

The Homeless youth and their exposure to Hepatitis B and Hepatitis C among in Tehran, Iran

Research Article

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Key words: Hepatitis B, Hepatitis C, street children

Abbreviations: Antibodies against HCV, (HCV-Ab); *Hepatitis B Virus*, (HBV); *Hepatitis C Virus*, (HCV)

Received: 29 April 2008; Revised: 2 June 2008

Accepted: 4 June 2008; electronically published: July 2008

Summary

Nowadays the issue of street children is one of the most important issues of societies from industrial city to developing cities. There are approximately one hundred million children spending their life in streets. With regard to the last description of the International Organizations for street children, many of statistical result and numbers are reported less than the actual facts. many countries are suffering from an individual relation the issue of street children despite possessing common properties, and by controlling this phenomenon each country will get in specific picture. 203 street children picked up from different places of Tehran and settled at welfare center, where provides shelter for street children, were chosen for this study. These children were clinically examined by pediatrician and requested to answer the questionnaire (asking about their gender; age; birth place; educational status; the origin of the family; sleeping place; occupation, income and social security of parents; number of siblings; reasons for being in streets; period of living in the streets; street friends; means of earning money; substance use. Smoking level was classified as heavy (10 and more per day), medium (1-9 per day) and rare (1-2 per week).) In order to determine the existence of *Hepatitis B Virus* (HBV) and *Hepatitis C Virus* (HCV) infections, ELISA, PCR and RT-PCR methods were performed on serum samples. Among 203 street children studied in this research, 196 children were boys and 7 children were girls. 6 cases (3%) were HBsAg positive, 54 cases were HBsAb positive (26.6%) and 16 cases were HBeAb positive (8%). 7 cases (3.5%) were HCV Ab positive. All of the positive cases were boys. There were 3 Iranian and 3 Afghanian kids among HBsAg positive cases. They did not smoke, they did not have tattoo, all of them had family that one of them lived alone and other 5 cases lived with their family and their average age was <14. In HCV Ab positive cases there were 5 Iranian and 1 Afghanian kids. 3 children did not have family, 6 children did not smoke and one of them was addicted to crack and had tattoo on his body. The average age of this group in three cases was 14> and in four cases 14 < years. 4 cases were HBV PCR positive and 6 cases were HCV RT-PCR positive. According to this results, additional laboratory examination for screening of acquired infectious disease such as *Hepatitis* seem to be necessary. Although in this type of infection clinical symptom may appear a few months after exposure to the virus, it can be transmissible in this latent period.

I. Introduction

According to the last description of Human Rights Organization Watcher, the children and youth under 18 years old living and working in the streets are called street children. In fact these children spend most of their time in the street and work either to become their family's source

of income or to settle their personal needs. These children spend considerable time in the street without any supports, supervisions, and inspections of manager (UNICEF, 29 March 2004).

Nowadays the issue of street children is one of the most important issue of societies from industrial cities to

developing cities. there are approximately 100 million children in the world that spending their life in the street (Turkmen et al, 2004). million of these children are dispersed in the streets without any protection and supervision with the last description of International Organization for street children, many of documental statistics and numbers are counted less than the actual figures. many countries are suffering from an individual reality on the problem of street children despite possessing common properties and by controlling this phenomenon each country will get an specific picture. Unfortunately, only a few numbers of countries have investigated this problem and published some reports.

One of the most important problems which street children are facing with it, is health situation. Many children suffer from malnutrition, anemia, and respiratory infections (because of spending most of their time in the street and breathing polluted air), gastrointestinal infections, skin infections, and acquired infectious diseases such as *Hepatitis B, C*, HIV and tuberculosis (Kipk et al, 1997; Inciardia and Surrat, 1998; Lajor, 1999; UNICEF, 29 March 2004).

Studies in Turkey show that 5% of street children were HBs Ag positive and 24 % of cases were anti-HBs Ag positive (Turkmen et al, 2004). In research performed on street children in south of Tehran in 2004-2005, 2.9% were HBs Ag positive and 14.5 % were HBs Ab positive (Vahdani et al, 2006). 13.5% of street children in Brazil were anti-HBc Ag positive and 2% were HBs Ag positive (Porto et al, 1994).

