Cord and Maternal Blood Essential and Toxic Elements Levels in Occupationally Exposed Pregnant Women - Possible Implications in Asd Aetiogenesis

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Research article

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Abstract

Background: Incidence of Autism Spectrum Disorders (ASD) in children as a neurodevelopmental abnormality is growing. Compounded by equivocal deductions from several genetic and environmental studies aimed at establishing its aetiology, it is becoming a global medical challenge. This work investigated placental transfer of some micronutrients (Cu, Zn, Ca, Mg, Se) and toxic (Cd, Pb) metals in occupationally vulnerable pregnant mothers as possible basis of neurodevelopmental abnormalities in children with ASD.

Method: 105 third trimester pregnant women comprising 50 occupationally exposed (cases) (27.68±5.57 years) and 55 non-occupationally exposed age-matched pregnant women (28.84±5.37 years) (controls) were recruited by convenient sampling method for this study. Blood (including cord blood) was collected from all participants. Trace and toxic elements levels were determined in the blood samples using Induction-Coupled Plasma-Mass Spectroscopy; anthropometric and sociodemographic data of the women along with the developmental milestone indices of the baby at infancy were also recorded.

Results: Levels of trace elements were 328.02±109.99mg/L, 370.82±192.97umol/L, 8.61±0.89mg/dl, 1.52±0.26mg/dl and 10.17±1.22mg/L; 348.27±150.61mg/L, 416.80±276.73umol/L, 8.61±0.86mg/dl, 1.46±0.35mg/dl and 8.96±1.15 mg/L for Cu, Zn, Ca, Mg and Se in cases and controls respectively. The differences were not significant. Less than 10% of participants samples (maternal and cord blood) had detectable toxic metal levels. However, cord blood trace elements concentrations were 125.07±24.66mg/l, 525.38±45.86umol/L, 8.44±0.15mg/dl, 1.51±0.31mg/dl and 7.02±0.72mg/dl in cases and 91.05±13.27mg/l, 591.22±44.62umol/l, 1.63±0.15mg/dl and 8.19±0.78mg/L in control for Cu, Zn, Ca, Mg and Se respectively. Only cord blood Mg level was significantly different (p=0.013). Baby weight and head circumferences also correlated significantly with cord Zn and Cu levels (r=0.293, p=0.039), (r=0.478, p=0.010) respectively.

Discussion: The observed downregulation of Mg and Se may have initiated a prooxidant reaction of the upregulated Cu in the foetus overwhelming the protective effects of Zn in scavenging the ROS produced by the combined effects of Cu and the toxic metals to which the cases were occupationally exposed. Our hypothesis is that given the role of Se, Cu and Mg in neurodevelopment, this may be the basis of the abnormal developmental milestones characteristic of ASD.

Conclusion: The need to monitor environmental exposure in pregnancy may be an imperative step in stemming the growing incidence of neurodevelopmental disorders in this environment.

Introduction

The increasing pollution from industrialization exposes the entire population to several substances like micronutrients and heavy metals. Exposure to some environmental substances particularly heavy metals causes disease in humans. Heavy metals are systemic toxicants which can induce multiple organs effects even at low level of exposure [1], some micronutrients are also known to induce prooxidative reactions when present in excessive amounts [2]. Environmental toxicants like cadmium and lead have the potential for adverse fetal effects as a result of maternal exposure. They have been identified by World Health Organization as among the priority environmental metals of public health importance [3]. Exposure to one or more toxic elements in pregnancy causes not only concern for the production of disease and disability in the mother but major concern for the safety and well-being of the fetus [4]. They cross the human placenta and pose a risk of adversely impacting the fetus during sensitive stages of development affecting health throughout life [5]. Prenatal exposure to these heavy metals is a growing concern because they have been identified as the leading causes of prenatal stress [6]. A number of neurological diseases and disorders like impaired cognition, attention deficit, vasomotor problems in children are thought to be caused by prenatal stress from exposure to environmental toxic heavy metals [7]. These environmental toxicants alter the developmental plasticity often and affect the endocrine control of development in fetus. The most sensitive period that humans are sensitive to environmental toxicants is when organs are developing utero and this has been reported to be associated with low birth weight, neuro-behavioural functions and adverse neurodevelopmental outcomes [8][9][10]. Lead (Pb) and Cadmium (Cd) with their effect in the body particularly in children are gaining attention as environmental toxicants that have no safe level. In pregnancy, even low lead concentration could have adverse effects such as developmental delays, low birthweight, and miscarriage. Lead and Cadmium are actively transported across the placenta and impairs fetal neurodevelopment [11][12][13].

Micronutrients are essential trace elements needed in minute quantities for human health. They are also called essential trace elements in the human body. They include calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu) and selenium (Se). These trace elements are involved in so many metabolic reactions and enzymes activities that deficiency of a single trace element is often not associated with specific clinical manifestation but may manifests as a combination of various symptoms among which may be neuro-behavioural malfunctions and adverse neurodevelopmental outcomes. Micronutrients deficiency or excess could lead to detrimental pregnancy outcome and neurological disorders in the fetus. Because micronutrients also play important roles in the division and differentiation of fetal cells and their development [11][14], trace elements are closely associated with fetal growth and development during pregnancy. Micronutrients levels in humans are affected by dietary habit, lifestyle, and environmental toxicants. Cd has been reported to have a direct interaction with the membrane transporters for iron and zinc by reducing the efficiency of transport of the minerals. It may also influence Zn transport indirectly by increasing metallothionein production in the placenta which in turn reduces the efficiency of Zn transfer to the fetus [15][16][17] with its consequent deleterious effect on Zn function.

Micronutrients have been known to freely cross the placenta through the umbilical cord [18]; hence their roles in the division and differentiation of fetal cells and their development in-utero may not be unconnected with their concentration in the developing fetus. There is paucity of data on the possible roles of trace and toxic elements in the neurodevelopmental processes of fetus in-utero. This becomes important in the wake of increasing incidences of neurodevelopmental diseases like ASD. This work was thus aimed at investigating the possible roles of variation/alteration in levels of essential and toxic elements derived through in the pathogenesis of ASD in pregnant women occupationally exposed to these elements.

