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Biochemical and Haematological Abnormalities in Factory Workers Exposed to Hexavalent Chromium in Tanneries of Kasur District*

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Abstract.- The values of liver function tests of factory workers in tanneries of Kasur were found to be within the normal range in all age groups except for the albumin content and alkaline phosphatase activity, which were higher in younger age group. The total chromium content in the blood of workers of age group 1 – 20 years was significantly higher (24%), when compared with the same age group amongst the non-workers. The mean cell volume, packed cell volume and platelet counts in workers were generally lower, whereas the haemoglobin, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were higher in workers than in non-workers. The white blood cell counts decreased in workers up to the age of 40 years, whereas in older population it showed slight increase. The albumin, alkaline phosphatase, alanine aminotransferases, aspartate aminotransferases and total protein showed higher values, whereas the total bilirubin, direct bilirubin and blood serum glucose contents showed lower values in blood sera of factory workers, both male and female, when compared with those of the control population. The total chromium (569%) and hexavalent chromium (78%) were significantly higher in the blood of exposed male and female worker population as compared with the control population. No definite pattern was observed in different haematological and biochemical parameters, when comparing workers with non-workers, normal male with exposed males and normal females with exposed females. Slight variation may be due to multitude of factors in addition to possible effects of chromium toxicity.

Key words: Liver function tests, haematological changes, tannery workers, chromium toxicity**.**

INTRODUCTION

Industrial wastes contain a variety of toxic chemicals, including heavy metals, which are carcinogenic and mutagenic (Gilberg, 1974; Degraeve, 1981; Moore and Ramamoorthy, 1984; Chang *et al.,* 1998; Hamilton *et al.,* 1998; Zhitkovich *et al.,* 1998). Heavy metals when present beyond traces are toxic to humans. Initially they may combine with proteins and may not cause any poisoning but when their concentration exceeds the threshold level, they become a real health concern (Jaffer*,* 1988). These toxic metals interact with essential cellular components through covalent and ionic bonding. At high levels, both essential and non-essential metals can damage cell membrane, alter enzyme specificity, disrupt cellular function and damage the structure of DNA (Bruins *et al*., 2000; Blasiak *et al.,* 1999). On a global basis, chromium discharge to aquatic systems is mostly from the metal industries followed by domestic wastewater sources. The estimated maximum worldwide aquatic discharge is reported to be $239x10^3$ t $year^{-1}$ (Nriagu *et al.,* 1988). Hexavalent chromium is used extensively in the chrome plating, manufacture of the dyes, and pigments, leather tanning and wood preservation industries. Elevated levels of Cr in anthropogenically-polluted ecosystems are of serious human concern.

Chromium was first determined to be essential for animals by Schwartz and Mertz in 1963. It is thought to potentiate the action of insulin by facilitating insulin-receptor binding at cell surfaces, thus enhancing insulin sensitivity and responsiveness in peripheral tissues and reversing the effects of clinical hyperglycemia (Mooradin and Morley, 1987; McCarty, 1993; Morris *et al.,* 1993). Chromium increases the absorbability of insulin, therefore helping to reduce body fat and help build lean muscle (Davis and Vincent*,* 1997; Jeejeebjhoy *et al.,* 1977). The essentiality of dietary Cr has been demonstrated in numerous species (Gurson and Sauer, 1973). For optimal health, as a supplement,

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women should take between 200-400 µg daily and men should take between 400-600 µg daily. On the other hand, elevated amounts of chromium may be hazardous to fauna and flora (Nriagu *et al.,* 1988; Bartlett and Kimble, 1976; Losi *et al.,* 1994). Several toxic effects are associated with exposure to hexavalent chromium compounds, including increased incidence of certain cancers, toxic towards living cells, tissue and organisms (Becker *et al.*, 1991; Deschamps *et al.,* 1995; Langaard and Norseth, 1986; Langaard' 1990; Leonard and Lauwerys, 1980), serious damage to such major organs as lung, liver and kidneys (Kim and Na, 1991; Landgaard and Norseth, 1986; Tandon, 1982), pulmonary fibrosis and chronic bronchitis, skin ulcers, allergic dermatitis, lung cancer and mutagenic effect on bacteria (Baruthio, 1992; Nishioka, 1975; Nestmann *et al.,* 1979; Green and Muriel, 1976; Petrilli and DeFlora, 1977) and impairment of primary immune responses (Graham *et al.,* 1978). Occupational exposure to chromium VI compounds has been related to an increased risk of lung cancer (Koponen *et al.,* 1981; Kiilunen, 1994; Huvinen *et al.,* 1996; Goyer, 1986; Baruthio, 1992; Ptashekas, 1992; Gurjar *et al.,* 1996; Costa, 1997).

Industrial wastes laden with heavy metal are posing serious problems in Pakistan where the environmental awareness is abysmally low. Waste recycling treatments and disposal of effluents is not according to world standards. In the province of Punjab, there are about 46,000 industrial units of various categories, out of which 4,600 units are considered to be the major contributors of pollution (Khalil *et al*., 1991). In Kasur the effluents from tanneries are discharged in open fields, rendering the agricultural land into waste land and the atmosphere absolutely smelly and the air unbreathable. The water of Bangla Kamboan (Kasur) has been reported to be harmful even for irrigation (personal communication). The present project aims at (1) a assessing and evaluating the biochemical and haematological abnormalities in tannery workers rexposed to chemicals including chromium used for leather processing in tanneries, and (2) estimating chromium in the blood serum of workers of factories of Kasur.