Routine screening for *HBV* requires assessment of at least two serologic markers. HBsAg is the first appeared serologic marker of infection and it is found in almost all infected patients. Because HBs Ag levels fall before the end of symptoms, presence of anti-HBs Ag also indicates the previous infection or immunity reactions against *Hepatitis*. Anti-HBcAg is the most valuable single serologic marker of acute *HBV* infection because it is present almost as early as HBsAg and continues to appear. Anti-HBsAg and anti-HBcAg are detected in persons with resolved infection. In order to identify *HCV* different kinds of serology methods are available, but with this method distinguishing of acute, chronic and asymptomatic infections is impossible. Antibodies against *HCV* (HCV-Ab) appear at the beginning of clinical symptoms in 50-70% of cases and in other cases can be identified in 3-6 weeks.

Different statistics have been reported from worldwide studies concerning this issue. With Regard to the prevalence of *Hepatitis* in Iran and lack of identification of infected people, particularly street children, we decided to determine the prevalence of *Hepatitis B and Hepatitis C* among street by ELISA and PCR method.

II. Materials and methods

In This "prospective study ", 203 street children with the average age 7-18 years old picked up from different places of Tehran and settled at welfare center, have been chosen. They were collected from terminals and stations by Welfare

Organization Officers. Welfare Organization has justified 3 keeping centers for boys and 1 keeping center for girls in Tehran.

We used ELISA method for detecting of HBs Ag, HBc Ab, HBs Ab and HCV Ab. 3ml of blood sample was taken in the sterile tube and then transported to the laboratory. In the laboratory, after separating the serum, for long maintenance, all samples were kept at -20 C.

Thus, we evaluated *HCV* infection base on the existence of HCV-Ab. All serum specimens were investigated by ELISA method using Diapluse Inc kits and PCR/RT-PCR methods using CinnaGen Inc kits.

A. DNA extraction from specimen

100 ml Proteinase K and 16 ml DNX tm solution was added to each tubes. Then 100 ml of each patient specimen (serum) was added to the tubes and vortex 3-5 seconds. incubated for 10 min at 72 °C and then cooled in 4 °C for 5 min. 200 ml Equilibrated Phenol was added and vortex 3-5 sec, then centrifuge at 12000 rpm for 5 min. Transferred the upper phase to new tube and added equal volume of Chloroform, vortex it for 3-5 sec and centrifuge at 12000 rpm for 5 min. Transferred the upper phase to new tube and added 1/10 vol of 3M Sodium Acetate on ice. 3 vol of 96% Ethanol was added, invert it 10 times and put on ice or -20 °C for at least 30 min, then centrifuge it at 12000 rpm for 5 min and decant it. 500ml 70% Ethanol was added to the pellet and invert it 10 times and centrifuge at 12000 rpm for 5 min. Decant it and dry the pellet 10-20 min at 65 °C (up to dry). 30 ml DNase free deionized water was added and stored at -20 °C.

Then added the following reagents for each tube on ice: 1XPCR MIX 15 ml, Taq-DNA polymerase 0.4 ml.. Mixed the mixture thoroughly by shaking and spin. To each tube added one drop (20-25ml) mineral oil. Cap the reactions tubes or place the tube in a resealable plastic bag and seal the bag securely, don't cap tubes at this time. Then added 10 ml DNA, closed tubes, spine the mixture on microfuge for 3-5 sec. Transferred the tubes to preheated thermocycler. Reaction was run for 35 cycle under following condition: 20s at 93 °C, 20s at 61 °C, 40 s at 72 °C. After the last cycle. a final elongation step of 10 min at 72 °C was performed to complete the elongation. Amplification products were visualized and photographed under UV light after electrophoresis for 45 min at 100 V through a 1% agarose gel, containing ethidium bromide.