Materials And Methods

Location /Site of Study

This study was conducted on willing pregnant women attending ante natal clinic at Adeoyo Maternity Teaching Hospital and University College Hospital, Ibadan, Oyo State, Nigeria. The two hospitals are secondary and tertiary medical centres respectively established over 5decades ago to cater for the medical needs of Ibadan and her environs. Ibadan is the largest and a cosmopolitan city with about 5 million inhabitants (2014 Nigeria census figure) essentially made up of civil servants as elites and small-scale entrepreneurs. The large population of inhabitants of the city and those of small-scale industries may account for the level of environmental pollution in the city.

Recruitment

A total number of 105 participants in the third trimester of pregnancy were recruited by convenience sampling method from antenatal clinics of Adeoyo Maternity Teaching Hospital and University College Hospital, Ibadan, Oyo State, Nigeria for this study. This comprised fifty (50) occupationally exposed clinically pregnant women with mean age 27.68 ± 5.57 years and fifty-five (55) non-occupationally exposed pregnant women with mean age of 28.84 ± 5.37 years. The occupationally exposed pregnant women recruited for this study were women working in milling, smelting and grinding industries, wives of auto-mechanics, painters, battery chargers and others routinely exposed directly or indirectly occupationally exposed to metals and toxic elements. The non-exposed participants recruited for this study were pregnant women working as civil servants in ministries and hospital staffs not routinely exposed to these metals and toxic elements.

All participants recruited for this study received routine pregnancy vaccinations.

Ethical Approval: Ethical approval was obtained from the UCH/UI joint Ethical Committee (UI/EC/15/0087) and Oyo State Ministry of Health Ethical Board (Informed consent was obtained from each participant after due explanation of the project in local dialect).

Inclusion criteria: Pregnant women in third trimester that gave informed consent were recruited for the study.

Exclusion criteria: Pregnant women in first and second trimesters and those in third trimesters with complications like anaemia, diabetes and hypertension in pregnancy were excluded from the study.

Method: History of all participants in the study was taken, these included anthropometric and socio-demographic characteristics, environmental exposure factors, dietary history, and health issues.

Blood collection: About 5mls of venous blood was collected from each participant from the ante-cubital vein and 5mls cord blood after delivery. Blood was carefully dispensed into lithium heparin bottles to avoid haemolysis. Blood samples were centrifuged at 3000 r.p.m. at room temperature for 10 minutes using Centeur 2-centrifuge (Fiston centrifuge, manufactured in England) to obtain plasma which was promptly separated into another clean plane bottle. All samples were kept frozen at -20^{o} C until they were ready for analysis.

Biochemical Analysis:

Analysis of samples was done in the Department of Environmental and interdisciplinary Sciences, Texas Southern University, Houston, Texas, USA.

Samples were analyzed for Pb, Cd, Ca, Mg, Zn, Cu and Se using Induction Coupled Plasma-Mass Spectrometry (ICP-MS) (Inductively Coupled Plasma Mass Spectrometer – Agilent model 7500ce, equipped with an Octapole Reaction System (ORS). Inductively Coupled Plasma Spectrometer – Perkin-Elmer, model 5300DV) based on standard laboratory procedures.

Samples were digested with concentrated nitric acid in a microwave digestion apparatus, the sample digest was diluted, fortified with internal standards and analysed using inductively coupled plasma mass spectrometry (ICP-MS) (Pb, Se, Zn, Mg, Mn, Ca) and inductively coupled plasma optical emission spectrometry (ICP-OES) (Cu, V, Al). The procedure for sample preparation was according to the instructions of manufacturer of the equipment as contained in its manual of operation.

Appropriate in-built software in the instrument was used to calculate the result of the analysis with inclusion of standards and controls. The results were converted to SI units from part per billion (ppb) using appropriate conversion factors.

1. To validate results of analysis, samples were carefully prepared for analysis as directed in the ICP-MS instrument.

i. Sensitivity of the instrument. ICP-MS instrument used for the analysis of trace metal contents in the samples has inbuilt characteristics that ensures sensitivity, selectivity, accuracy and reproducibility. These indicators of validity as stated by the manufacturer was noted and found to be suitable and acceptable.

ii. Selectivity was ensured with the principle of the method of operation of the instrument which was based on induction of the metal of interest and identifying the metal producing the evaluated emission produced using mass spectroscopy

iii. Accuracy of results was ensured by calculating the SD of each metals and introduction of both blind and labelled control specimens results which were all correlated and found to show variations within internationally accepted ±2SD.
iv. Reproducibility of the results was ensured by running the samples in duplicate.

2. Sample preparation

This was done based on the analyte in view; plasma was the sample of choice for metals like Zn, Se, as some metals use plasma while some use whole blood for analysis.

3. Method of sample digestion; Samples were acid digested using a mixture of HNO_3 and perchloric acid. The digestion mixture was in the ratio of 1 to 10 (blood:acid)

4. The instrument has an inbuilt standard calibration curve which was checked at regular intervals to ensure reliability of final results

5. Method of selecting participants:

“Recruitment: A total number of 105 participants in the third trimester of pregnancy were recruited by convenience sampling method from antenatal clinics of Adeoyo Maternity Teaching Hospital and University College Hospital, Ibadan, Oyo State, Nigeria for this study. This comprised fifty (50) occupationally exposed clinically pregnant women with mean age 27.68±5.57 years (Cases) and fifty-five (55) non-occupationally exposed pregnant women with mean age of 28.84±5.37 years (Controls). All participants recruited for this study received routine pregnancy vaccinations”.

Statistical analysis: Data were reviewed, coded, tabulated and analyzed using statistical package for social science (SPSS 20.0) Mean standard error (MEAN ± S.E) was used to express descriptive statistics of the results while student t-test was used to assess the statistical significance of the difference between the two groups. Pearson’s correlation was used to determine relationship between studied trace elements while Chi-square test was used to determine relationship between non-parametric data in the study.

P value of 0.05 was regarded significant.

Results

Analysis of the structured questionnaire administered to the participants is summarized as below:

Table 1:

This is a summary of mean ages of the participants (pregnant mothers) (27.68±5.57 years and 28.84±5.37 years) and their spouses (33.40±6.54 years and 35.58±6.38 years) for cases and controls respectively. The gestation periods and age at first pregnancies for the mothers were 37.58±1.20

months and 23.72±4.48 years for cases and 37.55±2.00 years and 25.40±4.14 years for controls respectively. Although there was a slight difference in the ages at first pregnancy, the similarities in age and gestation period may ensure that this could not confound pregnancy outcomes in the participants. That the pregnancy outcome was not confounded may be inferred from the similar head circumference of the babies as recorded in Table 1.