MATERIALS AND METHODS

Sampling sites

A small industrial town Kasur, about 50 Km in the east of Lahore, known for the tanneries was selected as a site for investigation. This city and its population were taken as experimental sites because of the environmental problem due to unprocessed and indiscriminate disposal of industrial effluents. Chromium is used during the treatment and softening of raw leather to give a shine. The washings are ultimately drained through open channels, which end in big ponds in and around the industrial area and hence are hazardous for the inhabitants of adjoining residential areas as well as factory workers. Tanneries workers come in direct contact with chemicals used in this industry and hence are directly exposed to the chromium. The segments of population of the area, which have other professions and do not work in tanneries and hence are not directly exposed to the chromium, were considered controls for tanneries workers.

Blood samples

Blood samples of 600 tannery workers from Niaz Nagar and Din Ghar areas including businessmen dealing with supply of dyes and chemicals used for leather processing residents, school children and teachers of Kasur Public High School and Sir Sayed High School, the ages of whom ranged between 5-60 years, both males and females, were taken. The blood samples of people not in proximity of the tanneries and on the out-skirts of Kasur were taken as control.

Blood (5ml) was drawn from the brachial vein in 5 cc disposable (BD) syringe of which 2.5 ml was dispensed in a 5 ml sterile glass test tube containing 3.75 mg of dipotassium salt of Ethylenediamine tetra-acetic acid (EDTA) as an anti-coagulant, for the analysis of different haematological parameters. The remaining 2.5 ml blood was used for the biochemical analysis. These samples were transported to the Punjab University Laboratory within 45 minutes in cool box with minimal vibration. The blood samples were centrifuged at .5664 g for 30 minutes to separate the serum for biochemical analysis.

An aliquot of serum samples (300µl) was stored in a freezer (-20°C) for the estimation of total and hexavalent chromium estimation.

Haematological analysis of blood

The various haematological parameters, such as white blood cells (WBC) count, haemoglobin (Hb) count, red blood cells (RBC) count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (Plt), erythrocyte sedimentation rate (ESR) were determined according to the procedures given in Dacie and Lewis (1950). Haemoglobin was estimated according to VanKampen and Zijlstra (1961) and Eilers (1967).

Biochemical analysis of blood serum

The blood serum was used for the estimation of albumin according to Doumas *et al.* (1997) and Grant (1987), alkaline phosphatase according to King and King (1954), alanine aminotransferase and aspartate aminotransferase according to Reitman and Frankel (1957), direct bilirubin and total bilirubin according to Lo and Wu (1983), total protein according to Henry *et al.* (1969), serum glucose according to Barham and Trinder (1972), total chromium and hexavalant chromium according to Humphries (1958) and Rand *et al.* (1979).

Estimation of chromium in blood samples

The blood serum was digested according to Humphries (1958) and as described previously (Ahsan and Shakoori, 2004).The digested samples were used for total and hexavalent chromium estimation (Rand *et al.,* 1979).

Statistical analysis

For statistical analysis Jerrold (1996) was followed. The data was subjected to Student's `t' test in order to determine the significant differences between workers and non-workers.

RESULTS

Factory workers vs non-workers

Haematological parameters

Table I shows the heamatological values of factory workers and non-workers of different age groups. There is general trend of decreased MCV, PCV values and Plt counts in worker as compared with non-worker population, while the Hb, MCH and MCHC values were higher in workers as compared with that of non-workers. The WBC counts decreased in workers up to the age of 40 years, whereas in older group it showed slight increase. WBC, RBC, PCV and ESR showed slight fluctuation in their values among the different age group of both workers and non-workers.

Age group 1–20 years: The non-worker population had 9.92±0.28 $x10^3$/µl and RBC 6.33±0.94 $x10^6$ RBCs /µl, whereas in the workers the WBC and RBC counts decreased 7% and 15%, respectively. The values of Hb, MCH and MCHC of non-workers were 13.53±0.26 g/dl, 85.02±1.03 fl and 29.63±0.18 g/dl, respectively, whereas the workers showed 4%, 4% and 5% increase, respectively. The PCV, MCV and Plt count in non-workers are respectively 45.58±0.75%, 85.02±1.03 fl and 289.06±9.70 $x10^3$/µl, respectively. The worker population on the other hand showed 1, 1 and 7% decrease, respectively. The ESR of workers was slightly higher when compared with the non-workers.

Age group 21–40 years: In the age group 21-40 years of non-workers the WBC, MCV and Plt count were respectively 9.36±0.22$x10^3$/µl, 85.23±0.61 fl and 275.60±7.78$x10^3$/µl, respectively, whereas the worker population showed 7, 1 and 8% decrease, respectively. The RBC count, Hb content and PCV in non-workers were respectively 5.57±0.06 $x10^6$/µl, 14.21±0.18, g/dl and 47.32±0.49%, while the above-mentioned parameters of workers showed 2, 6 and 1% increase with reference to non-workers. The MCH and MCHC in non-workers were respectively 25.55±0.25 pg and 21.96±0.16 g/dl. The workers of tanneries showed 4% and 5% highly significant increase when compared with the non-workers. There was no change in ESR value of both non-worker and worker population.

