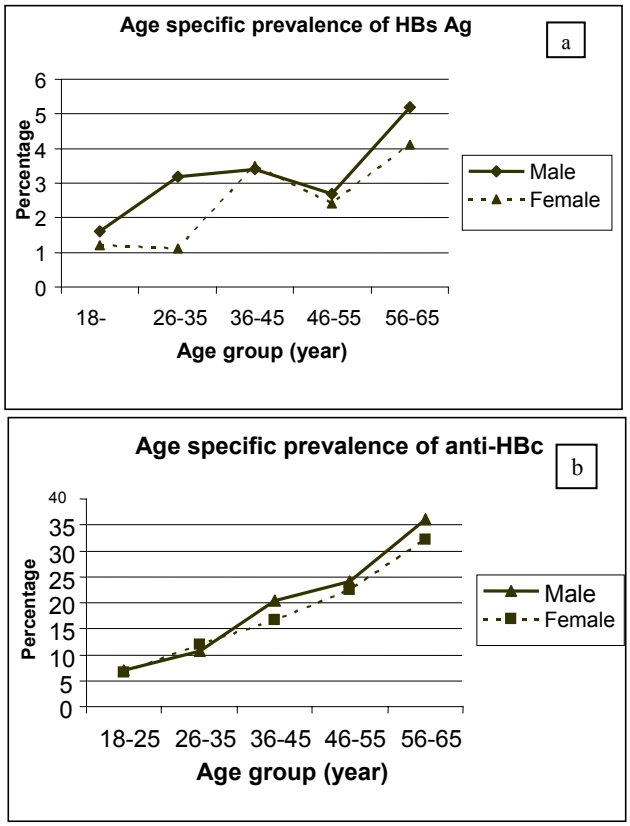

Figure 1 (a and b). Age specific prevalence of HBsAg and anti-HBc in the study population.

Table 4. Risk factors associated with HBsAg and anti-HBc positivity in Tehran and Hormozgan provinces in univariate analysis.

	Anti-HBc	Odds ratio (95%CI)	*P* value	HBs Ag	Odds ratio (95% CI)	*P* value
History of blood transfusion						
Yes	17%	1.4(0.9–2.0)	0.1	5%	2.2 (1.1–4.4)	0.03
No	13%			2%		
Nonintravenous drug abuse						
Yes	18%	1.5(0.9–2.6)	0.1	1%	0.5 (0.1–3.7)	0.5
No	13%			2%		
History of surgery						
Yes	16%	1.5(1.2–1.8)	0.001	3%	1.4(0.8–2.3)	0.2
No	12%			2%		
History of traditional phlebotomy						
Yes	18%	1.5(1.1–2.0)	0.006	4%	2.1(1.1–3.9)	0.02
No	13%			2%		
History of tattooing						
Yes	18%	1.3(0.9–2.0)	0.2	4%	2.2(1.0–4.7)	0.04
No	13%			2%		
Family member with liver disease						
Yes	21%	1.8(1.2–2.5)	0.002	6%	3.3(1.7–6.2)	<0.0001
No	13%			2%		
Rural*						
Yes	39%	2.5(1.9–3.1)	<0.0001	6%	2.7 (1.6–4.5)	<0.0001
No	17%			3%		
Less than 12 years education*						
Yes	28%	2.5 (2.1–3.1)	<0.0001	4%	1.8 (1.1–3.0)	0.015
No	12%			3%		

* In all three provinces.

tattooing, cupping, or even drug abuse is not associated with HBV transmission.

In the earliest studies on the prevalence of HBV infection in Iran, the rate of HBsAg positivity among healthy nonprofessional blood donors was 3.4%. Our study indicated a rate of 2.6% in the general population, i.e. a decrease of 0.8%. Interestingly, recent reports from blood donors show a much greater decrease from almost 3% in early studies to 1.79% in 1998 and 0.41% in 2007,[18] and in the case of Tehran, 0.51%.[19] This indicates the effectiveness of the donor selection procedure being practiced by the IBTO during the recent years. In this procedure, donors having certain risk factors are excluded from the blood donor pool.

We observed a prevalence of 2.6% for HbsAg; however, another recent study performed on the general population reported a very different prevalence of 1.7%.[8] By reviewing the current literature on the prevalence of HBV infection in Iran, it is seen that reports from different provinces vary widely.[20–24] This important phenomenon was also well-demonstrated in the present study where

Table 5. Logistic regression of predictors of HBsAg and anti-HBc seropositivity.*

	HBsAg positivity	
	Odds ratio (95% CI)	***P* value**
Age (for every 10 years)	1.3 (1.1–1.6)	0.001
Having family member with liver disease	3.0(1.6–5.9)	0.001
Living in rural area	3.0 (1.2–7.2)	0.01
	Anti-HBc positivity	
	Odds ratio (95% CI)	***P* value**
Age (for every 10 years)	1.6 (1.5–1.8)	<0.0001
Having family member with liver disease	1.7 (1.1–.4)	0.008
Less than 12 years education	1.5 (1.1–1.9)	0.005