There was a general non-significant reduction in mean maternal blood Cu and Zn in cases relative to those of controls while mean maternal Mg and Se levels in cases were higher than those of controls. Mean levels of few data obtained for the toxic metals - Pb and Cd - were also higher in cases compared to those of controls. However, while mean cord blood Cu level was higher in cases, mean cord blood Zn and Mg levels in cases were all lower in comparison to values obtained in controls; the difference in Mg level was also significant (P=0.013). Values obtained for maternal and cord blood Ca levels were similar in all cases. These observed variations in levels of the essential elements and toxic metals may be physiologically significant in neurodevelopmental processes in a developing foetus.

Figure I :

This is a summary of the various environmental factors and conditions to which the participants were exposed as represented in a bar chart. Environmental exposure of participants (cases and controls) occasioned by proximity to tarred road, house paints, insecticide and smoking was similar in the participants, this may further validate their comparison.

Figure II:

This is a bar chart summary of food and nutritional supplements consumption by participants. There was no difference in the consumption of fruits and vegetables, seafood and even nutritional supplements. This also validate their comparison.

Figure III: This is also a pie chart representation of consumption of nutritional supplements by the mothers during pregnancy. This becomes imperative because of the prevalence of factors like anaemia, malnutrition which are common medical problems associated with pregnancy in this environment. That there was no significant difference in the summary of consumption of these supplements in pregnancy by the participants may be further proof of validity of cases and control participants in this study.

Figure IV:

This is a chart summary of occupational exploits of participants and their spouses. Majority of cases were unskilled workers with its attendant lack of necessary public health education. This was worsened by the preponderance of their husbands working as artisans or traders compounding the lack of necessary public health awareness.

Table 2:

Data from maternal (cases and controls) and cord blood samples (cases and controls) were correlated using Spearman's Correlation towards establishing a statistical relationship among them. The summary of the results is as shown in Table 2. Maternal Zn and Se levels correlated significantly with cord Mg and Cu levels respectively in controls (r=0.266, p=0.045); (r=0.450, p=0.005**)**. This underscores the relative dependent disposition of these essential elements in foetal metabolic activities and may be indicative of their essentiality in normal neurodevelopment during foetal growth. In essence there was a direct relationship between these elements from mother through the umbilical cord and the foetus. The relative importance of this relationship is that sufficient Zn and Se were available to mitigate the possible anti-oxidative actions of Cu and Mg which their preponderance could have precipitated. However, maternal Ca level correlated significantly negatively with cord Se level (r= -0.349, p=0.022) in cases; this inverse relationship as against what was obtained earlier may precipitate an interplay between Mg and Ca in cases which may create an abnormal relationship between these two essential elements in comparison to what was obtained in controls.

Table 3:

Further correlating analysis as seen in table 3 showed that weight of babies of exposed mothers (cases) correlated significantly with cord Zn (r=0.293, p=0.039) while head circumference of babies of exposed mothers (cases) also correlated significantly with cord Cu (r=0.478, p=0.010). These may underscore the earlier deductions on relationship of these essential elements especially in foetal neurodevelopment.

Discussion

Placenta transfer of elements and other materials from maternal into the foetal circulation of the baby in-utero has been well documented especially based on studies from the developed parts of the world [19][20]; however, whether such movement may precipitate or exacerbate abnormal neurodevelopment in the developing baby in-utero remains contentious. Understanding this becomes imperative especially in the aetiology of neurodevelopmental diseases which are generally initiated in early life of the baby in-utero [21]. The development is more worrisome in pregnant mothers occupationally exposed to some of the known essential or toxic elements which as a result of their placenta transfer to the baby may create an elemental imbalance in the baby with possible disruption of sensitive metabolic functions and the attendant systemic dysfunctions. The focus of this work was thus the attendant implications of such transfer on the delicate balance of these elements and its possible influence on the aetiopathogenesis of autism spectrum disorders in children of mothers occupationally exposed to such metals.

In recruiting participants for this project, various environmental peculiarities including anthropometric and anthropogenic factors were considered to ensure that such did not confound the credibility and appropriateness of findings. These anthropometric and anthropogenic factors are known sources of exposure to various contaminants and toxicants [22]. Occupational exposure to them have been established as veritable sources of organophosphates, organo-pesticides and arsenate (insecticides), cadmium (smoking and seafoods) and lead (leaded paints and environmental dust) [23][24][25][26][27].

Hence, the similarities in participants' exposure in this study in the use of insecticides, passive/active smoking and exposure to various environmental contaminants derivable through dust by virtue of the type of residence and proximity to tarred road may be said to validate appropriateness of the recruited participants. Also, consumption of nutritional supplements and sea food especially during pregnancy was similar in all the participants as derived from the administered structured questionnaire. This was to moderate the possible exposure to toxic metals including cadmium which may be derivable from sea foods and other essential elements that are often constituents of the common nutritional supplements voluntarily administered by pregnant mothers especially in this clime. Inappropriate consumption of nutritional supplements during pregnancy may precipitate alteration in levels of some essential elements resulting in an imbalance in levels of pro- and anti-oxidant elements in the system. The observed similarity in the consumption of these items and the comparative educational levels of mothers and their spouses in this study may also be indicative of similarities in the social and educational standards of participants thus validating their comparison. In summary, similarities in anthropometric and anthropogenic factors by participants in this study (cases and controls) may have ensured appropriateness of the choice of participants and hence validity of deductions from various biochemical data obtained.

In considering the focus of this work which was to examine the possible implications of placenta transfer of various elements from mother to the foetus and their possible involvement in the development of autism, results obtained in this study showed that essential (zinc, selenium, copper, calcium, magnesium) and toxic (cadmium, lead) metals were transferred from maternal to foetal circulation. Levels of these elements and metals in maternal and fetal circulation as observed in this study may be seen as an indication of placental transfer of the elements in-utero. Findings in this study were similar to previous ones [5][10][28]; hence, variations in levels of these elements in cord blood of cases and controls may be seen as evidence of trans-placenta transfer of the elements from the mother to the developing baby in-utero. A closer look at the degree of transfer which affected all elements under consideration showed that Zn was particularly heavily transferred from maternal blood circulation into that of the foetus. The observed level of blood Zn also showed that it accumulated more in the baby than in the mother from which it was transferred. This observation was similar to previous works which documented that cord blood Zn level was elevated in babies than in mothers. (Irwinda *et al.,* 2019).