Age group 41–60 years: In 41 to 60 years old population of non-workers the WBC count, RBC count and Hb concentration have 8.80±4.2 $x10^3$/µl, 5.49±0.01$x10^6$/µl and 14.24±0.28 g/dl, respectively.

Table I.- Haematological analysis of blood of factory workers from tanneries of Kasur industrial area. They have been compared with the residents from other parts of Kasur town where no factories are located.

Haematological Parameters	Age group 1-20 years		Age group 21-40 years		Age group 41-60 years	
	Non workers (n = 48)	Workers[c] (n = 111)	Non workers (n = 100)	Workers (n = 256)	Non workers (n = 38)	Workers (n = 47)
WBC[b] ($x10^3/\mu l$)	9.92±0.28[a]	9.23±0.19*	9.35±0.22	8.72±0.1*	8.80±0.42	9.45±0.43
RBC ($x10^6/\mu l$)	6.33±0.94	5.39±0.06	5.57±0.06	5.69±0.04	5.49±0.10	5.62±0.12
Hb (g/dl)	13.53±0.26	14.08±0.15	14.21±0.18	15.01±0.08***	14.24±0.28	14.73±0.25
PCV (%)	45.58±0.75	45.13±0.45	47.32±0.49	47.67±0.29	47.52±0.72	46.96±0.77
MCV (fl)	85.02±1.03	84.02±0.66	85.23±0.61	84.11±0.42	86.96±1.13	84.31±1.08
MCH (pg)	25.21±0.35	26.20±0.26*	25.55±0.25	26.55±0.16***	26.05±0.47	26.53±0.18
MCHC (g/dl)	29.63±0.18	31.17±0.21***	29.96±0.16	31.58±0.13***	29.91±0.27	31.41±0.34***
Plt ($x10^3/\mu l$)	289.06±9.70	269.07±7.66	275.60±7.78	252.82±5.23*	253.45±11.3	239.64±10.3
ESR (mm/hr)	6.52±0.51	7.41±0.39	6.87±0.68	6.86±0.31	8.66±0.54	7.70±0.53

a. Mean ± SEM; *P<0.05; **P<0.01; ***P<0.001

b. Abbreviations used; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, pecked cell volume; Plt, platelets count; RBC, red blood cells; WBC, white blood cells.

c. Population living in industrial area of Kasur, which predominantly comprises tanneries.

The workers of tanneries showed 7, 2 and 3% increased when compared with the non-workers. The Plt count and ESR value of non-workers had 253.45±11.63 $x10^3/\mu l$ and 7.7±0.54 mm/h, whereas the workers showed 5% and 9.1% decrease in these parameters, respectively. The PCV, MCV, MCH and MCHC of non-workers and workers showed very negligible change in their values.

Biochemical analysis of blood serum

Except for the albumin content and activity of alkaline phosphatase - that too in younger group, all the liver function tests were normal in the workers as well as non-workers The total chromium content of blood of workers of age group 1 – 20 years showed significant increase, while the remaining two age groups showed non-significant increase, when compared with the non-workers.

Age group 1-20 years: The non-workers in this age group had 5.39±0.09 g/dl albumin, while the workers of the same age group showed 6% decrease. The workers showed 34% decrease in the AP activity when compared with the non-workers. The ALT, AST, D.Bili, T.Bili, T.Prot and Cr.VI had non-significant changes among the workers and non-workers. The total chromium in the blood sera of the workers showed 24% increase, whereas glucose content showed 21% increase when compared with the non-workers.

Age group 21-40 years: The workers showed 2% significant decrease in albumin content, whereas D.Bili and Cr.VI of workers showed 8% and 6% decrease when compared with the non-workers of the same age group. The AP, ALT, and AST activity, and T.Bili, T.Prot. Glu and total chromium content of blood serum of workers are 6, 5, 5, 10, 1 and 7% more respectively, when compared with the non-workers.

Age group of 41 - 60 years: The AP, ALT, D.Bili, T.Bili and Glu of workers showed in the age group 41 – 60 years 4, 11, 5, 4 and 16% , respectively, decreased when compared with the non-workers of the same age group. The Alb, AST, total protein and CrVI of both workers and non-workers had no change in their values. The total chromium of workers showed 20% increase when compared with the non- workers.

Female vs male workers population

Haematological studies

Table II shows haematological values of male and female factory workers. There is general trend of increased WBC count, RBC count, PCV, and platelets count in the female and male workers as compared to non-workers. The other parameters such as haemoglobin content, MCH and MCHC

Table II.- Biochemical analysis of blood of factory workers form tanneries of Kasur industrial area. They have been compared with residents from other parts of Kasur town where no factories are located.

