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Endocrine abnormalities and DNA damage in welders exposure to Cr and Fe

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Abstract
The present experiment was conducted to study the endocrine abnormalities and DNA damage in welders exposure to Cr and Fe on 60 individuals including control and affected. Blood sampling was conducted by categorizing the individuals into two age groups and also according to exposure period. DNA damage was measured by single gel electrophoresis. The assessment of selected endocrine hormonal parameters were determined by using Sysme model KX-21 automated haemotology analyzer, and biochemical parameters were determined by using Abbott Diagnostic. The present study results clearly showed that blood elevation of hexavalent chromium and iron significantly caused DNA damage and effected on endocrines.

Keywords: Endocrine abnormalities, DNA damage, welders exposure, Cr and Fe

1. Introduction
Human activities have increased the concentrations of several toxic metals and metalloids. Sedimentary rocks have maximum concentrations of chromium manganese, cobalt, nickel, cadmium and Iron [1]. Moreover, volcanoes also release great levels of aluminum, manganese, nickel, copper and iron along with poisonous and harmful gases [1]. The major path of entry of heavy metals in the human body is through inhalation. Urbanization, traffic, agricultural and industrial activities has remarkably contributes to the access of heavy metals through breathing in human body. Cadmium, nickel and chromium role as a health hazards to humans [2].
However, the severity of toxicity diverge with the type of heavy metal involved, its contact level, chemical and valence states, mode of contact and the age of the individual. Therefore, children with their rising nervous systems are primarily rare to heavy metal toxicity [3].
Gases released contain CO, CO2, Fe and HF, which exposure lead toward numerous hazardous effects depending upon the types of welding and of materials which are used. However, both the electric arc and the oxyacetylene flame give high temperatures [4].
However, Cr^{3+} is the most general type known to be important for certain physiological functions and suitable to its deprived inclusion and incapability to transverse cell membranes and does not show health hazards to humans [5]. In contrast to Cr^{3+} the Cr^{6+} has much greater human health hazards due to strong oxidizing agent. Cr VI compounds are approximately 1000-fold more mutagenic and cytotoxic than trivalent compounds [6]. According to Deloughery [7] in 2014 reported that DNA damage by (Cr^{6+}) result of its reductive metabolism in the living cells. Contact to Cr^{6+} causes point mutations in genome and DNA damage and protein oxidative change [8]. DNA damage cause by chromium (Cr^{6+}) such as chromosomal aberrations and by reactive oxygen species cellular oxidative DNA damage produce by Cr^{6+}. Moreover, Cr6+ exposure causes DNA lesions and gene alteration. According to Tsou *et al.*, [9], Cr causes DNA damage by generating 8-hydroxydeoxyguanine form of oxidative DNA damage. According to Trenton, (2003) [10], chromium compounds have hazardous effect on thyroid functions. Many studies revealed that vitamin C has protective role against ovotoxicity produce by $Cr6^{+}$ [11]. $Cr6^{+}$ can cross the placental membrane and origin wide range of abnormalities in fetal growth [12]. In pituitary gland and hypothalamus the Cr6+ accumulates and mimics prolactin levels but no effects on luteinizing hormone levels. Lactotrophs is more susceptible to the toxicity of the Cr^{6+}. Which result oxidative stress, by nuclear division. Cr6+ toxicity also disturbing the normal endocrine function [13].
Iron is very essential component of living organisms as well in humans in the oxygen transfer

proteins, such as hemoglobin in blood and myoglobin in muscles [14].

In biological metabolic processes iron can inter-convert between the ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions. Biologically iron is a most important nutrient for many living things in many vital proteins and enzymes; it also acts as the cofactor. If iron is not protecting suitably, it can catalyze the reactions and can create free radicals which can damage biomolecules and the entire organism. In addition, children are highly susceptible to iron toxicity or iron poisoning, because they bare the maximum iron containing environment [16]. Many studies reveals that the oxidation of PUFA, loose intracellular iron which support DNA damage [17]. Superoxide liberates the protein bound with iron result formation of $H_2O_2$and Fe^{2+}correspondingly. This reacts to form OH oxidant. This oxidant may lead toward DNA damage [18]. Iron load cause the endocrine system toxicity, most widely in pituitary gland and thyroid gland. The main secondary outcome of iron over loading is gonadal damage in the anterior lobe of pituitary gland [5]. The aim of the present research work was to find out the Endocrine abnormalities and DNA damage in welders exposure to Cr and Fe.

