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BLOOD DONATION AND HEAVY METAL POISONING IN DEVELOPING NATIONS: ANY LINK?

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Abstract

Long term health effects of heavy metal exposure from both occupational and environmental settings involve multi-organ toxicities including but not limited to disturbances of neurological, cognitive, and metabolic processes, immune system dysregulation, carcinogenesis and sometimes permanent disabilities. Humans are exposed to toxic metals through various sources and routes of entry. The risk of heavy metal poisoning from donor blood has been the subject of many scientific investigations. In this review we highlight how the access to a safe and adequate blood transfusion with minimal risk of toxic metals to recipients is a public health challenge, especially in developing nations. For quality assurance purposes, blood donors are screened for various blood-borne pathogens, but screening for toxic metal levels is not routine. Evidence from scientific studies used in this review lends credence to the risk of heavy metal poisoning from donors with high blood concentrations of these heavy metals. The risk of toxicity is exceptionally high in vulnerable populations such as neonates and preterm infants, as well as in pregnant women and other individuals with conditions requiring multiple blood transfusions. This is worse in developing countries where some members of the population engage in illegal refining and artisanal mining activities. In order to reduce toxic metal exposure in vulnerable populations, blood meant for transfusion in vulnerable subjects, e.g. children, should be routinely screened for heavy metal concentrations. Patients receiving multiple blood transfusions should also be monitored for iron overload and its attendant toxicities.

Keywords: Heavy metals toxicity; blood transfusion; health risk; developing nations; neonates.

1. Introduction

Several environmental toxic metals such as Lead (Pb), Arsenic (As), Mercury (Hg), and Cadmium (Cd) accumulate in living organisms without any degradation or catabolism [1]. They interact with biological molecules and other essential trace metals as a result of metal exposure from environmental pollution and also compete for absorption, dispersion, and accumulation stages in mammalian tissues [2]. Several countries have carried out studies to determine community exposure to environmental toxic metals to

examine changes over time, and establish reference values [3-6]. The adverse effects of some heavy metals are mainly seen during periods of life involving rapid growth and development, for example, *in utero* life and childhood [7]. Even low amounts of toxic metals transferred via maternal blood during pregnancy or by subsequent exposure may disturb neurological and cognitive development, resulting in permanent disabilities [8], and impair the immune system [9].

Blood transfusion is a life-saving procedure but may also represent a hidden source of heavy metal exposure for the recipients, particularly in vulnerable groups such as premature infants and neonates (first four weeks of life) who receive multiple blood transfusions. The various indications for transfusion in neonates include prematurity, neonatal jaundice, sepsis, and perinatal asphyxia [10]. For quality assurance purposes, blood donors are screened for various infections such as hepatitis B, hepatitis C, cytomegalovirus, syphilis, human immunodeficiency virus (HIV), blood grouping, and compatibility testing. However, screening for toxic metal levels is not part of the routine process [11]. In developed countries, recombinant human erythropoietin (rHuEPO) in replacement therapy is the ideal and commonly used alternative protocol to blood transfusion [10, 12]. However, in resource and technologically-deficient developing countries, blood transfusion is mostly used because the ideal is unaffordable and unavailable [13-14]. Aside from using erythropoiesis-stimulating agents, 85% to 90% of extremely low birth weight (LBW) infants receive blood transfusions [15-16]. There is still no universal screening of blood for heavy metals such as Pb before transfusion in vulnerable populations like extremely LBW infants and children receiving recurrent or massive blood transfusions (e.g., in trauma, extracorporeal membrane oxygenation, haemoglobinopathies, chronic hemolytic anaemias) [17]. Some institutions may require additional blood processing methods such as irradiation [18] and leukoreduction [19] before blood transfusion to neonates. Premature babies are at increased risk for anemia of prematurity due to reduced gestational age and resultant underdevelopment of the hematopoietic system [20]. Red blood cells (RBCs) transfusions are often used to manage the anaemia by increasing oxygen delivery to tissues [21]. They are given during the early weeks of life, which is a period of limited excretion via urine and stool and are associated with several adverse effects, including intraventricular

hemorrhage, necrotizing enterocolitis, retinopathy of prematurity and bronchopulmonary dysplasia [22-23]. This review aims at discussing how the access to a safe and adequate blood transfusion with minimal risk of toxic metals to recipients is a public health challenge, especially in developing nations.