Biochemical Parameters	Age group 1-20 years		Age group 21-40 years		Age group 41-60 years	
	Non-worker (n = 48)	Worker [c] (n = 111)	Non-worker (n = 100)	Workers (n = 256)	Non-worker (n = 38)	Workers (n = 47)
Alb [b] (g/dl)	5.39±0.089 [a]	5.05±0.05***	5.15±0.04	5.04±0.03*	5.13±0.05	5.05±0.05
AP (U/l)	524.63±20.06	347.25±16.17***	233.23±5.99	247.25±4.42	268.24±13.03	258.68±10.01
ALT (U/l)	30.02±2.46	31.31±2.13	34.85±2.93	36.41±1.56	39.21±5.17	34.77±2.59
AST (U/l)	29.48±2.45	31.95±1.20	32.21±1.71	33.88±1.00	31.03±3.34	30.92±1.79
D.Bili (mg/dl)	0.21±0.02	0.23±0.01	0.26±0.01	0.24±0.01	0.22±0.01	0.21±0.01
T.Bili (mg/dl)	0.53±0.05	0.57±0.03	0.52±0.02	0.57±0.02	0.51±0.02	0.49±0.03
T.Prot (g/dl)	8.05±0.10	7.85±0.08	7.91±0.06	7.99±0.04	7.84±0.12	8.00±0.07
Glu (mg/dl)	69.60±8.45	84.17±2.60	72.90±6.69	74.13±1.45	89.78±15.38	75.77±3.42
T.Cr. (µg/l)	64.67±5.35	76.62±3.36*	59.60±3.75	63.83±1.78	58.68±6.18	70.53±4.32
Cr. VI (µg/l)	11.25±1.99	12.34±1.12	8.72±1.10	8.28±0.53	8.55±1.72	8.62±1.07

a. Mean ± SEM; *P<0.05; **P<0.01; ***P<0.001

b. Abbreviations used; Alb, albumin; ALT, alanine aminotransferases; AP, alkaline phosphatase; AST, aspartate aminotransferases; Cr. VI, chromium VI; D.Bili, direct bilirubin; Glu, glucose; T.Bili, total bilirubin; T.Cr, total chromium; T.Prot, total protein;

c. Population living in Industrial area of Kasur, which predominantly comprises tanneries.

decreased in workers, while the MCV and ESR remain unchanged.

Female: The female control population has WBC and platelets count of 7.26±0.67 $x10^3$/µl and 262.00±15.83 $x10^3$/µl, respectively, whereas in the factory workers the WBC and platelets were 31.8% and 16.8%, respectively, higher. RBC count and PCV were 23.7% and 8.5% higher in the female workers. The control non-workers showed MCH and MCHC values of 28.25±0.94 pg and 32.62±0.58 g/dl, respectively. The working female population showed 11.1% and 10.9% lower MCH and MCHC values, respectively. The haemogolobin content of workers showed 3.5% decrease in the female.

Male: The male workers showed the same trend as shown by the female workers. The RBC count and PCV of the control blood were 5.16±0.05 $x10^6$/µl and 43.45±0.39%, respectively, whereas theses parameters were 9.1% and 8.8% higher in the male workers. The WBC and platelets count also showed 3.6% and 4.1% increase in the workers. The control males had MCH 28.75±0.24 pg and MCHC 34.12±0.14 g/dl, whereas these values were respectively 2.1% and 8.5% lower in the workers. Although slight decrease of 0.4% was observed in haemoglobin content, the MCV and ESR remained un-changed in male workers.

Biochemical analysis of blood serum

Table III shows the comparison of biochemical analysis of blood serum of male and female workers and non workers. Almost all the biochemical parameters *viz.* albumin, alkaline phosphate, alanine aminotransferases, aspartate aminotransferases and total protein of liver were raised in the male and female workers. The total bilirubin, direct bilirubin and blood serum glucose contents were however lower in the workers as compared with those of non workers. The total chromium and hexavalent chromium were significantly higher in the blood of workers as compared with the non workers.

Female: The concentration of total chromium and hexavalent chromium in the blood of female workers exposed to industrial waste hazards was 569% and 78% higher than in the control population. The serum albumin and total protein contents were significantly higher *viz.* 26% and 27.2% respectively, in the female workers as compared with the control population. The other parameters such as AP, ALT and AST showed an increase of 21.5%, 19.8% and 13.9% respectively, when compared with control. The total bilirubin, direct bilirubin and blood serum glucose level in the non worker were 1.01±0.14 mg/dl, 0.38±0.04 mg/dl and 106.75±4.48 mg/dl respectively, whereas the

Table III.- Haematologicl analysis of blood of male and female population working in tanneries and residing in the Kasur industrial area. the population of Murree hills, Ayubia and Khanaspur has been taken as control population, as there are no tanneries and other industries.

