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Incidence of Hepatitis B and C in Industrial Areas of Sheikhupura and Kasur

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Abstract.- There is strong evidence that humoral and cell mediated immune responses are involved in the clearance of viruses including hepatitis virus B and C, on the other hand exposure to industrial pollutants, including heavy metals and organic chemicals, are known to cause immunosuppression. Present study was conducted to find any association between the industrial pollution and incidence of hepatitis B and C. Three population groups, two from industrially polluted areas and one from non-industrial area, were included for this study. A total of 300 subjects in age group between 10-49 years were selected. Their blood samples were screened for hepatitis B and C antigen with ELISA kits. No significant association could be observed in industrial pollution and incidence rate of hepatitis B and C. Relative risk for HCV was found to be slightly higher in residents of polluted area but the difference was not statistically significant.

Keywords: Hepatitis B, hepatitis C, industrial pollution, immunosuppression.

INTRODUCTION

Industrial pollution can cause widespread environmental problems through its impact on air quality, soil and food chain. It is considered as a serious problem for entire planet especially in rapidly industrializing areas. According to an estimate 50% of pollution is caused by industry and heavy metal pollution has been associated with small scale industry, it includes the iron, manganese, zinc, copper, cadmium, nickel, lead and chromium (Rawat *et al*., 2009). The chemicals and the heavy metals of industry after penetrating body may bind to vital proteins and hormones and affect the body systems, thus making it more susceptible to pathogens (Gleichmann *et al.,* 1999). Mercury is a pervasive environmental contaminant and well documented immunosuppressor (Farahat *et al*., 2009; Hawley *et al*., 2009). Cadmium is a potential carcinogen and potent immunotoxicant. It is documented to disturb CD4+/CD8+ ratio along with inhibition in INFγ (Pathak and Khandelwal, 2008).

In Pakistan, Sheikhupura district has number of small industries related to shoe making, textile and paper manufacturing, while Kasur is the area of tannery industry. The effluents of these industries are known to contain heavy metals which exert both sublethal and lethal effects on the organisms (Boyd, 2010). Solvents involved in shoe making have been documented to possess genotoxic potential (Lladó *et al*., 2008). On the other hand textile industries use Azo dyes during dying processes which have mutagenic potential and are reported to increase the micronuclei frequency in human lymphocytes (Chequer *et al*., 2009; Kwon *et al*., 2008). Paper mill effluents have been reported to induce generalized stressed response leading to potential immunosuppression in fish model (Sepúlveda *et al*., 2004; Baer *et al*., 2009). The pollutants generated in tannery industry contain high concentration of chromium and aluminum which directly affects human health. Ahsan *et al*. (2006) reported significantly higher concentration of chromium along with decreased number of leukocytes in tannery workers.

The immune system protects organism by precluding the entrance and proliferation of pathogens in body. Humoral and cellular branches of immune system coordinate in

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restricting pathogen. In the presence of immunosuppression body has higher chances of being colonized by pathogens. Viral hepatitis is one of the major causes of chronic liver disease, cirrhosis and liver cancer. Cellular immunity plays important role in the control of HCV and HBV (Cooper *et al*, 1999; Willberg *et al*., 2003; Thimme *et al*., 2008). With reference to a case report the recurrence of HCV has been reported to be associated to leukocytopenia (Gruener *et al*., 2007). The concern of the effects of environmental contaminants on immune function of humans is growing, particularly nothing is known about its impact on the spread of viral hepatitis (Sorvari *et al*., 2007). A number of studies have been conducted to find the prevalence of HCV and HBV in different areas of Pakistan including Lahore, Karachi and Islamabad (Ali *et al*., 2009; Waheed *et al*., 2009). Least information is available about industrially polluted areas like Sheikhupura and Kasur. Current study was therefore planned to find prevalence of hepatitis B and C in these areas along with its association with industrial pollution.