B. RNA extraction from specimen

50ml serum was added to 450 ml cold RNX TM-plus solution. Vortex the sample to dissolve the clamps. Incubated for 10 min on ice. 100 ml of Chloform was added, vortex (3-5 sec) and centrifuge it at 12000rpm for 5 min. Transferred the aqueous phase to new tube and added equal volume of Isopropanol (250-300ml). Invert the tube 10 times and then incubated at -20 °C for at least 20 min. Centrifuge at 12000 rpm for 15 min. Discard aqueous phase and added to pellet 200ml 70 % Ethanol and invert 10 times, and centrifuge it at 12000rpm for 5 min. Discard aqueous phase and incompletely dry the pellet (RNA) for 20-30 min at room temperature. Dissolved RNA in 30 ml DEPC treated water, then cDNA synthesis protocol within 3hours of specimen preparation or store the processed specimens frozen at -70 °C or colder for up to one month with no more than one freeze-thaw. Then following reagents for each tubes on ice was added: Mix1 39 ml, RT Enzyme 1 ml, Mineral oil 40 ml, mixed the mixture thoroughly by shaking and spin. Place RNA tube at 95 °C, 1 min and then place on ice.5 ml RNA was added to each tube and positive control to pos and DEPC water to negative tube. Closed tubes, spin the mixture on microfuge 3-5 sec and transferred the tubes to preheated thermocycler and Reaction was run for 20 cycle under following condition: 40s at 93 °C, 40s at 60 °C, 40s at 72 °C. After this protocol following reagents was added :1X PCR

Mix 11, Taq DNA Polymerase 0.2 ml, Mineral oil 20 ml. then 3 ml PCR product from first round was added. Reaction was run for 35 cycle under following condition: 40s at 93 °C, 40s at 60 °C, 40s at 72 °C. Amplification products were visualized and photographed under UV light after electrophoresis for 45 min at 100 V through a 1% agarose gel, containing ethidium bromide.

C. Questionnaire

After clinical examination by Pediatrician, these children were requested to answer the questionnaire. This form included questions about: age, sex, nationality, educational status (elementary, guidance, and high school), smoking status (heavy 10 or more per day, medium 1-9 per day, rare 1-2 per week), having tattoo, type of occupation, having family, the period of time that they spend in the street and the period of time that they sleep in the street.

D. Statistical analysis

Statistical analysis was performed by SPSS Statistical software package version 12, using T-test (Post Hoc multiple comparison test) and Chi-square test. Statistical significance was assumed at the $P < 0.05$ level.

III. Results

Among 203 street children, 196 cases (96.6 %) were boys and 7 cases (3.4%) were girls. 129 cases (63.5%) were Iranian, 71 cases (35%) were Afghanian, 2 cases (1%) were Pakistanian and 1 case (0.5%) was Iraqi.

181 cases (89%) had care taker and family, 22 cases (11%) were unsupervised.

177 cases (87%) did not smoke and 26 cases (13%) were smokers [18 cases (9%) rare, 2 cases (1%) medium, 5 cases (3%) heavy].

22 cases (11%) had tattoo and 181 cases (89 %) were without tattoo.

78% lived without family, 71 cases (35%) were with elementary education, 62 cases (5.30%) were with guidance school education, and 9 cases (5.4 %) were with high school education and 61 cases (30%) were illiterate.

Among 203 street children, 6 cases (3%) were HBsAg positive, 54 cases were HBs-Ab positive (26.6%), 16 cases were HBc-Ab positive (8%) and 7 cases were HCV-Ab positive (3.5%).

1 case was HBc-Ab, HBs-Ag and PCR positive. That result shows acute or chronic infection.

9 cases were HBc-Ab, HBs-Ab positive and they were PCR negative. That shows recovery from acute or chronic infection.

44 cases were only HBs-Ab positive and PCR negative. That shows recent vaccination.

6 cases were HBc-Ab positive and PCR negative. That shows false positive or infection in many years ago or window period or passive Ab transports.

5 cases were HBs-Ag positive that 1 case was PCR negative and 4 cases were PCR positive.

Characteristics of this group are summarized in **Tables 1-2**.

5 cases were HCV-Ab and RT-PCR positive. This result shows acute infection.

1 case was HCV-Ab negative and RT-PCR positive. This result shows acute infection before stimulating the immune system.

1 cases was HCV-Ab positive and RT-PCR negative. This result shows false positive or recovery from infection.

From the occupation and income point of view, 76 cases (37.4%) neither had income nor job. The other 127 cases had jobs such as flower selling, fall selling (selling peace of papers that different fortunes have been written on them and people pick them up by chance), music playing and etc. Among these occupations the highest percentage belonged to Fall selling (16%).

Table 1. HCV-Ab positive children.