Haematological parameters	Female		Male	
	Control (n = 8)	Treated [c] (n = 67)	Control (n =92)	Treated (n =533)
WBC [b] ($x10^3/\mu l$)	7.26±0.67 [a]	9.57±0.25**	8.70±0.22	9.01±0.10
RBC ($x10^6/\mu l$)	4.66±0.18	5.78±0.38	5.16±0.05	5.63±0.03 ***
Hb (g/dl)	13.24±0.76	12.77±0.20	14.80±0.13	14.74±0.06
PCV (%)	40.40±1.82	43.84±0.57	43.45±0.39	47.30±0.21 ***
MCV (fl)	86.50±1.69	86.27±0.86	84.33±0.67	84.31±0.29
MCH (pg)	28.25±0.94	25.12±0.32**	28.75±0.24	26.31±0.11 ***
MCHC (g/dl)	32.62±0.58	29.08±0.15 ***	34.12±0.14	31.20±0.09 ***
Plt ($x10^3/\mu l$)	262.00±15.83	306.04±10.48*	245.82±7.65	255.93±3.36
ESR (mm/hr)	8.88±1.86	9.01±1.06	6.46±0.44	6.81±0.19

a. Mean ± SEM; *P<0.05; **P<0.01; ***P<0.001

b. Abbreviations used; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, pecked cell volume; Plt, platelets count; RBC, red blood cells; WBC, white blood cells.

c. Population living in industrial area of Kasur, which predominantly comprises tanneries.

Table IV.- Biochemical analysis of blood serum of male and female population exposed to industrial waste from tanneries of Kasur industrial area. the population of Murree hills, Khanaspur and Ayubia has been taken as control population, as there are no tanneries and other industries.

Biochemical parameters	Female		Male	
	Control (n = 8)	Treated [c] (n = 67)	Control (n =92)	Treated (n =533)
Alb [b] (g/dl)	4.22±0.16 [a]	5.33±0.06***	4.77± 0.03	5.06±0.02 ***
AP (U/l)	251.00±31.07	305.03±27.06	281.45±20.98	284.03±6.36
ALT (U/l)	24.50±4.04	29.37±2.88	23.36±1.45	35.21±1.08***
AST (U/l)	25.63±4.51	29.19±1.82	27.37±1.33	32.89±0.69***
D.Bili (mg/dl)	0.38±0.04	0.22±0.01 ***	0.42±0.03	0.24±0.01 ***
T.Bili (mg/dl)	1.01±0.14	0.49±0.02 ***	1.02±0.07	0.55±0.01 ***
T.Prot (g/dl)	6.36±0.09	8.09±0.08 ***	6.91±0.06	7.93±0.03 ***
Glu (mg/dl)	106.75±4.48	78.51±2.49 ***	112.98±1.49	64.12±1.6 ***
T.Cr (µg/l)	8.75±0.82	57.31±4.75 ***	22.83±2.56	66.55±1.40 ***
Cr. VI (µg/l)	0.00±0.00	15.60±1.34 ***	1.79±0.48	9.39±0.44 ***

a. Mean ± SEM; *P<0.05; **P<0.01; ***P<0.001

b. Abbreviations used; Alb, albumin; ALT, alanine aminotransferases; AP, alkaline phosphatase; AST, aspartate aminotransferases; Cr. VI, chromium VI; D.Bili, direct bilirubin; Glu, glucose; T.Bili, total bilirubin; T.Cr, total chromium; T.Prot, total protein;

c. Population living in industrial area of Kasur, which predominantly comprises tanneries.

workers showed 42.1%, 51.5% and 26.5% decrease respectively.

Male: The pattern of changes in the various biochemical components of blood serum in the male workers was almost the same as described above for female workers. The alkaline phosphatase activity however generally remained unchanged. The Alb, ALT, AST, total protein, total chromium, Cr VI values increased 6.1%, 50.7 5, 20%, 14.7%, 184.5% and 424.5% respectively, in the male workers. The non workers showed 0.42±0.03 mg/dl of direct bilirubin, 1.02±0.07 mg/dl of total bilirubin and 112.98±1.49 mg/dl of glucose, respectively in the blood serum. These parameters were significantly decreased in the workers *viz.* 42.8%, 46% and 43.3%, respectively.

DISCUSSION

The present study deals with approximately 1/4th population of Kasur city, residing in industrial area comprising tanneries. Six hundred individuals were included in the study, out of which 500 were those who worked in the tanneries and had direct contact with the chemicals used in tanneries, where as 100 were those who lived in the area, but had no direct exposure or contact. The present study showed that workers had total and hexavalent chromium. The Kasur's drinking water had 250µg/l hexavalent chromium, compared to the control area drinking water, which has 22µg/l. It is obvious that Kasur water is highly contaminated.

Liver function tests

Present study showed no significant or adverse effect on liver function tests and haematological parameters of occupationally exposed human . Present data principally showed insignificant change in the total proteins (mainly Albumin), AP, ASAT. Chen *et al.* (2001) reported that dietary chromium supplementation did not significantly influence serum constituents, including insulin, HDL, VLDL, total protein, albumin and gamma globulin. Uyanik (2001) had reported that no significant differences were found in total protein, albumin, ALAT, and ASAT. He also reported that chromium supplementation may affect carbohydrates and lipid metabolism and lipid deposition in lamb. Albumin, total proteins and ALAT were significantly higher in the workers when compared to the control population. ASAT remained unchanged in the I and III age group, but increase was observed in the II age group. Bilirubin (Total and direct) and glucose contents were lower in the workers when compared to non workers. Similar decreasing trend in bilirubin (total and direct) and glucose was observed both in the workers

Albumin

The tannery workers however, showed a significant decrease of 7% in the albumin of age group I and 2% significant decrease in the age group II when compared with the non-workers. When the data was considered sex wise, the females showed 26% increase in the female and only 6% in the males. The factory workers are directly exposed to chromium by physical content and hence show lower albumin content as compared with the control population. Uyanik (2001) has reported a non-significant decrease in the albumin in sheep because of dietary chromium. Chen *et al.* (2001) on the other hand have not shown significantly effect on serum constituents, including total protein, albumin, gamma globulin, insulin, HDL, VLDL, HDL-C, and VLDL-C, after dietary chromium supplementation. The higher albumin level I the wortkers may actually reflect the increased production of albumin to accommodate increased transport of chromium in them. Ananth *et al.* (2000) have postulated molecular interaction of bovine serum albumin with potassium dichromate to form relatively stable chromium (V) and as well as Cr (III) mediated cross-links in the proteins.

