Determination of Trace Elements in Blood by Total Reflection X-ray Fluorescence (TXRF) Analysis

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Abstract—**Baseline elemental concentrations in blood samples from healthy Jamaicans were determined using total reflection x-ray fluorescence (TXRF) spectroscopy. The mean concentrations of Cu, Fe, P, Rb, S, Se and Zn of the cohort were found to be 92, 36045, 21930, 199, 147106, 10, 1211 µg/dL respectively. Except for Rb (lower) and Zn (higher) these are similar to values observed worldwide. The mean concentrations of P, S, Fe, Se and Rb in males were significantly different from those in females with males having higher concentrations of all elements determined except Rb. TXRF has demonstrated its ability to measure trace concentrations with accuracy and precision high enough to identify differences within a seemingly similar population of men and women and to identify sub populations within each of these. This technique promises possible studies into the effect of trace elements on various medical disorders such as cancer, diabetes, hypertension and heart diseases among others in Jamaica and other countries.**

Index Terms— **whole blood, trace elements, TXRF, gender.**

I. INTRODUCTION

People are exposed to trace elements in the environment in many different ways but primarily from foods we eat. These elements can be considered as essential, non-essential or toxic. The symptoms of mild to severe toxicity of trace elements are readily recognizable in humans. However, milder deficiencies and imbalances often times go un-noticed until they become irreversible or life threatening. Trace element information on soils [1] and foods [2] in Jamaica have led to an interest in elemental transfers to humans and possible links with diseases such as hypertension, cancers, renal maladies, diabetes and cardiac related illnesses which are of high incidence in Jamaica [3-6]. There have also been noted gender differences within the population. From 2004 to 2007 female death rate from cardiovascular diseases, diabetes mellitus and hypertension were on average 12%, 68% and 35% respectively higher than that of males [7]. The effects of trace elements, especially copper, selenium and zinc, on Jamaican children suffering from kwashiorkor and marasmus received some attention in the 1980's [8]. Since then with exception of lead [9,10] and to a lesser extent cadmium [11], very little is known about the levels of trace elements in the Jamaican population.

This study was undertaken to investigate the concentration of trace elements in whole blood samples of male and female blood donors in Jamaica and to determine the relationship, if any, between these elements and gender. The technique utilized was total reflection x-ray fluorescence (TXRF) spectroscopy [12], a sophisticated analytical technique with capabilities of multi-elemental analysis, low limits of detection, high accuracy and relatively high throughput rates. TXRF is an ideal technique for the large scale analysis of blood and other biological samples such as biopsy or autopsy tissue samples that are often available in minute quantities since only a few µL of sample is required per analysis.

II. MATERIALS AND METHODS

This study was approved by the Ethics Committee of the University Hospital of the West Indies and The University of the West Indies Faculty of Medical Sciences. The cohort consisted of 100 adults – 50 males and 50 females – ranging in age from 18-51 yrs for males with a mean of 31.67 yrs and 18-70 yrs for females with a mean of 33.24 yrs. Blood samples were collected by venipuncture into 5 ml tubes, containing 9 mg of K3-EDTA, from blood donors at the University Hospital of the West Indies (UHWI) and the Jamaica National Blood Transfusion Service (Blood Bank). The samples were stored at 4ºC prior to preparation and analysis.

III. SAMPLE PREPARATION AND ANALYSIS

All reagents used were analytical grade purity; the distilled de-ionized water was obtained using a Barnstead E-pure system. The calibration standards were prepared by serial dilutions of 10,000µg/mL CertiPrep single element solutions in 5% HNO3.

A 200 µL aliquot of whole blood sample was added to 400 µL of concentrated, ultra-pure HNO_3 and digested in a water bath for 20 minutes as were the reagent blanks. The samples and blanks were made up to 2 mL with distilled de-ionized water, after which a known volume of Co internal standard was added to achieve a final concentration of 10 µg/mL of Co in the sample and blank solutions. The samples were then homogenized with a vortex mixer and 10 µL of the solution was then pipetted onto a quartz sample carrier. Each sample carrier was then placed on a hot plate at low power to evaporate the solvent. Blanks were treated identically to the samples. The samples were irradiated under vacuum for 1000 seconds at 50 kV and 40 mA in a Wobistrax© TXRF spectrometer [13]. The accuracy of the results was assessed by

the analysis of the certified reference material IAEA A-13, Trace Elements in Freeze Dried Animal Blood.