Age	14 \geq	3	
	14<	4	7
Life style	To gathers	1	
	alone	2	
	family	4	7
Tattoo	yes	1	
	no	6	7
Smoking	smoker Heavy	1	(Addicted to crack)
	Moderate smoker	0	
	Rare smoker	0	7
	Non smoker	6	
Care taker	yes	4	
	No	3	7
Nationally	Iran	6	
	Afghan	1	7

Table 2. HBs Ag positive children.

Age	14 \geq	6	6
	14<	0	
Life style	To gether	-	6
	alone	1	
	family	5	
tattoo	yes	0	6
	no	6	
smoking	smoker Heavy		6
	moderate smoker	0	
	Rare smoker	0	
	No n smoker	6	
Care taker	yes	6	6
	No	0	
nationality	Iran	3	6
	Afghan	3	

IV. Discussion

Children living and working in the streets are an increasing problem in developing countries as well as in developed countries.

There is a few information about the prevalence of infectious diseases among street children in Iran. In this research, we have found that the majority of street children in Tehran are immigrants from other cities. Alone or with their families, they immigrate to big cities like Tehran and because of the poverty and lack of any job proficiencies, they are absorbed in fake jobs. During the day, they are vagabond in the street and face with dangers and social impairments. In order to earn more money, they usually involve in illegal activities such as transferring drugs. Parallel to our social structure, almost all were male, since the family expected boys to contribute to the family budget.

In study done in Turkey Struggles against poverty, and in some cases social unrest, have pushed many rural families in Turkey to the cities. Internal migration has increasingly become one of the main survival strategies of poor families, especially those from the eastern part of the country. These families come to the city and are challenged by lack of skills and unemployment. An outcome of this social situation is children working in the streets (Turkmen et al, 2004).

Another important finding of our study was about children's care taker. Most of the children (181/203 cases) had care taker and lived with their family. In 89.2% cases, these children were considered as the main source of income for their family.

In the current study, all children except one stayed with their families. However, some of these children might temporarily leave their homes. In a previous study of ours it was found that 78% of children had steady relations with their families (Vahdani et al, 2006).

In this study 3% cases were positive HbsAg and all of them were under 14. There was no evidence of children's vaccination and most of them did not have any information about Hepatitis and its transmission way. In order to follow treatment, children with positive HBs Ag were introduced to the infectious specialist.

In the same study performed in the neighbor country, Turkey, in Adana 2 cases (5%) among 38 street children under investigation were HbsAg positive (Turkmen et al, 2004).

which was more than our results. Sexual affairs and alcohol usage are risk factors that may cause these differences.

In another research performed in Istanbul, 4.3% of street children were positive HbsAg in the 10-14 years old age group, and 9.9 % of them positive HbsAg in the 15-19 years old age group (Pasha et al, 1999).

In this study 3.5 % of cases were HCV-Ab positive, 3 of them (4.2%) were under 14 and 4 cases (5.1%) were above 14.

In the study performed in Iran on southern Tehran kids in 2003, 3 cases (2.9%) were HbsAg positive and all of 103 kids were HCV negative (Vahdani et al, 2006).

From the acquired results in our study, the prevalence of HCV has been increased among street

children in comparison with the previous study performed on street children in south Tehran in 2004-2005 in which all of them were HCV-Ab negative. This difference can be explained by this fact that our study has covered all the four zones of Tehran and larger population.

In this study we used two methods (ELISA, PCR) to specify infected children. ELISA method is used to detect serological marker which appeared in serum 1-2 months after infection and PCR method to detect genome (Figure 1, HBV genome and Figure 2, HCV genome) of viruses. This method can detect DNA or RNA before clinical symptoms appear.

In all of study that performed on street children in other countries, they used only ELISA method to detect infected children. This method like all other serological methods has false positive or negative results. For avoiding false positive or negative results and increasing specificity and sensitivity we used PCR method. Using this method we could detect infected children base on presence of virus's genome before initiation of Immune response.