Materials and Methods

Sampling site

Sampling site in the present study was selected according to human maximum exposure to welding occupation. The different areas of Sargodha city and adjoining region of Sargodha city, pull 111 (13 km of south of Sargodha city) nearby village 46 sb were selected as control site for sampling. The peoples of these areas are mostly involved in welding occupation.

Bio monitoring the health status of the welders

1. Selection of individual for the study.
2. Grouping of the individuals.
3. Collection of blood sample from individuals.
4. Assessment of selected endocrine hormonal parameters.
5. Measurements of DNA damage.
6. Assessment of the selected hematological parameters.
7. Assessment of the selected biochemical parameters.

Selection of individual for the study

Total 60 individuals for each group were selected for study, all these individuals were welders. Group I was (subject) with the age ranging 20-35 years with the working experience of 5-10 years, and control group also consisted of 60 individuals from the same age group but never exposed to any type of welding pollution. In group II there were 60 individuals with the age of 35-50 years with the working experience of 10-20 years, and control was 60 individuals from the same age group (35-50 years old) but they were never exposed to welding pollution. The welders were worked for at least 8-10 hrs per day for six day per week. The permission was obtained from each individual and honors of workshops by explaining the aim of study and those individuals were included who agreed to aim of the study. A detailed medical questionnaire was filled by each worker who was involved in objective of study. In this questionnaires demographic data, past medical history and clinical manifestations of all participants were included. The workers were selected on the basis of following criteria.

Inclusion criteria

1. Worker had been associated with welding occupation.
2. They had not been worked in other kind of polluted occupation.
3. Workers had not suffered any serious disorder (Cancer, diabetes, angina)
4. Welding experience according to grouping.

Exclusion criteria

Diabetic, hypertensive, cancerous and cardiovascular disease workers were excluded from study to avoid other factors effecting blood samples.

Collection of blood samples

Visited the different sites of welding workshops and about 3cc blood sample was collected in a metal free safety vacationer blood collecting 1.5 mg EDTA K2 tubes. The samples were stored at -20 ºC until required for analysis.

a b

Fig 1: 3 cc blood samples of group I taken and preserved in EDTA vials at -20 ºC (a, b).

Fig 2: 3 cc blood samples of group II taken and preserved in EDTA vials at -20 ºC.

Assessment of selected endocrine hormonal parameters
Following hormonal parameters were determined in the blood samples.

Assessment of testosterone hormone:
The assessment of testosterone hormone in blood samples was determined by commercial kit, (CMIA) (Abbott-ARCHITECT). The Testosterone acridinium-labeled conjugate in citrate buffer surfactant stabilizer with minimum concentration of 0.1 nM was used. The micro particles which used for testosterone hormone analysis, contained 6.6 ml anti-testosterone coated in BIS-TRIS buffer with protein stabilizers. 5.9 ml testosterone assay diluents containing surfactant in citrate buffer. The trigger solution contained 0.35N NaOH and Pre-Trigger Solution contained 1.32% (w/v) H_2O_2. Phosphate buffered saline solution was used as a wash buffer. Anti-testosterone was coated with paramagnetic micro particles. Then serum was mixed with this anti-testosterone. To create a reaction mixture the testosterone acridinium-labelled conjugate and assay dilution were added. In serum testosterone compete with testosterone acridinium-labelled conjugate antigen-antibody complex is formed by bonding anti-testosterone coated micro particle. Washed with wash buffer, Trigger and pre-Trigger solution were added to reaction mixture. The resulting chemo luminescent reaction was measured as relative light units. Inverse relationship was found between the relative light unit detected and the amount of testosterone in the sample. Intra- assay coefficients of variations were between 2.3% and 6.2%, while inter-assay variations were between 1.4% and 4.7%. Detection limit was 10ng/dl. Calibration range was 20 to 1600ng.