IV. RESULTS

The comparison in Table I of the observed and recommended results for the reference material IAEA A-13, are in good agreement with certified values.

A total of 3% of the Cu results and 2% of Se results for the cohort were at or below the detection limit of the technique. For these samples the concentrations were set to half the detection limit [14], all other elements were above the detection limits.

Both normal and log-normal distributions were observed and because of this, median concentrations were used for comparisons within the datasets as shown in Table II. However, the majority of published data reported mean concentrations, therefore mean elemental concentrations for the cohort were used for inter-country comparisons.

Fe and P in both males and females were normally distributed along with Rb in females and S in males. S in females along with Rb and Zn in males were log-normally distributed. Cu in both males and females and Zn in females had discontinuous/bimodal distributions; however the continuous portions of the populations followed log-normal distributions. Se in both males and females appeared to be bimodal.

V. DISCUSSION

A. Comparison with other work

The mean trace elements concentrations obtained in this study are compared with the reported means in an extensive literature review [15] of 55 countries and more recent data reported from six countries, Table III.

The blood Cu levels in Jamaicans were within the literature range and similar to those of Sweden, Pakistan and Brazil and less than those found in Spain, Germany and Mexico. Se levels were comparable with other reported values. Jamaicans had the lowest Rb concentrations but was still within the literature review range; The Jamaican Zn levels were on average 42% higher in Jamaicans than in Swedes, Pakistanis and Spaniards, 20% higher than Brazilians, comparable to the Mexican population and approximately 21% higher than the upper limit of the literature range reported in a review article [15].

TABLE I. IAEA A-13, TRACE ELEMENTS IN FREEZE DRIED ANIMAL BLOOD (μg/kg)

Element	Recommended value ± Stdev	Measured value ± Stdev (n=10)
Cu	4.3 ± 0.60	5.07 ± 0.30
Fe	2400 ± 300	2323 ± 17
P	940 ± 250	988 ± 38
Rb	2.3 ± 0.60	2.29 ± 0.12
S	6500 ± 500	7474 ± 269
Se	0.24 ± 0.09	0.22 ± 0.07
Zn	13 ± 1	13.06 ± 0.23

This high concentration of Zn in Jamaicans was also observed in a previous study where the mean Zn content of the red blood cells was 17.6 % higher than that found in the literature for healthy adults [22].

B. Observed differences by gender

Males were found to have higher concentrations of trace elements than females with the exception of Rb. Males on average were 14% higher in trace element concentrations than females. A study conducted in Mexico [18] reported the opposite with females having on average 27% higher concentrations of the elements reported with lower concentrations of Rb than males. The mean concentrations of P, S, Fe, Se and Rb in males are significantly different statically at the 95% confidence interval from those in females which suggest that Jamaican males and females constitute two different populations.

C. Elemental Distributions

Both male and female distributions of Cu were discontinuous and log-normal. Separation of the male population based on the two identified sub-groups (Figs. 1 & 2) of the Cu distribution yielded some significant differences in elemental concentrations between the high and low blood Cu concentration males shown in Table IV. Most males with low blood Cu concentrations had low concentrations of P, S. All males with low Cu concentrations had low Se concentrations ranging from 2 – 7 μg/dL. These males also had higher concentrations of Rb and Zn highlighting the negative correlations between Cu and Zn. The differences in the mean male elemental concentrations of groups A and B for all measured elements were significantly different based on the two-sample t-test at the 95% confidence interval.