MATERIALS AND METHODS

Blood sampling and processing

The blood samples were collected from three hundred individuals; one hundred from non industrial areas (Babral a village in Tehsil Shakargarh), two hundred from industrially polluted area (Sheikhupura and Kasur). From Sheikhupura subjects working or living around paper mill, textile industry and shoe factory were selected, in Kasur subjects working and living around tannery industry were included and from non industrial area (Babral a village in Tehsil Shakargarh) blood samples were collected at random by door to door visit. The blood was collected by authorized technician, kept in heparanized tubes and transported to laboratory in the insulated ice boxes. The plasma was separated by centrifugation (10 min, 4000 rpm/ 1,359 x g) and stored at -20°C till further use.

Enzyme linked immunosorbent assay (ELISA) for hepatitis B

The HBsAg kit (Equipar, Itali) based on one-step "Sandwich" ELISA was used for detection of hepatitis B antigen following the protocol given by manufacturer. Briefly, the system uses two highly specific monoclonal antibodies, directed to different epitopes, one absorbed onto the walls of microplate and second labeled with the enzyme horseradish peroxidase (HRP). The sample and the conjugate were added simultaneously to the plate and incubated at 37^{o}C. The presence of a specific immunocomplex on the solid phase was detected by the action of the captured conjugate on the chromogen in the second incubation. The intensity of the color generated by the enzyme was proportional to the amount of antigens in the samples which was determined by ELISA Reader.

ELISA for hepatitis C

The Hepatitis C kit (Equipar, Itali) contained the microplate that is coated with HCV-specific recombinant protein analogues of antigens of hepatitis C virus, in which solid phase is first treated with the diluted sample and HCV antibodies are captured if present, by the antigens. In second incubation bound HCV antibodies are detected by the addition of highly specific monoclonal anti H-lgG antibody, labeled with horseradish peroxidase (HRP). The enzyme captured on solid phase acts on chromogen and generates color that is proportional to the anti-HCV antibodies present in the sample which was determined by ELISA Reader.

Statistical analysis

The data was analyzed by χ^2-test to find out the effect of industrial pollution on the incidence of hepatitis B & C. Relative risk of viral hepatitis in the inhabitants of industrial area was calculated following Gardner and Altman (1994). Age of the subjects varied from 10-49 years, age wise comparison was made by dividing subjects from each group into four age groups *i.e.*, ≤ 19 years, 20-29. 30-39 and ≥40 years. Age wise comparison was made to identify any association

between different age groups and incidence of hepatitis.

RESULTS

Incidence of hepatitis B

In Babral, 9% subjects were positive for hepatitis B, while 12% and 14% were positive in Sheikhupura and Kasur, respectively. No association between hepatitis B and pollution could be observed. The relative risk was also not higher in subjects living in polluted areas (Table I).

The incidence of hepatitis in non polluted (Village Babral), and polluted areas (Sheikhupura and Kasur) was 7.7%, 9.1% and 10.5% in age group ≤19 years, 12.5%, 14.3% and 16.0% in age group 20-29 years, 5.0%, 13.0% and 15.4% in age group 30-39 years and 7.1%, 10% and 13.3% in age group ≥40 years, respectively (Table II). The differences in incidence rates in different age groups were not statistically significant.

Incidence of hepatitis C

The hepatitis C was detected as 4% in village Babral, 7% Sheikhupura and 9% in tannery polluted area (Kasur) (Table I). The χ^2-test showed no association of hepatitis C and pollution. Relative risk was slightly higher (statistically non significant) in subjects inhibiting polluted areas. Just like hepatitis B, age wise comparison of incidence of hepatitis C was also performed. It was 3.8%, 4.5% and 5.3% in age group ≤19 years; 5.0%, 8.6% and 12.0% in age group 20-29 years; 0.0%, 4.3% and 11.5% in age group 30-39 years and 7.1%, 10% and 6.7% in age group ≥40 years for non industrial area (Village Babral), and industrially polluted areas, Sheikhupura and Kasur respectively (Table II).