Alkaline phosphatase

The tannery workers in age group I and III showed highly significant decrease (34% and 4%, respectively) in AP activity, whereas group II showed 6% increase. When considered sex wise, the females showed 22% rise in AP activity as compared to none in the males. Behari *et al.* (1978) have reported inhibition of acid phosphatase, adenosine triphosphatase and succinic dehydrogenase after administration of trivalent and hexavalent chromium. Nehru and Kaushal (1993), on the other hand, reported significant increase in AP activity after lead intoxication.

Alanine and aspartate aminotransferases:

The tannery workers showed no change in the ALAT activity. Considering the two sexes, the male showed 51% increase and the female showed 20% increase in the activities of aminotransferases when compared with the control population. The tannery workers showed negligible increase (8% and 5%) in the first two age groups and no change in the age group III. The male members of the population showed a significant increase of 20%, whereas females showed 14% increase in ASAT activity when compared with the control population. The present studies showed that the liver function tests as reflected by the enzyme activities (ALAT and

ASAT) have variable patterns, with an overall increase in all age and sex groups when compared with the control population. This increase may be statistically significant, but are of no clinical significance as the obtained values remained well with in the normal range.

The chromium and other heavy metals have been reported to raise the level of aminotransferases. Awadallah and Hanna (1980) have reported that the serum ASAT was significantly higher in animals injected with chromium than cobalt, zinc and manganese; while serum ALAT levels were higher in cobalt than in chromium, zinc and manganese. Bavazzano *et al.* (1981) reported that ALAT and ASAT enzymatic activities are higher in tannery worker as compared to workers in the shoe factory. Kim and Na (1990) reported significant increase in serum lactate and pyruvate after intraperitoneal injection of sodium dichromate in rate (20 or 40 mg/kg). Vaglio and Landriscina (1999) reported increased ALAT and ASAT in the serum after intoxication of cadmium. Wu *et al.* (2000) reported that a 33 years old white woman taking 6-12 time more chromim picolinate s compared to recommended dose, presented with weight loss, anemia, thrombocytopenia, hemolysis and raised liver enzymes (15 to 20 times) as compared to the normal values. Guha (2001) reported hepatic membrane damage due to continuous arsenic feeding, probably due to reduction of glutathione and anitoxidant enzymes through drinking water. Fatty liver with elevated serum aminotransferases has also been reported by Sclove (1997). Kumar *et al.* (1985) reported alteration in the distribution of alkaline phosphatase, acid phosphatase, glucose-6-phosphatase and cholinesterase after chromium poisoning. Chromium also been reported to induce inhibition.

Total bilirubin

In the present study a significant decrease in the total bilirubin was observed *i.e.* 33% in the age group I, 48% in the age group II and 58% in the age group III. The total bilirubin did not show any appreciable change in the different age groups of industrial area population. The females and males, however, showed a decrease of 52% and 46%, decrease respectively as compared with the control population. Wu *et al.* (2000) have reported that the 6-12 time increased dose of chromium picolinate resulted weight loss, anemia, thrombocytopenia, hemolysis, liver dysfunction and renal failure. It is obvious that the chromium supplementation may cause serious liver and renal impairment when ingested in excess.

Direct bilirubin

The tannery workers did not show any significant difference in the direct bilirubin content when compared with their controls. The female and male members of the industrial area population showed 42% and 43% decrease, respectively, in the direct bilirubin content when compared with that of the control population. The total and direct bilirubin was found to be higher in control population as compared with the industrial area population. One of the possible factors may be a higher haemoglobin concentration at high altitudes (control population) resulting in high haemoglobin break down products like bilirubin. This however may be one of the contributing biochemical factors. Frank *et al.* (2000) reported increased concentration of total bilirubin in goats, deficient of Cu^{2+} and Cr^{6+}.

Total protein

The tannery workers did not show any appreciable change in the total proteins, when compared with the control group. Considering sex wise, the females showed 27% increase and males 15% increase as compared with the control population. Comparatively low level of total protein and albumin in tannery workers as compared to control group could be because of decreased protein synthesis due to chromium intoxication. The possible variation may relate to different levels of exposure and liver toxicity (Uyanik, 2001; Chen *et al.,* 2001; Canli, 1995; Shrivastava and Nair, 2000).