Zn was distributed log-normally in both males and females and also appeared to be bimodal in females, Fig. 1f, sub groups C & D. Table V shows the separated female population into low and high Zn concentrations, groups C and D respectively. It is observed that the mean Zn concentration in group C is 64% lower than that of group D. Females with low blood Zn concentrations had lower concentrations of other elements but based on the two-sampled t-test the difference was not statistically significant with exception of Zn.
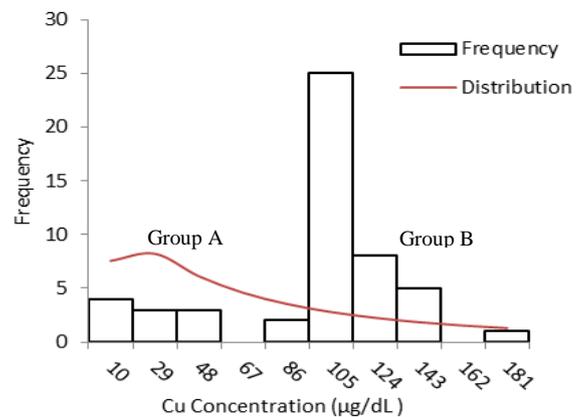

Fig. 1. Cu distribution in males

TABLE II. MEAN AND MEDIAN TRACE ELEMENT CONCENTRATION IN JAMAICAN PEOPLE (µg/dL)

Elements	All		Males		Females	
	Mean ± StDev	Median	Mean ± StDev	Median	Mean ± StDev	Median
Cu	92 ± 39	100	97 ± 36	106	82 ± 36	73
Fe	36045 ± 5582	36279	39790 ± 4392	39256	32126 ± 24790	31605
P	21930 ± 7730	22520	25146 ± 5797	25194	18817 ± 8017	17190
Rb	199 ± 65	183	171 ± 41	163	229 ± 72	235
S	147106 ± 31454	149746	161239 ± 22905	158143	132020 ± 32604	124889
Se	10 ± 5	10	11 ± 5	11	9 ± 4	8
Zn	1121 ± 301	1149	1167 ± 217	1152	1074 ± 364	1145

TABLE III. COMPARISON OF MEAN CONCENTRATIONS OF TRACE ELEMENTS IN WHOLE BLOOD FROM DIFFERENT COUNTRIES (µg/dL)

Element	Reference Values[15]	Jamaica (this study)	Brazil[16]	Germany[17]	Mexico[18]	Pakistan[19]	Spain[20]	Sweden[21]
Cu	80 - 130	92	92	104	158	93	118	95
Fe	30900 - 52100	36045	-	-	-	44310	-	-
P	35000 - 45000	21930	-	-	-	-	-	-
Rb	117 - 598	199	664	241	471	-	-	290
S	168000 - 193000	147106	-	-	147895	-	-	-
Se	5.8 - 23.4	10	-	13	-	-	11	11
Zn	480 - 930	1121	895	-	1090	641	691	610

TABLE IV. SEPARATED MALE POPULATION BASED ON BLOOD Cu CONCENTRATION (µg/dL)

Males	Group A	Group B	All data
Cu	**23.6 ± 18.1**	**115.1 ± 17.9**	97.2 ± 40.8
Fe	37692 ± 5085	40302 ± 4114	39790 ± 4392
P	18021 ± 3554	26883 ± 4835	25146 ± 5797
Rb	206.1 ± 62.0	162.3 ± 3.6	170.9 ± 40.8
S	144660 ± 19601	165282 ± 21998	161239 ± 22905
Se	3.6 ± 1.7	12.6 ± 3.6	10.8 ± 4.9
Zn	1389.7 ± 265.7	1112.5 ± 165.3	1166.8 ± 216.7

TABLE V. SEPARATED FEMALE POPULATION BASED ON BLOOD Zn CONCENTRATION (µg/dL)