2. Methodology

Multiple online searches were carried out in the databases of Medline, Pubmed and Google Scholar using terms like "heavy metals and blood transfusion," "toxic metals in blood," "metal toxicity and blood donation," "blood heavy metals," "blood transfusion and metal toxicity in developing nations," "blood metal overload," "heavy metals in sickle cell disease," and "heavy metal poisoning from blood donors." Sourced works of literature were screened, full texts were obtained, and inclusion and exclusion criteria were used to determine the suitability of articles used in this review. Studies included in this systematic evidence-based review were those published between 2010 and 2020, and transfusion carried out using only whole blood or other blood components like red cells, platelets, plasma, and serum in human subjects. Articles were excluded if toxicity was assessed using non-blood parameters, such as urine and if the articles were unavailable in English.

3. Results & discussion

3.1 Search results

One hundred and eleven studies were found in the initial search. After screening their titles and abstracts, 37 articles were excluded, leaving 74 articles for subsequent review. Exclusion of articles was based on being relevant or not, 23 articles were not relevant (n = 23), non-availability of full texts (n= 10), not available in English (n = 2), and duplications (n = 2). Application of exclusion and exclusion criteria for further review of the full texts of the remaining articles resulted in the removal of 13 additional articles, leaving 61 studies included in this review (Figure 1).

3.2 Evidence-based studies associating blood donation with heavy metal poisoning (2010-2020)

The concept "blood donors" includes donors of whole blood, red cells, platelets, plasma and other blood components, donated as whole blood and/or through apheresis [24]. In blood, metals can be found both in the non-cellular fraction (plasma and serum) and intra-cellular compartment (especially, erythrocytes) [25-27]. The affinity of metals for each component varies depending on their chemical properties, for example, Pb has strong affinity for erythrocytes [28] and 75% of Pb present in whole blood has been reported to be in the red blood cells [29]. Significantly higher concentrations of heavy metals in whole blood compared to that in serum have been reported in many studies [27, 30]. Table 1 summarizes some evidence-based studies linking blood donation with heavy metal poisoning. Red blood cells (RBCs) transfusions are often used to manage anemia but they may latently increase blood lead level (BLL) delivered to preterm infants and other vulnerable population [21].

Table 1. Studies linking blood donation with heavy metal poisoning.

A study involving 352 participants measured the concentrations of three heavy metals namely Pb, Hg and Cd alongside other environmental pollutants in donor blood from three Norwegian blood banks, and also evaluated the potential risk for exposure via blood transfusions in premature infants [7]. Estimations of tolerable intravenous doses of Pb in this study were based on the maximum concentration of 3.1 μg/dL for Pb in donor blood acceptable in neonates [31] and secondly on the lower concentration of intravenous Pb exposure for extremely LBW infants of 1.84 μg/dL [32]. This second threshold 1.84 μg/dL was predicated on i) the conservative assumption that except for Hg which is highly absorbed in the GIT, only 10% of an oral dose of heavy metal would be absorbed and converted to intravenous dose [33, 34], ii) the assumption of no other Pb exposure despite known Pb transfer through the placenta in utero and through breast milk, and iii) a provisional tolerable weekly intake (PTWI) of 25 μg/kg based on the 1996 WHO guidelines for drinking-water quality [17]. There is currently no safe level of Pb in children, and even the PTWI has been linked with the adverse health effect of a significant decrease of IQ in children [17]. Since only 10% of Pb is absorbed from the gastrointestinal system, a daily permissible dose from the weekly intake of 25 μg/kg will be 0.36 μg/kg or 1.84 μg/dL for the donor blood unit of 20 mL/kg commonly used in many neonatal

