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Baseline blood levels of manganese in resident of Tripoli region, Libya

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Abstract: *A Baseline blood concentration of metals is an important indication of observing metals exposure in environmental and occupational settings. On this regard, a study was conducted on 116 men and 84 women 20-65 years of age in Libya. Its aim was to determine the blood plasma levels of manganese for those who live in the inner city of Tripoli. Blood plasma samples were extracted from 200 participants between April and July 2013.Manganeselevels in blood plasma were determined using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). There was significant age-related correlation in blood plasma levels of manganese which correlated positively with age (P for trend = 0.05). The mean (SD) of manganese was 2.14 µg/l (0.37) with a peak value reached 3.02 µg/l.*

Keywords: *manganese, blood plasma, GFAAS, Tripoli region, Libya*

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I. Introduction

As it is known to enter the environment through a number of different methods, such as air and water, manganese (Mn) is recognised as fundamental, important for development, growth, and preservation of good health for animal, human and plant life [1]. In the aquatic environment, Mn occurs in the forms of Mn(II) and Mn(IV), which notably change in relation to abiotic or microbial mediators [2]. Also, earth is known to comprise high concentrations of Mn, which markedly travels to air, soil or water. The manganese solubility is established according to redox potential and pH in soil [3].

Principally, Mn facilitates a number of fundamental metabolic functions in the human body. These include energy metabolism, enzyme activation, immunological and nervous systems. Additionally, Erikson &Aschner (2003) [4] highlighted those functions to be essential in blood clotting, the control of cellular energy as well as tissue growth. Nevertheless, the three main metabolic functions associated with manganese are: primarily, to stimulate the gluconeogenic enzymes pyruvate carboxylase and isocitrate dehydrogenase. Secondly, to protect the mitochondrial membranes and finally, the activation of glycosyltransferase, which is involved in saccharide synstudy (Zlotkin*et al.*, 1995) [5]. Accordingly, the consumption of Mn is important, with diet playing the main role. With this in mind, the deficiency of Mn has undergone much research in the context of animals, with the finding established that diet is able to impact on the production of both hyaluronic acid and heparin (Zlotkin*et al.*, 1995) [5]. It has also been stated that Mn is known to be a neurotoxic element: for instance, in France, a number of employees who exposed to Mn in great concentrations, such as smelting and mining, have experienced various ailments. Symptoms of limb tremor, muscle weakness and salivation (Santamaria, 2008) [1] were believed to have resulted of such exposure. it was recognised then that the Mn toxicity has the potential to cause brain damage, and was referred to as Manganism with recognition of being similar to Parkinson's disease (Santamaria, 2008) [1].

There are studies in literature to establish the reference values for Mn element in various developed countries such as in Italy [6-8], Canada [9], Spain [10, 11]and Sweden [12]. However, the reliable reference values among Libyan general population remained uncertain. The purpose of the present study was to establish background blood plasma levels of Mn in the healthy people living in Tripoli region (Libya) as a snap shot and to compare the values with literature data obtained from other developed countries. The outcomes shall be of importance for nutritional and environmental monitoring of Mn in human populations.

II. Materials and methods

2.1. Blood sample collection and study population

An Ethical approval from the biotechnology research centre (Tripoli, Libya) was obtained for a study to investigate the dietary and lifestyle habits of Tripoli residents. The Ethical approval was obtained for a questionnaire regarding diet as well as exercises and the volunteers were also asked to provide blood samples. The collected blood samples and serum was separated using centrifuge to be kept at - 20°C until further analysis. Prior to sample collection and questionnaire completion,consents were obtained from 200 volunteers 20 – 65 years of age. 104 volunteers are reported to be non-smokers (n=104) whereas the rest were smokers (n=96). The male percentage was 58% for all samples (see Fig. 1). These samples were divided into three groups as an age factor for male and female (see Fig. 1).

2.2. Reagents

Deionized water was used for the preparation of reagents and standards. Analytical grade HNO_3Nitric acid 63% v/v (BDH, UK).A commercial (Fisher) standard solution of manganese (1000 mg/L) was used to prepare the standards. Hydrogen peroxide (W-WLAB, UK), ammonium dihydrogen phosphate (Merck, Germany) and Triton X -100 were used in the current study.

2.3. Sample preparation

A Selected volume (about 5 ml) of blood was taken from each person andtipped in an unclothing tube (lithium heparin tube). All tubes were kept in refrigerator at $4C^{o}$ for analysis. 2 ml from the blood sample and 2 ml of diluted (1%) triton X-100 solution were mixed in a centrifuge tube (Eppindorf tube) and the mixture was homogenised using a shaker for several seconds. the homogenised solutions was then transferred to a 10000 R / min centrifuge for four minutes with. The upper layer (plasma) was transferred to a small cup using Pasteur pipettes. Mn concentrations in the blood plasma samples were analysed using graphite furnace atomic absorption spectrometry.

2.4. Digestion procedure

Blood plasma samples were digested as a follow: 8 ml of concentrated nitric acid (63%) and 2 ml of hydrogen peroxide (30%) were mixed and then added to 1.5 ml of blood sample (plasma) in a Teflon container. The mixture was digested in a 1000 Watts microwave digester for complete digestion. The used microwave digester is capable to achieve a maximum temperature of $230^{\circ}C$ (Ethas plus, pty, Italy). Mn concentrations in the digested samples were measured using graphite furnace atomic absorption spectrometry (GFAAS) model AA-8600FS Shimadzu Corporation with GTA-110 Graphite furnace.