DISCUSSION

Viral hepatitis is a transmittable disease and many risk factors have been associated to its spread. The reported factors include use of infected syringes, unhygienic dental procedures, contaminated blood transfusion and sexual contact with infected partners. The industrial pollution has been least studied as a risk factor for viral hepatitis. There is enough evidence to believe that cell mediated immune responses are responsible for curtailing viral hepatic infections. The cross talk among dendritic cells, natural killer cells and Natural Killer T (NKT) cells have been reported to be crucial in shaping subsequent adaptive immune response against HCV and HBV (Kanto and Hayashi, 2007; Dolganiuc *et al*., 2008). This cross talk among different partners is through chemical mediators, the cytokines. On the other hand industrial pollutants have been documented to be responsible for immuno-suppression through disturbances in cytokine levels and development of special cell subsets required for the virus clearance in exposed subjects. Current study is an effort to find any relationship between viral hepatitis and industrial pollution.

A large variation in the incidence rate of hepatitis B and C occurs in Pakistan and incidence rate varies over time (Ali *et al*., 2009; Waheed *et al*., 2009). There was no previous information on the incidence rate of HBV and HCV in industrially polluted areas like Sheikhupura and Kasur. The incidence rate of hepatitis B and C in non polluted area was 9% and 4% in contrast to polluted area (Sheikhupura and Kasur) 13% and 8%, respectively. Waheed *et al*. (2009) have reported 4.95±0.53% incidence of hepatitis C in the general adult population of Pakistan. In present study it was observed to be higher in polluted area. On the other hand no association between viral hepatitis and industrial pollution could be observed. Similarly in age wise comparison, slightly higher rate of incidence of HBV and HCV was observed in polluted areas in all age groups but the differences were not statistically significant. These finding are in incongruity with an online report by Jordan (2010) in which it is mentioned that people living in polluted areas have higher frequency of viral hepatitis but no scientific evidence could be found with such conclusion except one. Bashir *et al*. (2005) correlated the HCV with pollution using indirect method in which they detected higher incidence of co-infection of typhoid and HCV in the subjects who had no past one year

Table I.- Comparison of the incidence rate of hepatitis B and C in polluted and non-polluted area.

Disease	Comparison groups	P (χ^2)	Relative risk	Confidence interval
HBV	Control vs Polluted*	0.49 (0.47)	1.44	0.70-2.96
	Control vs Sheikhupura	0.64 (0.21)	1.33	0.58-3.02
	Control vs Kasur	0.38 (0.79)	1.55	0.70-3.42
HCV	Control vs Polluted*	0.69 (0.15)	2.00	0.68-5.82
	Control vs Sheikhupura	0.53 (0.38)	1.75	0.52-5.79
	Control vs Kasur	0.28 (0.59)	2.25	0.71-7.06

* Pooled data of Sheikupura and Kasur was compared with Control.

Table II.- Details of subjects investigated for hepatitis B and C in non polluted and industrially polluted areas.

Age Groups (Years)*	Non polluted			Industrially polluted					
	Shakaragh			Sheikhupura			Kasur		
	Subjects	Positive cases		Subjects	Positive cases		Subjects	Positive cases	
	Total	HBV	HCV	Total	HBV	HCV	Total	HBV	HCV
≤19	26	2	1	22	2	1	19	2	1
20-29	40	5	2	35	5	3	25	4	3
30-39	20	1	0	23	3	1	26	4	3
≥40	14	1	1	20	2	2	30	4	2
Total	**100**	**9**	**4**	**100**	**1 2**	**7**	**100**	**14**	**9**

history of exposure to any known risk factor of hepatitis C and concluded a relationship in the occurrence of hepatitis and water pollution. The hepatitis C virus can live for 16 hours to maximum 4 days while HBV, can remain infectious for up to a week outside the body (Daniel, 2009), however it can cause infection only if enters in body through wounds. Current study did not show any correlation in industrial pollution and incidence of viral hepatitis. The risk of exposure through wounds may be similar in polluted and non polluted areas. In conclusion present study indicates that industrial pollution may not be responsible for increasing incidence of hepatitis B and C. The adaptation of the subjects with polluted environment with out immunosuppression could be the possible explanation of insignificant variations observed in current study. The validation of this statement requires further studies on the incidence of immunosuppression among inhabitants of polluted areas.

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