It could also be seen that Cu level was higher in cord blood of exposed babies than in non-exposed babies as against the picture obtained in maternal circulation where Cu was higher in non-exposed mothers than in exposed mother's circulation. Although this has also been the findings by previous works (Hu *et al.,* 2015; Irwinda *et al.,* 2019), the import and concern about this is that cord blood Cu would be expected to follow the same pattern since the baby derived her nutrients from that of the mother while still in-utero. Zn and Cu have also been reported to have an inverse relationship especially when one out-number the other (Halsted, *et al.,* 1968). There is therefore the need for a plausible explanation for this phenomenon especially as it may possibly explain some of the issues associated with neurogenesis and its disorders.

Associating differences in the clinical and cognitive functions of children from the two groups of mothers (cases and controls) with alterations in these elements as an aetiological basis of the pathogenesis of autism in early life of the children requires examining the established physiological and biochemical roles of the various elements/metals in neurodevelopmental processes.

Zinc as an essential element is known to be involved in several metabolic activities while its anti-oxidant activities and that of Se especially in the nervous system has been well documented [29][30][31].Zn, Se and Mg are known to be very crucial in the metabolic processes of neurogenesis; their roles in axon development and antioxidation in neurogenesis have been established (Adamo & Oteiza, 2010). Cu, on the other hand, is said to contribute in a number of neurodevelopmental processes and is needed for numerous proper enzymes functioning in the brain. Copper deficiency and toxicity can interfere with brain developments and functions. Zn is known to couple Cu in the Cu/Zn enzyme dismutase which along with glutathione scavenges toxicants in form of ROS that may disturb the oxidant/antioxidant balance in the brain. The observed increased cord Cu level may then be examined in context of its relationship with both Zn and Se that complimentarily maintain the oxidant/antioxidant balance in the brain (de Lucia *et al.,* 2020). Secondly, Zn is known to influence the absorption of Cu in the intestine, a definite relationship (1:1) is known to exist between Zn and Cu in ensuring appropriate biologic functions of the two essential elements [17][19] under normal circumstances. A disturbance of this delicate balance as reflected by an increase in Cu in the foetal circulation emanating from the cord blood (altering the Zn:Cu ratio) has the tendency of converting the Cu into an electron acceptor (from Cu to Cu^{2+}) thus becoming an oxidant. Normally, Zn and Cu are directly associated with the metallothionein formation necessary for scavenging toxic metals; however, a protraction of this disturbance as may be seen in pregnant women exposed to these toxicants (excessive toxic metals and Cu) may exacerbate the condition thereby increasing the toxic burden on the system and its attendant effect on the limited antioxidant pool.

However, the observed high Zn level in cord blood in this study may be likened to the typical scenario in diabetes mellitus where in spite of a high glucose level in the blood, the cells are deprived of the glucose necessary to provide energy that will drive the expected metabolic processes. A plausible inference from this may therefore be that although there was a large amount of Zn transferred from the maternal to the foetal circulation as seen in the cord blood of the babies (exposed and unexposed), it may be inferred that the Zn ions needed in neurogenesis were not available at the cellular level to perform the needed co-enzymatic activity. This may be due to non-development of the various Zn-related enzymatic pathways as a consequence of age which may have precipitated non-availability of Zn at the cellular level to compliment Se both of which constitute the major antioxidant pool in the neurons. Alternatively, it may be that Zn-related enzymes are not involved in the necessary metabolic activities of a developing baby in-utero possibly because of their relative immaturity at that stage or that Zn is non-consequential in that process at that stage. Perhaps, the antioxidant mechanism in the brain of babies at this stage revolves round blood Se and Mg more than on Zn, Se and Mg concentrations. That this is plausible may be seen from the fact that even a higher cord blood Zn level in non-exposed babies than in exposed babies did not result in any neurodevelopmental abnormality in the former. This raised a basic question of whether Zn is actually essential for oxidative balance in the brain of babies in-utero. It is a known fact that for adequate metallothionein activity, blood Zn and Cu levels must be equivalent to ensure the scavenging activity of metallothionein against excess ROS by metal toxicants. Although this antioxidative activity at the neuronal level has been documented to be largely dependent on Zn and Se, its non-effectiveness has been proposed to be marked by an increase blood Cu or a decrease in blood Zn level (Pokusa & Trančíková, 2017). Hence, increase in Zn level as recorded in cord blood of exposed babies in this study should expectedly bring about a corresponding lowering of blood Cu level in those babies (just like relationship between Ca and inorganic phosphorus) if the metallothionein system was functional. The deleterious effect of reduction in metallothionein formation may be exacerbated by the reduced Se level in the cord blood since the latter is also a strong antioxidant against toxic metals. Hence, the reduced Se level observed in cases in this study may be accentuating a reduction in antioxidative capability of the system. Several works have corroborated this theory that reduction in blood Zn and in particular with a concurrent reduction in blood Se may enhance Cd and Pb toxicity [32].

Thus, it may therefore be inferred that absence of this complimentary effect of Zn with Se may overwhelm the capability of Se alone as may be seen in the reduced level of exposed cord blood Se which is grossly low in comparison to the exposed, non-exposed maternal and cord blood Se levels.

The roles of calcium and magnesium especially in modulating movement across cells are interwoven. The biological function of Mg is closely related to that of Ca. Magnesium has also been reported to protect the brain from toxic effects of chemicals while its deficiency has been postulated to facilitate heavy metal toxicity and may be associated with the aetiology of learning disorders in children (Drybanska-Kalita,1995). Optimal Mg level is needed for the formation of glutathione, an important antioxidant system of the body. The observed significant reduction in Mg level in cases in this study may be exacerbating a depletion of the antioxidant pool of the body especially the glutathione level. Hence, a continuous depletion of the glutathione level which a sustained deficiency of Mg may precipitate in occupationally exposed pregnant women may have reversed the functional GSH/GSSG ratio in the developing foetus precipitating an abnormal neurodevelopmental process in the latter. Mg deficiency has been demonstrated in cases of ASD in previous works [33][34]. This shortfall in anti-oxidative capability induced by low Se and low/nonconsequential Zn levels may have overwhelmed the capability of Mg in exposed babies with the increasing antioxidant activity resulting in a decrease of cord blood Mg in exposed babies in comparison to others in the study.

Thus, the interplay of Se and Mg as the main metal antioxidant at the uterine level in exposed babies may also be inferred since even in the maternal circulation (exposed and unexposed), the concentrations of Mg were quite similar.