Assessment of Growth hormone
Growth hormone in blood sample was determined by commercial kit, (chemo-luminescent micro particle immunoassay on an IMMULITE 200 Analyzer).
Step I: Murine monoclonal anti- hGH antibody was used to coat sample and solid phase bead. The reagent contained alkaline phosphatase conjugate with anti hGH polyclonal antibody. The 23 reagent and hGH in the sample were incubated together with bead coated along with murine anti-hGH monoclonal antibody to form an antibody sandwich complex. By centrifugation unbound enzyme conjugate was removed.
Step II: chemo-luminescent substrate was mixed with bead and signal was generated in proportion to the bound enzyme. Calibration range was up to 40ng/ml. Detection limit was 0.01 ng/ml. Results were expressed in ng/ml.

Assessment of Thyroid stimulating hormone (TSH)
TSH in blood sample was determined by commercial kit, (CMIA) (Abbott-ARCHITECT). In reagent kit, 5.9ml /26.3ml anti-a TSH acridinium-labeled conjugate in MES buffer with protein stabilizer with minimum concentration 60ng/ml was used. Further, 6.6ml/27.0 anti-β TSH coated micro-particle in TRIS buffer with protein stabilizers was also used. The Preservation was in antimicrobial agent. Assay diluents with 8.0 ml/40.7 TSH in TRIS buffer that also preserved in antimicrobial agent. The trigger and pre-trigger solution contained 0.35N NaOH and 1.32% (w/v) H2O2 respectively. Phosphate buffered saline solution was used as a wash buffer. Anti-β TSH coated paramagnetic micro-particles; TSH assay diluent and sample were mixed. TSH present in sample binded with the anti-TSH antibody coated micro-particle then washed and anti-a TSH acridinium labeled conjugate was added. Trigger and pre-Trigger solution was mixed with reaction mixture. The resulting chemiluminescent reactions were measured as relative light units. Direct relationship was existed between the relative light units detected and amount of TSH in sample. Intra-assay varied from 2.41% to 2.48% and inter-assay varied from 2.05% -5.31%, respectively. Detection limit was 0.01 µIU/ml. Calibration range was up 75 µIU/ml.

Assessment of Triiodothyronine hormone (T3)
Free T3 in blood sample was determined by commercial kit, (CMIA) (Abbott-ARCHITECT). In T3 reagent kit, 5.9ml /26.3ml acridinium-labeled conjugate in citrate buffer with NaCl and Triton X-100 stabilizers having minimum concentration: 0.33ng/ml was used. The Micro particles 6.6ml/27.0 coated in MES buffer with IgG stabilizers. The preservation was in antimicrobial agent. 8.0 ml/40.7 TSH assay diluent in TRIS buffer, preservatives were also in antimicrobial agent. The trigger and pre-trigger solution contains 0.35N NaOH and 1.32% (w/v) H_2O_2 respectively. The wash buffer contained phosphate buffered saline solution. Following two steps were involved to determine free T3 in sample.
Step I: T3 coated paramagnetic micro-particles and sample were mixed. Free T3 present in sample binded with the anti-T3 coated micro-particle then washed and anti- T3 acridinium labeled conjugate was added.
Step II: Trigger and pre-Trigger solution was mixed with reaction mixture. The resulting chemiluminescent reactions were measured as relative light units. Inverse relationship was existed between the relative light units detected and amount of unbounded T3in sample. Intra-assay varied from 1.81-2.57% to 2.48% and inter-assay varied from 1.48-3.48%.