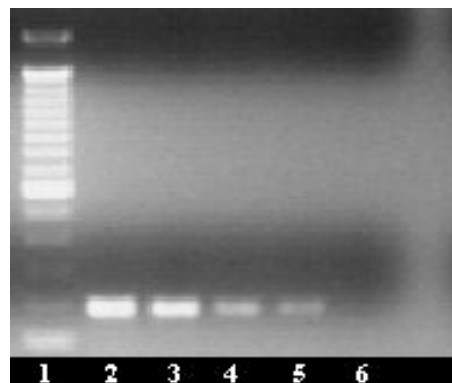


Figure 1. Detection of HBV genome by PCR. Lane1-100bp DNA ladder, Lane2- Positive control, Lane3- 5 Positive cases and Lane6- Negative control.

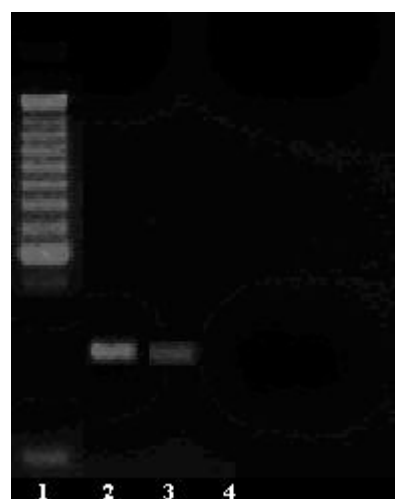


Figure 2. Detection of HCV genome by RT-PCR. Lane1-100bp DNA ladder, Lane2- Positive control, Lane3- Positive cases and Lane 4- Negative control.

Different factors are involved in the incidence of street children phenomenon what we attained from this study is: in Iran some factors like poverty and low income are the most important factors in the incidence of street children phenomenon. In other countries another factors are effective, for example in USA sexual relationship to gain food and shelter (Densley and Joss, 2000), in Sanpaolo lack of family and caretaker (Pancharoen and Thisyakorn, 2003) and in Thailand and Eastern Asian countries sex smuggling,are important factors (Marozzi, 1998).

IV. Conclusion

According to this results, additional laboratory examination for screening of acquired infectious disease such as Hepatitis seem to be necessary. Although in this type of infection clinical symptom may appear a few months after exposure to the virus, it can be transmissible in this latent period..

Acknowledgment

We would like to express our thanks and recognition to: Reverend research assistance of medical school and also welfare office of Tehran, specially reverend managers of associated center in children identification and also to:financial support of Dear manager of pediatric infectious research center,specially to Dr. Maham and dear statistics advisor Dr. Shamshiri

References

- Densley MK, Joss DM (2000). Street children: causes, consequences, and innovative treatment approaches. **Work** 15, 217-225.
- Inciardia JA, Surrat HL (1998) Children in street of Brazil: drug use,crime, violence, and HIV risk. **Subs Use Misuse** 33, 1461-1480.
- Kipk MD, Simon TR, Montgomery SB, Unger JB, Iversen EF (1997) Homeless youth and their exposure to and involvement in violence while living on street. **J Adolesc** 20, 360-367.
- Lajor KJ (1999) Street children :a comparative perspective. **Child Abuse Negl** 23, 759-770.
- Marozzi J (1998). Aiding the poorest. The Philippines: Children and Youth Foundation. Finance Times 4
- Pancharoen C, Thisyakorn U (2003) Stuart Gan Memorial Lecture 2002. HIV/AIDS in children. **Ann Acad Med Singapore** 32, 235 – 238.
- Pasha A, Uzsoy MF, Altunay H, Kocak N, Ekren Y, Cavuslu S (1999) Istanbul da hepatit B ve C Prevalansi. **Gulhane Tip Derg** 41, 325-330.
- Porto SO, Cardoso DD, Queiróz DA, Rosa H, Andrade AL, Zicker F, Martelli CM (1994). Prevalence and risk factors for HBV infection among street youth in central Brazil. **J Adolesc Health** 15, 577-581
- The state of the worlds children UNICEF, 29 March 2004.
- Turkmen M, Okyay P, Ata O, Kuyanglus O (2004) A descriptive study on street children living in the southern city of turkey. **Turk J Pediar** 46, 131-136.
- Vahdani P, Hosseini Moghadam S, Gachkar L, Sharafi K (2006) Prevalence of Hepatitis B, Hepatitis C, Human Immunodeficiency virus and Syphilis among street children Residing in the southern Tehran, Iran. **Arch Iran Med** 9, 153-155.