Glucose metabolism

Tannery workers, however, showed no significant change in the present study. When considered on sex basis, the males showed 43% decrease in contrast to 27% decrease in the females. The workers showed significantly lower random blood glucose levels in exposed and both in male and female population when compared with the

control population. This observation of lower glucose level in exposed population directly correlates with the already documented affects of chromium supplementation in improving glucose tolerance (Awadallah and Hanna, 1980; Vincent, 2000; Kim *et al.,* 2002; Appleton *et al.,* 2002). The difference of blood glucose level between tannery workers and non-workers or the vicinity of industrial site that affected area was found to be insignificant.

Chromium uptake and toxicity

Tannery workers showed a generalized increase in serum chromium level, when compared with the non-workers. From among the two sexes, females showed 569% increase in the chromium level as compared to 185% in the males.

The tannery workers of only the age group I showed an increase, as compared to the non-workers, while the other two age groups showed no change. Cumulatively the male members of population showed 425% rise, whereas females had a 185% increase, respectively. Hexavalent chromium in Kasure water was recorded 165 µg/l in comparison to 12 µg/l, of the controls. This raised level would be the result of long-term direct exposure to chromium, ingestion through the drinking water, and inhalation of air borne chromium particulates.

The intensive development of industries and water disposal without efficient emission control, to protect the ambient environment, may cause the accumulation of high amount of heavy metals in soils, which cannot be degraded by any natural biological process. Consequently these harmful substances have entered the food chain of man. These heavy metals have long half-lives and accumulate in the tissues (Moore and Ramamoorthy, 1984) and have varying toxic potentials in the soil (Mukherjee, 1998).

Chromium is the 3rd largest chemical, which is used in lather tannery. Male worker has more tendencies to accumulate chromium in their body tissue, blood and serum as well. Male workers have higher concentration of chromium in their serum, because almost 95% male works in tanneries and the chromium is accumulating with the passage of time, duration of exposure and concentration of chromium (Kornhauser *et al*., 2002).

With regard to toxicity, pure metallic chromium is reported to be nontoxic. Chromium III is poorly absorbed, and much less toxic than chromium VI. The industrial monitoring for toxicity is mainly related to total and hexavalent chromium. The effect of chromium related to different parameters is primarily based on data obtained from experimental animals, *viz.* mice (Bagchi *et al.,* 2002; Wunder *et al.,* 2002), cats (Appleton *et al.,* 2002), sheep (Uyanik, 2001), rats (Jarrar and Mahmoud, 2000, Sutherland *et al.,* 2000), *Limnodrillus hoffmeisteri* (Oligochaeta: Tubificidae) (Flores-Tena and Martinez-Tabchet, 2001) and guinea Pig (Mathur and Gupta, 1994).

Haematological changes

Chromium is a trace element and found in extremely low quantities in blood and urine. Among the different species of chromium hexavalent and trivalent forms are of special relevance to humans. The intermediate metabolites such as Cr IV and Cr V are toxic but fortunately they are highly unstable and are converted into Cr III rapidly.

Present study showed the biochemical and haematological defects in human population exposed to chromium in effluents of tanneries in industrial area of district Kasur. There are many other toxic agents/chemicals or heavy metals, which are related to this particular industry and affect the population under study. Arsenic is a common tannery chemical and has long been associated with lung cancer in workers who are exposed to it on a regular basis. Several studies have established links between sinus and lung cancers and the chromium used in tanning. Studies of leather-tannery workers in Sweden and Italy found cancer risks “between 20% and 50% above those expected (Hayes, 1997; Sclove, 1998; Labreche, 1997). The toxic affects of these agents are already established on the human bone marrow. Most important of these agents is benzene, which is related to many haematologic disorders including leukaemias, myelodysplasia and bone marrow depression. With this background the analysis of the haematological results need to be taken with utmost caution to avoid any misinterpretation.

White blood cell count:

Increasing trend in WBC count was observed in the industrial area population and decreasing trend was observed in tannery workers and non-workers. From amongst workers age group I and II showed 7% decrease, while age group III showed 8% increase, when compared with the non-workers. The females showed 32% increase as compared with the males which showed only 4% increase in the WBC count when compared with their respective controls.

Although a number of investigators (Paustenbach *et al.,* 1996; Minoia and Cavalleri, 1988) have examined the uptake and effects of chromium in the red blood cells, little is known about chromium uptake in the white blood cells. Suggestions have been made on the use of WBC as a target for the development of biomarkers for chromium exposure. This may be based on the investigations that WBC accumulates a greater extent of hexavalent chromium, than do the RBC's. WBC accumulates Cr III both *in vitro* and *in vivo.* In addition, white blood cells accumulate chromium to a greater extent than red blood cells (Coogan *et al.,* 1991).

Red blood cell count:

Determination of total chromium in serum and the red blood cells has showed a significant increase of chromium levels in erythrocytes of workers exposed to chromium VI (Cavalleri and Minoia, 1985). This increase was more in the younger age groups and seems to get lesser with the advancing age. The tannery workers showed 17% rise in the age group I and 2% in the age group II and III. The female members of the population showed 24% increase, while the males showed 9% rise.

Chromium concentrations of red blood cell and plasma returned rapidly to background levels within a few days after cessation of dosing since the concentration of chromium in the RBC would not decrease quickly if the chromium had entered the RBC as chromium VI. Concentration of Cr 10mg/l or less in drinking water of exposed humans appears to be completely reduced to Cr III prior to systemic distribution (Paustenbach *et al.,* 1996).