The apparent downregulation of Se, Zn and Mg in cord blood of cases may have created an imbalance and a shift in the relationship between Cu and Zn thus facilitating the pro-oxidative tendency of Cu thereby increasing the concentration of pro-oxidant and the consequent overwhelming of the antioxidative capabilities of the already reduced concentration of Se and Zn. The damaging effect of this phenomenon may be responsible for the significant reduction in the Mg level thus accentuating the dysfunction in neural activities as clinically manifested in autism at adolescence.

Mg has also been reported to protect the brain from toxic effects of chemicals while deficiency has been postulated to facilitate heavy metal toxicity. Optimal Mg level is also needed for the formation of glutathione, an important antioxidant system of the body (Drybanska-Kalita,1995), it has also been associated with the aetiology of learning disorders in children.

This shortfall in anti-oxidative capability induced by low Se and low/nonconsequential Zn levels may have overwhelmed the capability of Mg in exposed babies with the increasing antioxidant activity resulting in a decrease of cord blood Mg in exposed babies in comparison to others in the study.

Thus, the interplay of Se and Mg as the main metal antioxidant at the uterine level in exposed babies may also be inferred since even in the maternal circulation (exposed and unexposed), the concentrations of Mg were quite similar.

The observed significant reduction in Mg level in exposed babies in this study may be exacerbating a depletion of the antioxidant pool of the body especially the glutathione level.

Hence, it may be deducted that babies continuously exposed to these conditions may ultimately develop reduced metallothionein activity resulting in an imbalance oxidant/antioxidant pool leading to a derangement in many metabolic activities in the body. Such a derangement will be exacerbated and becomes fatal in a developing baby resulting in possible abnormal neurogenesis.

It may therefore be stated that at the uterine level (especially in pregnant mothers occupationally/environmentally exposed to these metals), results from this work showed that Se is the main antioxidant metal in the neuron, the deficiency of which may precipitate abnormal neurodevelopment which may ultimately result in neurodevelopmental disorders like ASD and CP in infancy.

The roles of calcium and magnesium especially in modulating movement across cells are interwoven. The biological function of Mg is closely related to that of Ca. Magnesium has also been reported to protect the brain from toxic effects of chemicals while its deficiency has been postulated to facilitate heavy metal toxicity and may be associated with the aetiology of learning disorders in children. Optimal Mg level is needed for the formation of glutathione, an important antioxidant system of the body. The observed significant reduction in Mg level in cases in this study may be exacerbating a depletion of the antioxidant pool of the body especially the glutathione level. Hence, a continuous depletion of the glutathione level which a sustained deficiency of Mg may precipitate in occupationally exposed pregnant women may have reversed the functional GSH/GSSG ratio in the developing foetus precipitating an abnormal neurodevelopmental process in the latter. Mg deficiency has been demonstrated in cases of ASD in previous works [33][34]. The apparent downregulation of Se, Zn and Mg in cord blood of cases may have created an imbalance and a shift in the relationship between Cu and Zn thus facilitating the pro-oxidative tendency of Cu thereby increasing the concentration of pro-oxidant and the consequent overwhelming of the antioxidative capabilities of the already reduced concentration of Se and Zn. The damaging effect of this phenomenon may be responsible for the significant reduction in the Mg level thus accentuating the dysfunction in neural activities as clinically manifested in autism at adolescence.

This abnormality which could be seen to have started from placental transfer of elements from mother to the foetus may also be exacerbated by the observed higher concentration of the toxic metals (Pb and Cd) which may further increase the ROS burden in the system and overwhelm the antioxidative capabilities of both Zn and Se. Findings in this study are similar to those of — who established in autistic children studied.

In clinical terms, abnormal head circumference of the newborn baby has been associated with neurodevelopmental disorders by previous workers [35][36] the observed abnormality in the head circumference of babies from cases in comparison to those of controls may be an indication of an ongoing neurodevelopmental disorder in these babies (cases) from birth giving a prognostic indication of an abnormality in neural development which in this case may result in autism syndrome disorders. Hence, in totality, the observed reduction in essential elements in this work in conjunction with elevated levels of toxic metals (Pb, and Cd) may be the basis of the anatomical abnormality as the abnormal head circumference which in adolescence may lead to the clinical symptoms of autism.

Conclusion: Familial imbalance in essential metal levels as indicated by abnormality in cord blood concentration of these metals derived from placenta transfer to the baby in-utero may be a strong indicator of abnormal neurodevelopment which may latter manifest as ASD in adolescence. The pathophysiology may be a gross deficiency in metallothionein formation with the attendant reduction in the scavenging capabilities of the system and a concurrent reduction in glutathione-based antioxidant pool which may also be further aggravated by a concurrent elevated level of toxic metals in the system. The need to monitor the principal metals (Zn, Se, Mg, Cu) associated with this mechanism in children with ASD becomes imperative.

Abbreviations

ASD
Autism Spectrum Disorder
Ca
Calcium
Zn
Zinc
Cu

Copper
Mg
Magnesium
Se
Selenium
Cd
Cadmium
Pb
Lead
ICP-MS
Induction-Coupled Plasma-Mass Spectroscopy
WHO
World Health Organization
UCH/UI
University College Hospital/University of Ibadan
ORS
Octapole Reaction System
ICP-OES
Inductively Coupled Plasma6 Optical Emission Spectrometry
SPSS
Statistical Package for Social Science
ROS
Reactive Oxygen Species

Declarations

Data Availability statement:

All Data associated with this work shall be made available on appropriate request

1. **Ethical Approval**: Approval was obtained from the UCH/UI joint Ethical Committee (UI/EC/15/0087) and Oyo State Ministry of Health Ethical Board (Informed consent was obtained from each participant.
2. **Consent for Publication:** All authors agreed on this publication
3. **Availability of supporting data:** All data in support of this manuscript are attached and available
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7. **I O Omotosho: corresponding and Principal Author:** He conceptualized and designed the project, he also prepared the manuscript
8. **A O Akinade:** carried out the analytical procedures, did the statistical analysis and also reviewed the manuscript

All Authors have read and approved the manuscript and ensured that this is the case

Conflict of Interest

The authors have no potential conflict of interest to disclose

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Tables

Table 1
Summary of Biodata (age), anthropometric and biochemical data of cord and mother's blood in cases and control.