2.5. Quality control and standard reference material

Standard solutions were prepared in a 10 ml measuring flask, by adding a volume of stock solution that is less than or equal to 50 µl to a 1% diluted triton X-100 solution. These standard solutions of Mn were prepared in the range of 3 – 50 µg/l. Manganese concentrations obtained by GFAAS technique were evaluated by the use of certified reference materials (QMEQAS 07B-06 and QMEQAS 07B-07, NIST, Canada) and it was found to be in good agreement with the certified values of the reference materials.

2.5. Statistical analysis

Means and standard deviations (SDs) of blood plasma Mn concentrations were calculated. All statistical analyses were conducted using SPSS software (version 17).

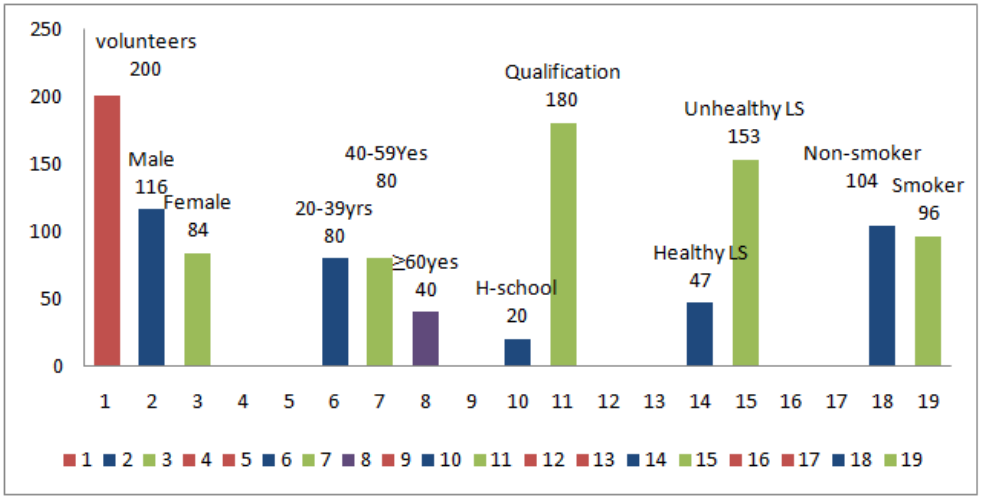

Figure1: Demographic characteristics of participants in the Libyan population aged 20 years orolder.

III. Results and discussion

The study involved 200 adults 20-65 years of age from Tripoli (Libya); their demographic characteristics are presented in Figure 1. The mean age and BMI of the study were 44.5 years and 21.4,respectively. The Descriptive statistics and outcomes of the study participants of blood plasma manganese concentration are presented in Table 2. The Mean (SD) and median for blood plasma manganese concentrations were 2.14 µg/l (0.37) and 2.11 µg/l respectively (see Table 1). However, minimum and maximum were 1.40 and 3.02 µg/l, respectively.

Table 1: Descriptive statistics of blood plasma Mn levels (µg/l) for Libyan aged 20 years orolder.

Descriptive	Statistic
Mean	2.143
Median	2.111
Variance	0.138
Std. Deviation	0.371
Minimum	1.40
Maximum	3.02
Range	1.62

Manganese is very important element for humans; it has some biological functions in human body. However, at high concentration of Mn can cause risk for mankind. From the current study, Mn concentration in blood plasma of Libyan population was at the upper levels of normal level of Mn in blood plasma (mean: 2.14 µg/L; median: 2.11 µg/L). Compared with other countries, some studies of Mn levels in blood have been reported such as Italy [8], Spain [11] and Canada [9]. Clark et al., (2013) [11] has determined Mn concentrations for Canada population, the mean and median of Canadian study were 0.81 and 0.67 µg/L, respectively (see Table 2) [9]. However, in Italian study, the mean (SD) were 0.63µg/L (0.16) (Table 2) [8] and in Canarian (Spain) study, the mean (SD) were 1.06µg/L (0.62) (Table 2). Interestingly, Mn level in blood plasma of Libyan population was 2 to 3 fold ofall studies.

Table 2: Mn levels for some countries

Country	Mean	Median	Reference
Canada	0.81	0.67	[9]
Canarian	1.06 ± 0.62	-	[11]
Libya	**2.14 ± 0.37**	**2.11**	**The current study**
Italy	0.63 ± 0.16	-	[8]

In our previous study (Al-Rmalli and Aboubaker, 2017) [13], the total daily intake of Mn from cereals consumption among Libyan population (Tripoli region) was determined to be 15.8 mg/day [13]. This daily intake is very high compared to that for both TDIs of Italian and Spain studies which were 2.23 and 1.38 mg/day of Mn, respectively [14, 15]. Good correlation was found between TDIs of Mn and Mn level in blood plasma for these study and the present study. It is very clear that Libyan population intake high amount of Mn from there food and this reflected on the Mn level in Libyan blood plasma in the present study. We recommended that bioaccessibility and bioavailability of Mn from Libyan food should

be studied to obtain an accurate estimation of exposure levels and risk assessments. On the other hand, further study for different parts of Libyan must be studied for evaluated Mn levels in blood serum.

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