Studies on human blood revealed that RBC fraction apparently has the capacity to reduce Cr VI at concentration in blood up to 15,000 µg/l and that the rate of Cr VI uptake into RBC may not exceed the rate of intracellular reduction at these concentration (Valeri *et al.,* 2002). Significant increase of Cr levels in erythrocytes of workers exposed to Cr VI was observed. Cr III was absorbed through respiratory tract and distributed in the body (Cavalleri and Minoia, 1985). Oxidation state of Cr largely influences uptake, mechanism of absorption, transport and organ distribution as well as toxicity of Cr containing compounds. Cr VI hence is more toxic to occupationally exposed subjects while Cr III has little effects.

Haemoglobin

The tannery workers showed 3%, 5% and 3% rise in the three age groups, respectively, while females showed decrease of 4% as compared to the males *i.e.* 0.4%. Flores-Tena and Martinez-Tabchet (2001) have reported that haemoglobin contents in *Limnodiluson* decreases significantly when Cr concentration increases above 1 µg/g dry weight.

Packed cell volume

The tannery workers however did not show any significant change in the PCV, when compared with the control population. From amongst the two sexes of the population, both the females and males showed same level of increase (8.5-9%) in the PCV values, when compared with the control.

Erythrocytic indices

Little or no change was observed in MCV in the industrial area population and tannery workers. The MCH and MCHC followed the same pattern of changes. From amongst tannery workers of age group I and age group II showed 4% increase, whereas workers of age group III showed 2% increase. Females showed a greater decrease of 11% while the males showed only a 2% decrease. Cells tend to remain hypochromic (low MCH) because of underlying nutritional deficiencies, which are considered to be quite common in these populations. A normal MCV with reduced MCH may be an indication of borderline iron deficiency state.

Platelet count

The tannery workers however showed a mild

decrease of 7%, 9% and 6% in first, second and third age groups, respectively. The decrease of 17% in females was more pronounced than 4% observed in the males.

Erythrocyte sedimentation rate

Chromium exposure seems to have no effect on ESR. Chromium concentration values are higher in plasma, erythrocytes and platelets 248%, 61% and 91%, respectively, and lower in the non nuclear leukocytes (35%). These are also age and sex dependent (Rukgauer and Zeyfang, 2002).

Concluding remarks

No definite pattern has been observed in different haematological parameters. The differences observed in various parameters are mostly insignificant. Even when the differences are significant the mean values obtained are well within the normal range. Slight variation may be due to multitude of factors in addition to possible effects of chromium toxicity. The factors involved may include the non-exposed population residents of high altitude where the haemoglobin concentration is known to be higher. Similarly a relatively raised red cell count observed in exposed population particularly in age group II may partially be explained on the fact that this age group included a high percentage of heavy smokers resulting in higher red cell count but because of possible underlying nutritional deficiencies, which are considered to be quite common in these population, cells tend to remain hypochromic (low MCH). PCV, which is higher in exposed population, relates to higher RBC count. MCV is raised insignificantly in exposed population but remains within the normal range. A normal MCV with reduced MCH may be an indication of borderline iron deficiency state. WBC shows no significant change in count. Platelets appear to be relatively increased in exposed population but are well within the normal range.

No adequate evidence of carcinogenicity has been found in classical epidemiological studies, in industries using mainly trivalent chromium, such as the tanning industry (IARC, 1990; Langård *et al.,* 1990). This industry uses trivalent chromium compounds in the processing of animal hides into leather. Although its carcinogenicity has been known for several years, there is still a considerable lack of knowledge of the mechanism(s) of toxic action of hexavalent chromium and the risks associated with various routes of exposure to both hexavalent and trivalent chromium compounds.

The significance of this research lies not on the results, which are by and large consistent with results from other parts of the world, but on the fact that human subjects were the focus of study. Such long term and consistent exposure to toxic elements are rarely found at any level in the western world. The enforcements of checks and balances on environmental pollution and the legalities of proper toxic disposals ensure that the residing populations are not exposed to such toxins. Pakistan being the under privileged of the developing countries, unfortunately lacks such essential checks, thereby the leverage of permissive dumping of chromium out in the open environment. Humans being are subjected to such this exposure, but this give us an opportunity to study the harmful and otherwise effects. Such a level of study has rarely been documented in Pakistan. Such evident adverse effects would also help stress the need for proper and more stringent procedures towards the free and liberal use of numerous toxins, their safe handling and proper disposal, leaving the environment and the world a cleaner and safer place to live in.

Epidemiological studies on occupational exposure to Cr compounds provide the primary source of information on Cr toxicity and carcinogenicity in humans (Costa *et al.,* 1996; Deschamps *et al.,* 1995; Ishikawa *et al.,* 1994a,b; Taioli *et al.,* 1995), and the route of exposure can influence which organs are most affected (Deschamps *et al.,* 1995; Ishikawa *et al.,* 1994a; Kirschbaum *et al.,* 1981; Tandon *et al.,* 1978). During the First World War, it was established that chromium compounds cause lung cancer (Shupack, 1991) and the carcinogenicity of chromium VI has been well established (IPCS, 1988; Baruthio, 1992).

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