	Exposed (n = 50)	**Unexposed (n = 55)**	**t**	**P**
Pregnancy age (weeks)	37.58 ± 1.20	37.55 ± 2.00	0.106	0.916
Age (years)	27.68 ± 5.57	28.84 ± 5.37	-1.082	0.282
Age at 1st birth (years)	23.72 ± 4.48	25.40 ± 4.14	-1.999	**0.048***
Husband age (years)	33.40 ± 6.54	35.58 ± 6.38	-1.730	0.087
Weight (Kg)	63.34 ± 9.99	63.33 ± 10.66	0.006	0.995
Height (Meters)	1.59 ± 0.07	1.59 ± 0.08	-0.032	0.975
BMI	25.09 ± 3.92	25.08 ± 3.99	0.014	0.989
Baby weight (Kg)	[a]2.98 ± 0.06	[a]3.60 ± 0.55	-1.326[b]	0.185
Baby height (Cm)	48.52 ± 2.38	47.45 ± 3.85	1.685	0.095
Headcircumference (Cm)	33.30 ± 1.42	33.47 ± 1.70	-0.563	0.575
Maternal Cu(mg/L)	328.02 ± 109.99	348.27 ± 150.61	-0.780	0.437
Maternal Zn(μmol/L)	370.82 ± 192.97	416.80 ± 276.73	-0.978	0.330
Maternal Ca(mg/dl)	8.61 ± 0.89	8.61 ± 0.86	0.034	0.973
Maternal Mg(mg/dl)	1.52 ± 0.26	1.46 ± 0.35	1.088	0.279
Maternal Se (mg/L)	[a]10.17 ± 1.22	[a]8.96 ± 1.15	-0.528[b]	0.597
Maternal Cd(μg/dl)	[a]96.67 ± 15.64	[a]70.00 ± 30.00	-0.519[b]	0.604
Maternal Pb(μg/dl)	11.00 ± 1.41	10.00 ± 1.85	1.000	0.423
Cord Cu(mg/L)	[a]125.07 ± 24.66	[a]91.05 ± 13.27	-0.807[b]	0.420
Cord Zn(μmol/L)	[a]525.38 ± 45.86	[a]591.22 ± 44.62	-1.028[b]	0.306
Cord Ca (mg/dl)	8.45 ± 0.15	8.50 ± 0.13	-0.291	0.772

*Significant at $p < 0.05$

[a]Mean±SEM

[b]Mann Whitney-U non-parametric test z-score

	Exposed (n = 50)	Unexposed (n = 55)	t	P
Cord Mg(mg/dl)	1.51 ± 0.31	1.63 ± 0.15	-2.531	**0.013***
Cord Se (mg/L)	[a]7.02 ± 0.72	[a]8.19 ± 0.78	-1.235[b]	0.217

*Significant at $p < 0.05$

[a]Mean±SEM

[b]Mann Whitney-U non-parametric test z-score

Table 2

Summary of Correlation analysis of maternal and cord blood essential element levels and anthropometric data

Correlating pairs		Total (r,p)	Cases (r,p)	Controls (r,p)
Maternal Cu	Baby weight	0.049, 0.725	-0.073, 0.613	0.049, 0.725
	Baby height	0.130, 0.344	0.014, 0.921	0.130, 0.344
	Head circumf	0.057, 0.679	-0.090, 0.533	0.057, 0.679
	Cord Cu	-0.034, 0.832	0.030, 0.881	-0.034, 0.832
	Cord Zn	-0.131, 0.341	-0.041, 0.775	-0.131, 0.341
	Cord Ca	-0.028, 0.838	-0.007, 0.962	-0.028, 0.838
	Cord Mg	0.136, 0.321	0.264, 0.064	0.136, 0.321
	Cord Se	0.114, 0.411	-0.105, 0.502	0.114, 0.411
Maternal Zn	Baby weight	0.070, 0.611	-0.148, 0.304	0.070, 0.611
	Baby height	0.253, 0.063	-0.091, 0.532	0.253, 0.063
	Head circumf	0.073, 0.597	-0.013, 0.928	0.073, 0.597
	Cord Cu	0.109, 0.497	0.213, 0.276	0.109, 0.497
	Cord Zn	0.163, 0.235	-0.015, 0.916	0.163, 0.235
	Cord Ca	0.018, 0.895	-0.044, 0.759	0.018, 0.895
	Cord Mg	**0.266, 0.045***	**0.274, 0.054**	**0.266, 0.045***
	Cord Se	0.058, 0.677	-0.089, 0.570	0.058, 0.677
Maternal Ca	Baby weight	-0.162, 0.236	-0.196, 0.173	-0.162, 0.236
	Baby height	0.118, 0.391	-0.036, 0.804	0.118, 0.391
	Head circumf	-0.022, 0.873	-0.011, 0.941	-0.022, 0.873
	Cord Cu	0.087, 0.587	-0.032, 0.873	0.087, 0.587
	Cord Zn	-0.232, 0.088	-0.105, 0.468	-0.232, 0.088
	Cord Ca	0.093, 0.500	0.204, 0.156	0.093, 0.500
	Cord Mg	-0.130, 0.345	-0.107, 0.462	-0.130, 0.345
	Cord Se	**-0.134, 0.335**	**-0.349, 0.022***	**-0.134, 0.335**
Maternal Mg	Baby weight	0.096, 0.484	0.072, 0.617	0.096, 0.484
	Baby height	0.101, 0.462	0.033, 0.822	0.101, 0.462

Correlating pairs		Total (r,p)	Cases (r,p)	Controls (r,p)
	Head circumf	0.052, 0.704	0.080, 0.580	0.052, 0.704
	Cord Cu	0.123, 0.443	0.247, 0.204	0.123, 0.443
	Cord Zn	-0.187, 0.171	-0.035, 0.808	-0.187, 0.171
	Cord Ca	0.200, 0.143	0.092, 0.526	0.200, 0.143
	Cord Mg	0.007, 0.958	0.263, 0.065	0.007, 0.958
	Cord Se	0.045, 0.745	0.149, 0.340	0.045, 0.745
Maternal Se	Baby weight	-0.150, 0.288	-0.098, 0.521	-0.150, 0.288
	Baby height	0.049, 0.729	-0.004, 0.977	0.049, 0.729
	Head circumf	-0.023, 0.871	-0.022, 0.884	-0.023, 0.871
	Cord Cu	**0.450, 0.005***	**0.041, 0.840**	**0.450, 0.005***
	Cord Zn	0.177, 0.210	-0.082, 0.591	0.177, 0.210
	Cord Ca	-0.105, 0.460	-0.090, 0.555	-0.105, 0.460
	Cord Mg	0.018, 0.900	-0.025, 0.868	0.018, 0.900
	Cord Se	-0.060, 0.677	-0.017, 0.920	-0.060, 0.677