Assessment of Tetraiodothyronine hormone (T4)
Unbounded T4 in blood sample was determined by commercial kit, (CMIA) (Abbott-ARCHITECT). The T4 reagent kit contained 5.9ml /26.3ml T4 acridinium-labeled conjugate in MES buffer with NaCl and Triton X-100 stabilizers, the minimum used concentration was 0.2ng/ml. The 6.6ml/27.0 anti T4 coated micro-particle in TRIS buffer with IgG stabilizers having minimum concentration 0.08% solids also with preservation in antimicrobial agent. 8.0 ml/40.7 T4 assay diluent in TRIS buffer with preservation in antimicrobial agent. Trigger solution contained 0.35N NaOH, pre-trigger solution contained 1.32% (w/v) H_2O_2 and wash buffer contained phosphate buffered saline solution.
Following two steps were involved to determine free T4 in sample.
Step I: T4 coated paramagnetic micro particles and sample was mixed. Free T4 present in sample binded with the anti-T4 coated micro particle then washed and anti- T4 acridinium labeled conjugate was added.
Step II: Trigger and pre-Trigger solution was mixed with reaction mixture. The resulting chemiluminescent reactions were measured as relative light units. Inverse relationship was existed between the relative light units detected and amount of unbounded T4 in sample. Detection limit: 0.18ng/dl and calibration range; 0.2 to 6ng/dl.

Measurements of DNA damage
Human lymphocytes were analyzed by single gel electrophoresis (comet assay). It performed by using blood sample of 20 µl. The duplicate slide was prepared for every person. Every slide was first coated with 50µl agarose (1% DW) to prepare a base layer and air dried. The second coat base layer was prepared by placing 50 µl regular melting point agarose (1% DW) on each slide and air dried. Each slide was then coated again with 200 µl agarose (1% in phosphate-Buffered saline (PBS)), and these slides were covered with glass. The slides were allowed to gel at 4 °C for 10 min. The cover slips were removed and second layer was prepared by mixing 20 µl of the whole blood with 80 µl low melting point agarose (0.5% in PBS). Covered with cover slip and stored at 4 °C for 10 minutes. The cover slip was removed and second layer was prepared by mixing 20 µl of the whole blood with 80 µl low melting point agarose (0.5% in PBS). Cover with cover slip and stand for 10 mints at 4 °C. Cover slip was removed and third layer of 100 µl low melting point agarose (0.5% in PBS) was pipette out on the second layer and covered them with cover glass. Cover slip removed and slides were submerged in a tank that filled with 50 ml cold lysing solution (2.5 M NaCl, 200mM Na_2 EDTA, 10mM tris-HCl, 10% dimethyl sulfoxide (DMSO) and 1% triton-X 100) for 24 hrs. The slide were submerged in alkaline Buffer (0.3 M NaOH, 1Mm Na 2 EDTA) at 12 pH for 20 min to allow unwinding of DNA. The slides were subjected to electrophoresis with 18 V (0.7-1.0 V/cm), 300 mA at 4°C for 20 min. The slides were placed in neutralizing with tris-HCl buffer (400 mM, pH 7.4) for 5 min and this step was repeated. In absolute methanol slides were dehydrated for 5 mints at 30 °C to dry and stained with 75 µlEtBr (Ethidium bromide). During screening of samples, undamaged cell appears as intact nucleus without a tail and damaged cell has the resemblance of comet. DNA damage visualized by fluorescent microscope equipped with excitation filter of 450-490 nm. DNA damaged was expressed in micrometer.

Assessment of the selected hematological parameters
Hematological parameters were determined at the department of biochemistry, Fatima Jinnah medical college, Lahore. Hemoglobin (Hb) count, Red blood cells (RBC) count, White blood cells (WBC) count, Packed volume cells (PVC) count, Mean corpuscular volume (MCV) count, Mean corpuscular Hemoglobin (MCH) count, Mean corpuscular Hemoglobin concentration (MCHC) count. Platelets (Plt) count, Erythrocyte sedimentation rates (ESR) were determined by using Sysme model KX-21 automated hematology analyzer.