*Area under curve for ROC=0.70 and *P* value for goodness of fit =0.5.

the prevalence of HBsAg in Golestan Province was shown to be almost two times more than those of the other two provinces. Iran is a large country with an area of approximately 1,700,000 square kilometers. The people residing in different parts of the country have different ethnicities and different lifestyles. Golestan is largely populated by Torkamans; a tribe which has limited contact with the rest of the country and rarely marries non-Torkamans. Torkamans generally follow their traditional lifestyle. Hormozgan is close to the Arab states south of the Persian Gulf and Oman Sea. Many Iranians living in Hormozgan have regular visits to countries like the UAE and Qatar. Tehran, the capital, comprises a mixture of various ethnicities. It is densely populated and western life-style is dominant. It is not appropriate to compare the results of studies performed in different provinces of Iran. Furthermore, due to the significant improvement in general health during the recent years and implementation of nationwide vaccination programs, the prevalence of HBV infection is changing rapidly, especially in younger Iranians. Thus, the age of subjects has an important influence on the rates reported by various studies. The rates reported by such studies, including ours, should be interpreted with caution and careful attention to the study population.

Hepatitis B infection is the most common cause of chronic liver disease in the world, including Iran.[1,5] Although vaccination against hepatitis B infection is becoming a standard practice, it will be decades before such vaccination programs actually decrease the burden of chronic hepatitis B infection. Governments should continue evaluating the epidemiology of HBV infection and take measures for infected individuals.

According to our results, an estimated two million Iranian adults over 18 years of age have chronic HBV infection that needs attention. HBV infection will remain the major etiology of end-stage liver disease for the next 50 years.[25,26] More than 90% of HBV infections in Iran are e antigen-negative precore mutants and almost 100% are of the D1 genotype.[27] There are very few studies on the natural history of e antigen-negative genotype D1 HBV infection but published studies clearly show a pattern different from the Asian genotypes B and C.[28] The incidence of HCC is much lower in D1 genotype, as is the case in Iran where HCC is not among the top 10 most common cancers in Golestan in spite of a 5.5% prevalence of HBsAg positivity.[29] Even the response to treatment is different and the D1 genotype responds poorly to interferon.[30] Thus, applying guidelines developed for Asian genotype B and C to the D1 genotype in Iran is not appropriate. In order to manage the two million Iranians infected with HBV, we need a guideline based on strong evidence. We should perform long-term studies on large cohorts of HBV-infected Iranians.

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References

1 Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004; **11:** 97 – 107.

2 Gust ID. Epidemiology of hepatitis B infection in the Western Pacific and South- East Asia. *Gut.* 1996; **38 (suppl 2):** S18 – S23.

3 Shamszad M, Farzadgan H. Hepatitis B- related cirrhosis and hepatocellular carcinoma in Iran. *J Irn Med Council.* 1982; **8:** 238.

4 Bagheri- Lankarani K, Saberi-Firoozi M, Nabipoor I, Fattahi F, Sarafraz-Yazdi M, Malekzadeh R. Reassessment of the role of hepatitis B and C viruses in post necrotic cirrhosis and chronic hepatitis in southern Iran. *Irn J Med Sci.* 1999; **24:** 117 – 121.

5 Merat S, Malekzadeh R, Rezvan H, Khatibian M. Hepatitis B in Iran. *Arch Irn Med.* 2000; **3:** 192 – 201.

6 Farzadegan H, Shamszad M, Noori-Arya K. Epidemiology of viral hepatitis among Iranian population: a viral marker study. *Ann Acad Med Singapore.* 1980; **9:** 144 – 148.

7 Farzadegan H, Harbour C, Ala F. The prevalence of hepatitis B surface antigen and its antibody in blood donors and high-risk groups in Iran. *Vox Sang.* 1979; **37:** 182 – 186.

8 Zali MR, Mohammad K, Farhadi A, Masjedi MR, Zargar A, Nowroozi A. Epidemiology of hepatitis B in the Islamic Republic of Iran. *East Mediterr Health J.* 1996; **2:** 290 – 298.

9 Jahani MR, Motevalian SA, Mahmoodi M. Hepatitis B carriers in large vehicle drivers of Iran. *Vaccine* 2003; **21:** 1948 – 1951.

10 Aali BS. The prevalence of HBsAg among pregnant women referred to Kerman maternity hospital in 1997. *J Kerman Univ Med Sci.* 1997; **6:** 89 – 96.

11 Mohammad-Alizadeh AH, Alavian SM, Jafari K, Yazdi N. Prevalence of HBsAg, HCV Ab, and HIV Ab in drug abuser prisoners in Central Hamadan Jail. *Res Med Sci.* 2002; **7:** 311 – 313.