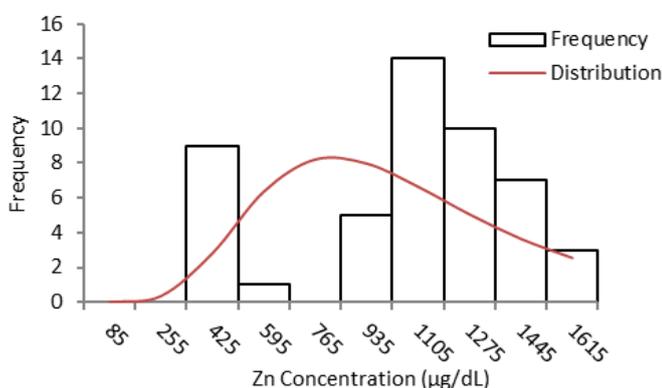| Female	Group C	Group D	All data
Cu	83 ± 31	83 ± 38	83 ± 36
Fe	30938 ± 3055	32416 ± 3876	32108 ± 3740
P	17592 ± 7540	19092 ± 8282	18780 ± 8079
Rb	266 ± 73	217 ± 68	227 ± 71
S	127643 ± 23776	134522 ± 34616	133089 ± 32551
Se	7.1 ± 5.1	9.4 ± 4.0	9.0 ± 4.3
Zn	**440 ± 49**	**1234 ± 190**	1069 ± 368

Fig. 2. Zn distribution in females

D. Element ratios

Though the absolute concentrations of trace elements in the human body are of great importance to health status, the ratios or balance in which these elements exist is also of great significance. The most commonly examined element ratio is the Cu/Zn ratio. Many studies have been conducted to determine relationships between this ratio and illnesses such as cancers [23, 24] and coronary heart diseases (CHD) [25]. In cancer cases the Cu/Zn ratios are generally higher than that of healthy people while for CHD the Cu/Zn ratios are lower than in healthy people. In this study it was observed that the mean and median Cu/Zn ratio for all male and female Jamaicans were lower than literature values from other countries as illustrated in Fig. 3.
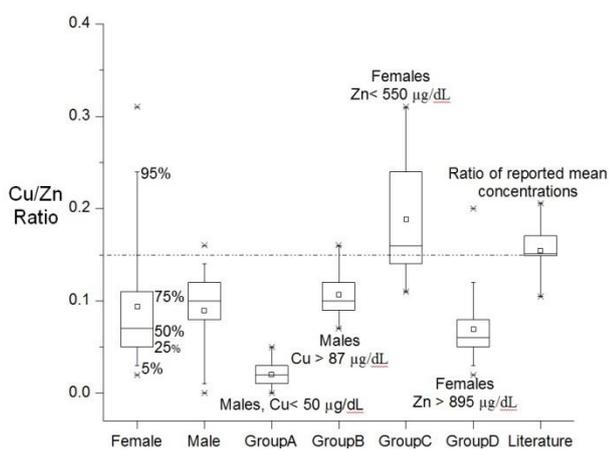
Fig. 3. Jamaican and Literature Cu/Zn ratios

The literature Cu/Zn ratios were obtained using mean Cu and Zn concentrations reported in other studies. Group A, males with low Cu and Group D, females with high Zn, represented 25% and 26% of the male female populations respectively. Only group C, females with lower Zn had Cu/Zn ratios consistently above those observed in the literature. In the Annual Vital Statistics Report of the Registrar General's Department of Jamaica it was reported that heart diseases, excluding hypertensive diseases, was the second leading cause of death, accounting for 19% of deaths in Jamaica in 2003.

VI. CONCLUSION

The trace element concentrations in blood are comparable in most cases to those of other populations. Zn concentrations were however significantly elevated and the relationship if any, with the relatively low level of impact of Cd in Jamaicans with elevated concentrations of Cd would seem worthy of further study [3]. Males generally had higher concentrations of traceelements than females and statistically could be considered to be a different population. There was no effect of age on elemental concentrations for most of the elements but Fe and Rb concentrations varied with age in both males and females albeit with contrary trends.

The implication of the relationship between CHD and the low Cu/Zn ratios have on the population where health is concerned is currently unknown but studies of this nature are of immense value to the population and is the type of study among others that will be undertaken in future work. If there is however a relationship between the Cu/Zn ratio and CHD then the Jamaican males with low Cu concentrations would be at very high risk of developing/having heart related diseases.

TXRF has demonstrated its ability to measure trace concentrations with accuracy and precision high enough to identify differences within a seemingly similar population of men and women and to identify sub populations within each of these. This technique promises possible studies into the effect of trace elements on various medical disorders such as cancer, diabetes, hypertension and heart diseases among others in Jamaica and other Caribbean countries.

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