Assessment of biochemical parameters
Biochemical parameters were determined at the department of biochemistry, Fatima Jinnah medical college, Lahore. Albumin, Total protein, alkaline amino transferase, alkaline phosphatase, Total bilirubin, Aspartate transaminase, Serum glucose, and direct bilirubin were determined by using Abbott Diagnostic.

Statistical analysis
The blood samples were analyzed (mean, percentage, and slandered deviation). For comparison of the two means, independent t-test was used, while to identify variations in the data ANOVA was applied. Pearson's correlation analysis was used to analyze DNA damage biochemical and hematological parameters.

Results
Effect of chromium and Iron metals on welder's endocrine
Effect of chromium and iron metals were investigated in welder's blood in occupational area of Sargodha city and nearby of Sargodha.

Testosterone Hormones
The effect of trace metals on testosterone hormone in control was found 583.23ng/dl, and effect of trace metals on testosterone hormone in affected age group I was found 421.54ng/dl and in affected age group II was found 368.95ng/dl (Fig. 3).

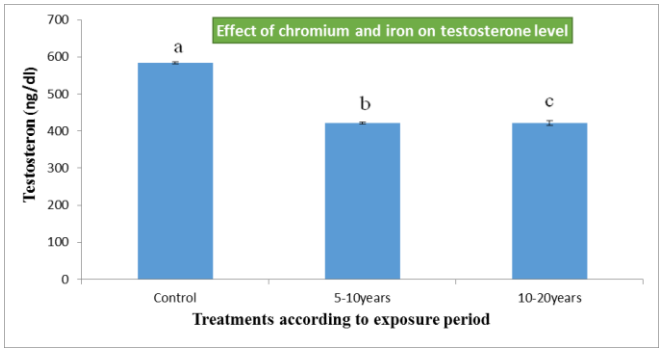


Fig 3: Variations of trace metals in testosterone hormone in control and affected workers.

Blood sample of 60 welders was collected at occupational site; they all were affected from occupational pollution. Affected were divided into two age groups, age group I was 20-30 years and exposure time was 1-10 years. Group II was 35-50 years old and exposure time was 10-20 years.

4.1.2 Growth Hormones The effect of trace metals on growth hormone in control was found 0.7688ng/ml, and effect of trace metals on growth hormone in affected age group I was found 0.1062ng/ml and in age group II was found 0.0829ng/ml (Fig. 4).

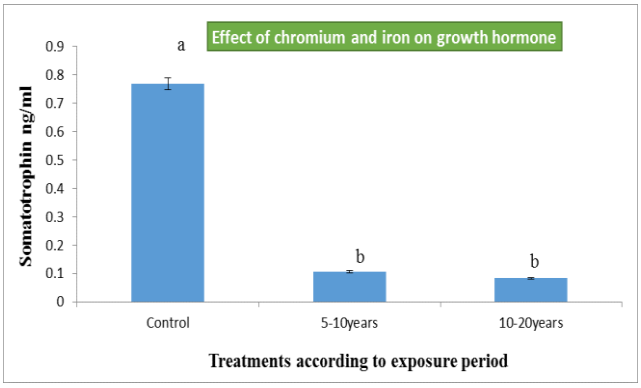


Fig 4: Variations of trace metals in male Growth hormone in control and affected workers.

Thyroid stimulating Hormones (TSH)

The effect of trace metals on male TSH in control was found 2.409 µIU/mL and effect of trace metals on male TSH in affected age group I was found 2.4802 µIU/mL and in age group II was found 2.492 µIU/mL (Fig. 5).

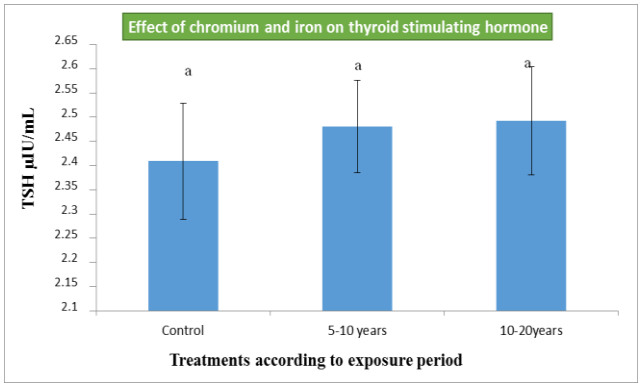


Fig 5: Variations of trace metals in male TSH in control and affected workers.