12 Khani M, Vakili MM. Prevalence and risk factors of HIV, hepatitis B virus, and hepatitis C virus infections in drug addicts among Zanjan prisoners. *Arch Iranian Med.* 2003; **6:** 1 – 4.

13 Talaie H, Shadnia SH, Okazi A, Pajouhmand A, Hasanian H, Arianpoor H. The prevalence of hepatitis B, hepatitis C, and HIV infections in non-IV drug opioid poisoned patients in Tehran, Iran. *Pak J Biol Sci.* 2007; **10:** 220 – 224.

14 Farajzadeh S, Shakibi MR, Moghaddam SD, Rahnama Z. Behcet disease: clinical spectrum and association with hepatitis B and C viruses. *East Mediterr Health J.* 2005; **11:** 68 – 72.

15 Ghanaat J, Sadeghian A, Ghazvini K, Nassiri MR. Prevalence and risk factors for hepatitis B virus infection among STD patients in northeast region of Iran. *Med Sci Monit.* 2003; **9:** CR91 – CR94.

16 Rezvan H, Abolghassemi H, Kafiabad SA. Transfusion-transmitted infections among multitransfused patients in Iran: a review. *Transfus Med.* 2007; **17:** 425 – 433.

17 Zali MR, Mohammad K, Noorbala AA, Noorinayer B, Shahraz S. Rate of hepatitis B seropositivity following mass vaccination in the Islamic Republic of Iran. *East Mediterr Health J.* 2005; **11:** 62 – 67.

18 Amini-Kafiabad S, Houri R, Abolghasemi H. Trends in prevalence of hepatitis B virus infection among Iranian blood donors, 1998 to 2007. *Transfus Med*.[in press]

19 Pourshams A, Malekzadeh R, Monavvari A, Akbari MR, Mohamadkhani A, Yarahmadi S, et al. Prevalence and etiology of persistently elevated alanine aminotransferase levels in healthy Iranian blood donors. *J Gastroenterol Hepatol.* 2005; **20:** 229 – 233.

20 Farhat A, Khademi G, Mazlouman SJ. The prevalence of hepatitis B carrier state in Khorassan Province of Iran. *Saudi Med J.* 2003; **24:** 549 – 551.

21 Ghavanini AA, Sabri MR. Hepatitis B surface antigen and anti-hepatitis C antibodies among blood donors in the Islamic Republic of Iran. *East Mediterr Health J.* 2000; **6:** 1114 – 1116.

22 Alizadeh AH, Ranjbar M, Ansari S, Mir-Arab A, Alavian SM, Mohammad K, et al. Seroprevalence of hepatitis B in Nahavand, Islamic Republic of Iran. *East Mediterr Health J.* 2006; **12:** 528 – 537.

23 Amini S, Mahmoodi MF, Andalibi S, Solati AA. Seroepidemiology of hepatitis B, delta, and human immunodeficiency virus infections in Hamadan Province, Iran: a population-based study. *J Trop Med Hyg.* 1993; **96:** 277 – 287.

24 Pourshams A, Nasiri J, Mohammadkhani A, Nasrollahzadeh D. Hepatitis B in Gonbad-e-Kavoos: prevalence, risk factors, and intrafamilial spreading [in Persian]. *Govaresh.* 2004; **9:** 222 – 225.

25 Ganji A, Safavi M, Nouraie SM, Nasseri-Moghadam S, Merat S, Vahedi H, et al. Digestive and liver diseases statistics in several referral centers in Tehran, 2000 – 2004 [in Persian]. *Govaresh.* 2006; **11:** 33 – 38.

26 Malekzadeh R, Dehpour AR. Report from Iran. *Liver Int.* 2008; **28:** 1136 – 1139.

27 Poustchi H, Mohamadkhani A, Bowden S, Montazeri G, Ayres A, Revill P, et al. Clinical significance of precore and core promoter mutations in genotype D hepatitis B-related chronic liver disease. *J Viral Hepat.* 2008; **15:** 753 – 760.

28 Zacharakis G, Koskinas J, Kotsiou S, Tzara F, Vafeiadis N, Papoutselis M, et al. The role of serial measurement of serum HBV DNA levels in patients with chronic HBeAg(-) hepatitis B infection: association with liver disease progression. A prospective cohort study. *J Hepatol.* 2008; **49:** 884 – 891.

29 Semnani S, Sadjadi A, Fahimi S, Nouraie M, Naeimi M, Kabir J, et al. Declining incidence of esophageal cancer in the Turkmen Plain, eastern part of the Caspian Littoral of Iran: a retrospective cancer surveillance. *Cancer Detect Prev.* 2006; **30:** 14 – 19.

30 Bonino F, Marcellin P, Lau GK, Hadziyannis S, Jin R, Piratvisuth T, et al. Predicting response to peginterferon alpha-2a, lamivudine and the two combined for HBeAg-negative chronic hepatitis B. *Gut.* 2007; **56:** 699 – 705.