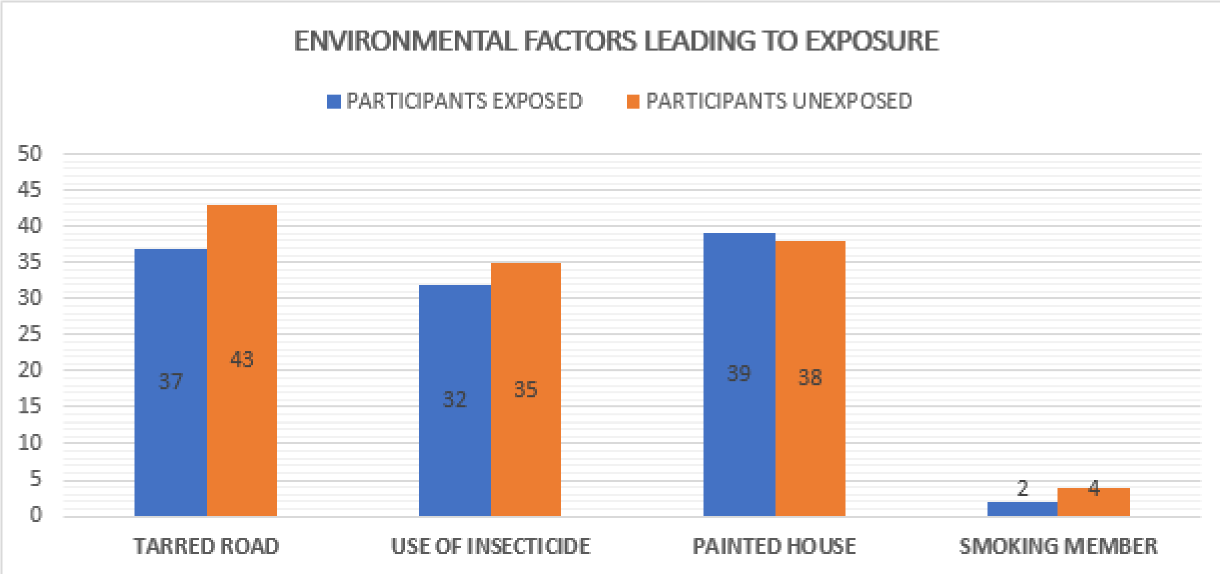
Table 3

Summary of correlation analysis of cord essential element and baby's anthropometric data

Correlating pairs		Total	Cases	Controls
		r,p	r,p	r,p
Baby weight	Cord Cu	0.113, 0.356	0.354, 0.065	-0.130, 0.418
	Cord Zn	**-0.002, 0.980**	**0.293, 0.039***	**-0.046, 0.740**
	Cord Ca	-0.062, 0.527	-0.071, 0.623	-0.087, 0.526
	Cord Mg	0.071, 0.470	0.013, 0.927	0.103, 0.453
	Cord Se	-0.031, 0.760	0.165, 0.290	-0.065, 0.643
Baby height	Cord Cu	0.145, 0.236	0.234, 0.230	0.030, 0.852
	Cord Zn	0.025, 0.797	0.181, 0.209	-0.032, 0.819
	Cord Ca	-0.036, 0.713	-0.089, 0.539	0.000, 0.999
	Cord Mg	-0.053, 0.591	0.016, 0.913	-0.054, 0.695
	Cord Se	-0.067, 0.512	0.097, 0.537	-0.133, 0.337
Head circumf	**Cord Cu**	**0.225, 0.063**	**0.478, 0.010***	**0.048, 0.766**
	Cord Zn	-0.023, 0.815	0.093, 0.522	-0.119, 0.386
	Cord Ca	-0.052, 0.595	-0.087, 0.550	-0.028, 0.837
	Cord Mg	-0.061, 0.539	0.091, 0.527	-0.072, 0.599
	Cord Se	-0.081, 0.433	-0.081, 0.604	-0.095, 0.494