Triiodothyronine T3

The effect of trace metals on T3 in male, control group was found 94.948 ng/dl, and effect of trace metals on T3 in affected age group I was found to be 94.046 ng/dl and in age group II was 93.272ng/dl (Fig. 6).

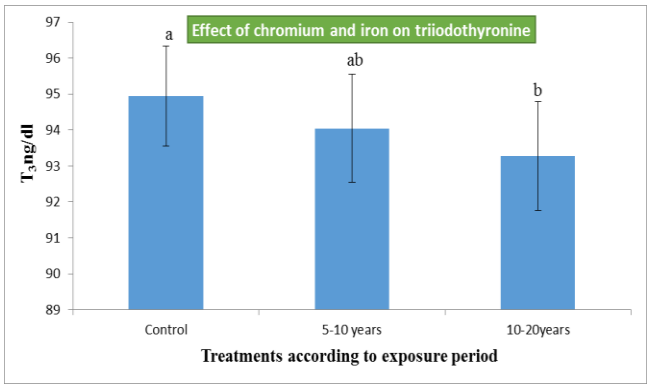


Fig 6: Variations of trace metals in male T3 in control and affected workers.

Tetraiodothyronine T4

The effect of trace metals on T4 in male, control group was found 5.724 ng/dl, and effect of trace metals on T4 in affected age group I was found 6.2962 ng/dl and in age group II was found 6.4318 ng/dl (Fig. 7).

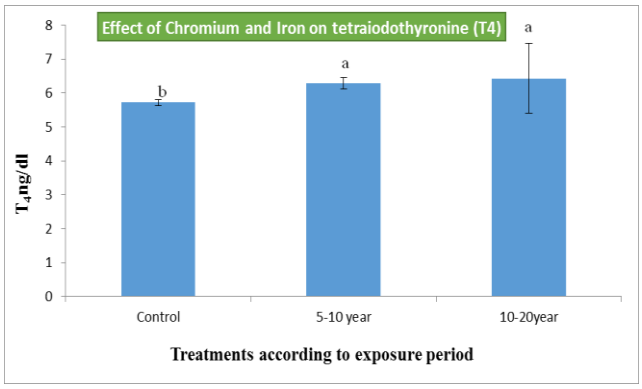
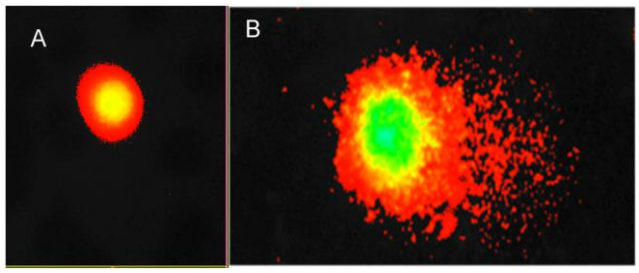
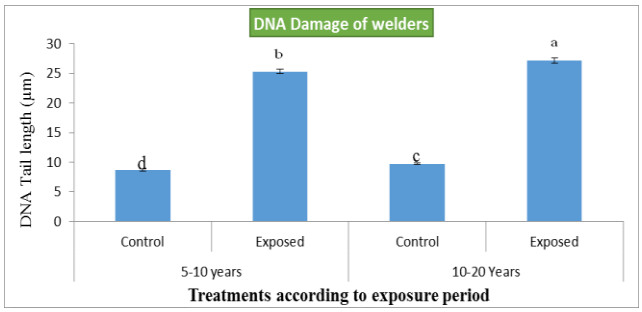


Fig 7: Variations of trace metals in male T4 in control and affected workers.

DNA damage by chromium and iron in welders

Blood samples of welding workers were collected at their work place the total number of individual in control was 60. They were divided into two age groups. DNA damage was measured in welders in 60 affected workers having two age group (20 to 35 years and 35 to 50 year) both having exposure period of 5 to 10 years and 10 to 20 years respectively. DNA damage was found in control age group I 8.685 µm and in control age group II 9.701 µm. In affected DNA damage was found age group I 25.303 µm and affected age group II is 27.174 µm. Fig. 8 A, B and C show the DNA damage in welders as compare to controls.





C

Fig 8: A, B, C Variations of DNA damaging in male welders by trace metals.

Discussion

Testosterone hormone

Testosterone is a male hormone and is responsible for the appropriate development of male sexual characteristics. It is also essential for maintaining muscular mass, sufficient levels of blood cells, bone growth, and normal sexual functions. The effect of chromium and iron metals on testosterone hormone in welder's blood was found in exposed age groups I and II. In Fig.3.1 the exposed groups I and II (421.54ng/dl, 421.54ng/dl respectively) showed significantly lower testosterone levels than that of control group (583.23ng/dl). In Fig. 4, control is significantly different from affected age group I and affected age group II of welders. Long-term

chromium exposed male welding workers in age group II exhibit significantly lower testosterone level than that of age group I with short term chromium exposure. It is observed that the duration of exposure has significant effect on Testosterone levels in blood of welders. Elevated levels of hexavalent chromium cause testicular degenerate and reduce in sperm count. Similar pattern was observed in previous studies by [19]. Low testosterone levels were associated with an increased mortality independent of numerous risky factors. Low testosterone levels are united with multiple risk factors for cardiovascular disease, with hypertension, abdominal obesity, and thrombosis, it is associated with mimic bone mineral density and osteoporosis, and muscles fatigue [20].

Growth Hormones

The effect of chromium and iron metals on growth hormone in welder's blood was found in exposed age groups I and II. In figure-5, the exposed groups I and II (0.1062ng/ml, 0.0829ng/ml) showed significantly lower testosterone levels than that of control group (0.7688ng/ml). In Fig. 2 shows that control is significantly different from affected age group I and affected age group II. It is observed that the exposure has significant effect on testosterone levels in blood of welders. Growth hormone (GH), also known as somatotropin, where the somatotropic cells are synthesize and secreted by the lateral wings of anterior pituitary gland a 191-amino acid in single chain polypeptide. Growth hormone is also called a stress hormone because it give raise the concentration of glucose and also free fatty acid in body. The adverse effects of growth hormone include fluid retention, hypertension, joint degeneration, and edema. According to Holmes *et al*., [21] Growth hormone deficiency has been associated with reduced lean muscle mass, changes in memory, processing speed and attention, decreased sweating and thermoregulation, Neuromuscular dysfunction, Increased low-density lipoprotein, anxiety and fatigue [22]. Patients suffering with diabetes, retinopathy, hypertension, and gynecomastia are rare [23].

Thyroid stimulating Hormones (TSH)

Thyroid-stimulating hormone also called thyrotropin, thyrotropic hormone and TSH is a pituitary hormone. TSH is a glycoprotein and consists of the alpha and the beta subunit. Meeker *et al*., [24] studied that contact to a number of metals (Cr, Cu, Pb, Fe, Mo) can affect neuroendocrine and thyroid stimulating hormone, cause adverse effects on development, behavior, reproduction, metabolism, and other functions. The effect of chromium and iron metals on thyroid stimulating hormone in welder's blood was found in exposed age groups I and II. Mean TSH values in control (2.409µIU/mL), affected group I (2.4802µIU/mL) and affected group II (2.4927µIU/mL) were not significantly different from each other as shown in figure-6. Hassanin *et al*., [25] studied that the thyroid gland is an important endocrine gland of human body, and metabolically its secretions are very important. K2Cr2O7 has a venomous effect on the thyroid gland structure which results of inducing a clear oxidative damage and liberate ROS. In the effluents the presence of chromium is harmful metal for animals and human beings.