*Significant at $p < 0.05$

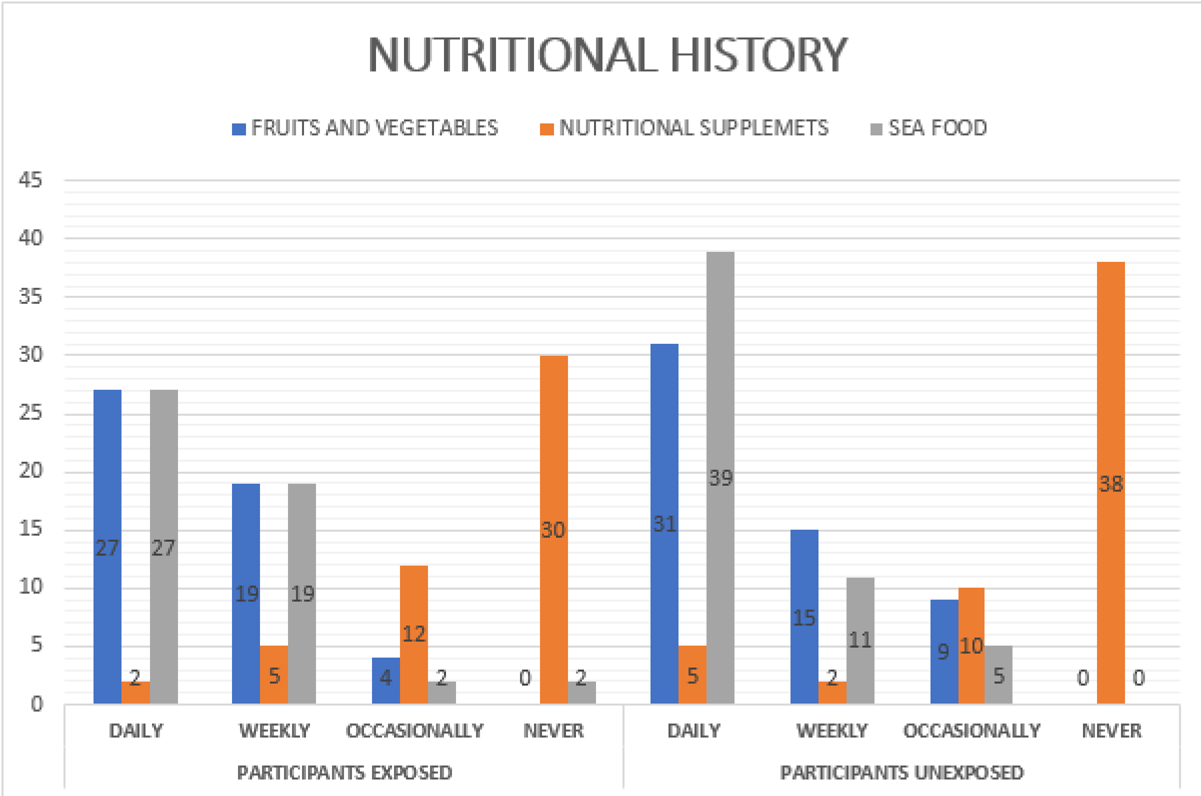
Figures



[Note: level of significance for all factors compared in the groups was P > 0.05 after subjecting to Chi-square test]

Figure 1

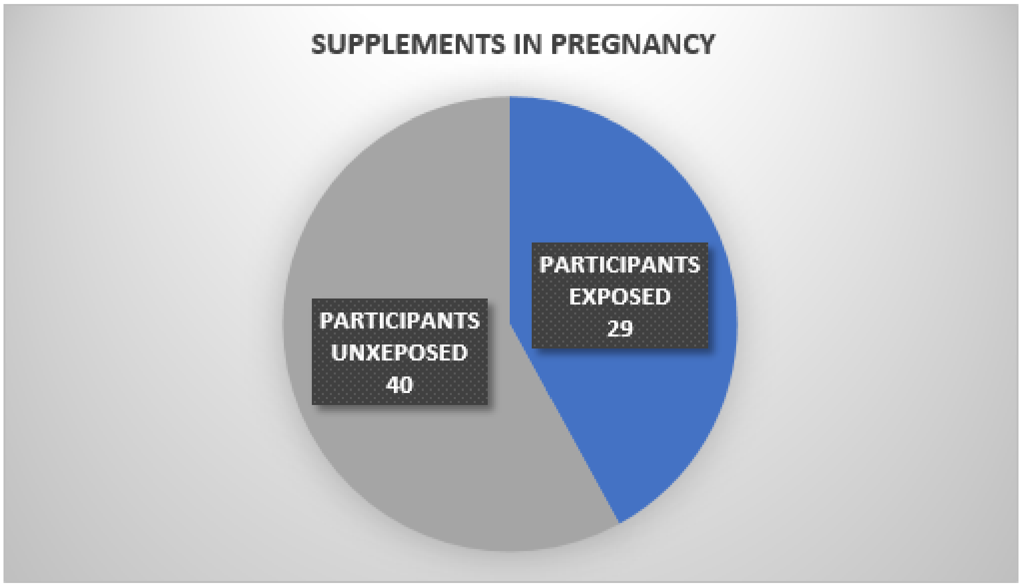
Bar chart representation of the environmental exposure to tarred road, house paints, insecticide and smoking



[Note: level of significance for all factors compared in the groups was P > 0.05 after subjecting to Chi-square test]

Figure 2

Nutritional history of participants (exposed and unexposed)



(Level of significance for all factors compared in the groups was not significant, P > 0.05)

Figure 3

Summary of consumption of supplements by the pregnant mothers

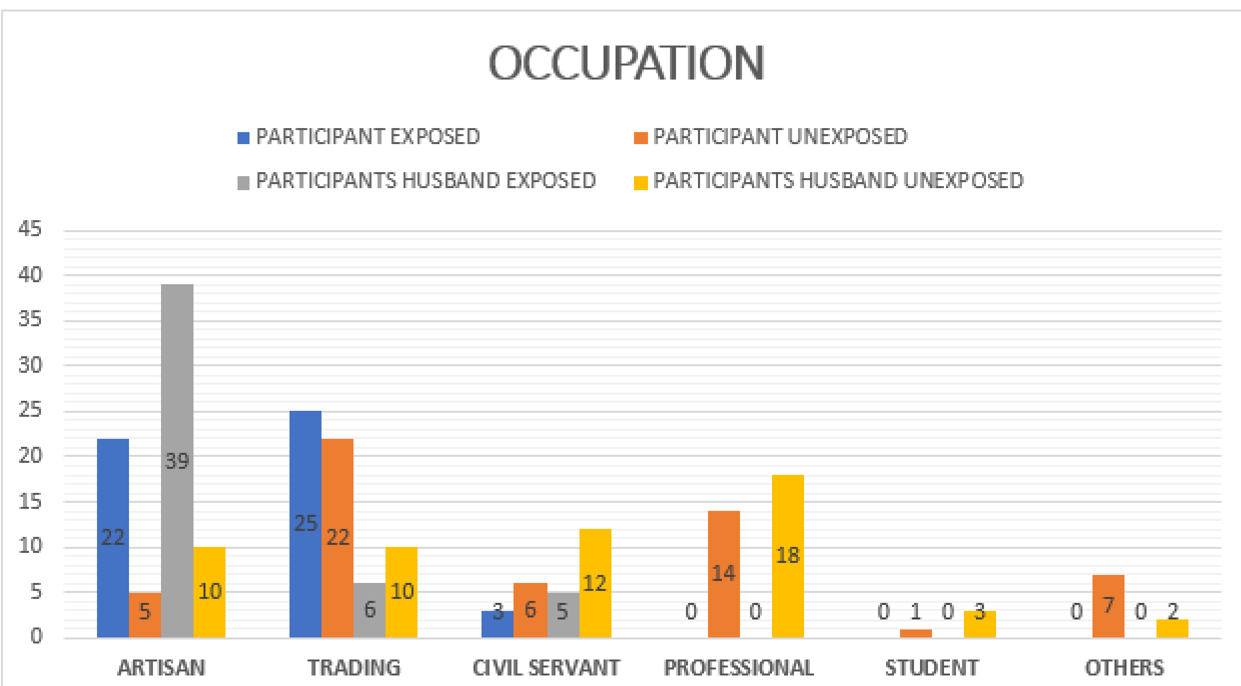


Figure 4

Chart summarizing occupation of participants in the study