Triiodothyronine

Triiodothyronine is a thyroid hormone. It affects every process in the human body. T3 are carried in the blood, attached to plasma proteins. T3 increases the metabolism and increases the body's oxygen and energy expenditure. The effect of chromium and iron metals on T3 in welder's blood was found in exposed age groups I and II. The mean T3 value in control was (94.948 ng/dl), in affected age group I (94.046 ng/dl) and in age group II was (93.272 ng/dl). Statistically control group is significantly not different from both affected group. The main function of the thyroid gland is to uptake iodine which found in many foods, and changes it into (T4) and (T3) form. These T3 and T4 are then released out into the blood stream and then they are transported all over the body wherever they control metabolism. In the body every cell depends upon thyroid hormones for their metabolism. The normal thyroid gland produces about 20% T3 this T3 contain about four times the hormone "strength" as T4 [26]. Misiewicz *et al*., [27] also studied the effect of metals on lower blood serum concentrations of triiodothyronine (T3). Rana, [28] studied that occupational exposure oftoxic metals iron and chromium is injuries to the pancrease [29]. Low concentration of T3 is result heart failure. Dillmann, [30] studied that T3 alter the cardiac function. T3 direct effects result of T3 action in the heart failure and are mediated extra nuclear mechanisms. Similarly, Zaidi *et al*., [31] also studied Cr, Mn, Co, Ni, Fe and other heavy metals have adverse effects on endocrine of welders.

Tetraiodothyronine T4

T4 are tyrosine based hormones which is secreted by the thyroid gland. It is generally dependable for regulation of metabolic process. T4 has more long life than T3. In our body every living cell depends on thyroid hormones for their metabolism regulation. Normally thyroid gland secretes about 80% T4 [32]. The effect of chromium and iron metals on T4 in welder's blood was found in exposed age groups I and II. The mean value of T4 in male, control group was (5.724 ng/dl), in affected age group I was (6.2962ng/dl) and in age group II was (6.4318ng/dl). Statistically, control is significantly different from both affected age group I and affected age group II. Both affected age group I and affected age group II are not significantly different from each other. Utiger, [33] also studied hormonal misbalancing effect on humans endocrine including T4.

DNA damaging in male welders by chromium and iron

The effect of chromium and iron metals on DNA damaging in welder's blood was found in exposed age groups I and II. DNA damage was found significantly high in group I affected (25.303 µm) than control group I (8.685 µm). DNA damage was also found significantly high in affected group II (27.174 µm) than in control group II (9.701 µm). Fig. 9 showed that control group was significantly different from exposed age group I with exposure period 5-10 years. Control is also significantly different from exposed age group II with exposure period 10-20 years. The DNA damage is defined as it is an alteration of chemical structure of DNA. DNA damage plays a key role in mutagenesis, carcinogenesis, cytotoxin and ageing. The enormous mutations in human tissues are certainly of endogenously. A careful knowledge of the types and occurrence of endogenous DNA mutation is thus crucial for an understanding of the interactions of endogenous processes and manipulate of damage of endogenous origin on cancer causing and other diseases [34]. Hengstler *et al*., [35] studied that occupational exposure to chromium, cadmium, iron, cobalt, lead and other heavy metals occurs which cause DNA single strand break. Chromium cause DNA damage in cultured human chronic myelogenous leukemia cells, promyelocytic leukemic HL-60 cells, and normalhuman

peripheral blood mononuclear cells [36]. Sugden, [37] also support current study according to Sugden chromium intermediates play role in the DNA damage produced by the human Cr (VI) is of rising attention for establishing a mechanism of genotoxicity and mutagenicity for chromium. Glei [38] also studied that Iron surplus produce oxidative DNA damage in the human. Filho [39] also support this study by reporting that intracellular iron causes cell damage and DNA damage.

Conclusion

From the current study it can be concluded that blood elevation of hexavalent chromium and iron significantly caused DNA damage and effected on endocrines.

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