intensive care units [7, 34]. The acceptable limit for Hg used in this study was based on the value given by the USEPA which estimated the maximum daily intake of Hg unlikely to cause harmful effects during a lifetime for dietary methylmercury to be 0.1 μg/kg/day [35, 36]. However, based on the assumption that about 95% of methylmercury is absorbed from the gastrointestinal tract, the acceptable limit for Hg will be 0.095 μg/kg/day or 23.7 nmol/L (4.75 ng/mL) for the donor unit of 20 mL/kg [7]. For Cd, the intravenous tolerable dose used was that given by the European Food Safety Authority (EFSA) which gave the value of 2.5 μg/kg/week (22.25 nmol/kg/week) as the tolerable weekly intake, giving an estimated tolerable daily intake (TDI) of 0.357 μg/kg/day (3.18 nmol/kg/day) [37]. Based on the assumptions that only 10% of the oral dosage is absorbed, the daily intravenous tolerable dose would be 0.32 nmol/kg or 16 nmol/L (1.80 ng/mL) for the donor unit of 20 mL/kg [7]. Results showed that 4.5% of all blood donors had Pb levels higher than the maximum concentration of 3.1 μg /dL for Pb in donor blood acceptable in neonates and about 18% of blood donors had Pb concentrations above the maximum limit of 1.84 μg/dL suggested for transfusions in LBW infants. Also, about 10.5% of all donors had Hg concentrations above the suggested limit of 23.7 nmol/L, while 4% of donors had Cd levels higher than the 16 nmol/L suggested limit. In their evaluation of age as a possible criterion to select blood with the lowest lead concentration, calculations by these researchers of the Norwegian donor study showed that the optimal age is 22 years or younger, and 0% of donors in this age group had lead concentrations over the 1.84 μg/dL limit. By using the age cut-off of 40 years or younger, They also established that 5.2% donors aged 40 years or younger had BLLs over this limit, while 28% of those over 40 years had BLLs above the limit. They concluded that blood levels of environmental pollutants tend to increase with the individual's age. Based on their findings from this study, the researchers suggested that selecting young donors for transfusions or measuring the concentrations of heavy metals in donor blood prior to transfusion may be a feasible approach to prevent the exposure of vulnerable patients like premature infants to the adverse effects of these metals. However, it may also be appropriate to ask donors for known sources of exposure (e.g. occupational, dietary habits, smoking etc).

Another study evaluated the BLLs in a representative sample of blood donors involving 3490 participants (1392 women and 2098 men) in the Hema-Quebec population as well as identified the risk factors associated with BLLs of 3.1 μg/dL or more [38]. Previously, BLLs of at least 3.1 μg/dL was identified as the maximum level of Pb in a blood donation that could safely be given to pediatric patients [31]. The study reported BLLs of more than 3.1 μg/dL in 15.5% of participants. The difference in the levels was primarily based on sex and age and secondarily on other parameters like education level, location of residence, dwelling age, occupational and leisure activities, and smoking and alcohol intake [29]. In answer to the authors' question, "what impact will this have on a vulnerable population?" the health effects of Pb in children especially during the first years of life makes neonates a vulnerable and "at-risk" population. Neonates are particularly vulnerable because their brains will undergo a significant amount of growth and development outside of the womb and they are also comparatively Fe-deficient which may worsen the long term health effects of Pb poisoning as Pb is known to compete with Fe, Zn, and Ca.

In another study, White et al. [17] reexamined the concentrations and variability of Pb, Cd and Hg in donor blood to determine if screening donor blood for heavy metals is still warranted in vulnerable populations. The assay involved 192 samples of packed red blood cell units randomly selected from the University of Nebraska Medical Center Blood Bank, Omaha, Nebraska, United States and donated through two American Red Cross regional centers, 74 (Midwest) and 118 (North Central). The concentrations of these metals in donor samples were compared to mean blood concentrations from the 2013–2014 National Health and Nutrition Examination Survey (NHANES), designed to generally assess the US population, including measurement of heavy metals. Results of the study showed that mean concentrations of Pb, Cd and Hg in donor blood were higher than mean blood concentrations in the US population. Lead, mercury, and cadmium concentrations from the 192 donor blood samples were determined via a NexION 300D inductively coupled plasma mass spectrometer (ICP-MS). Mean lead concentration in the donor blood was higher (1.11 ± 0.75 μg/dL) compared to the test value of 0.97 μg/dL. Cadmium concentration was 0.49 ± 0.46 μg/L compared to the test value of 0.30 μg/L, while

mercury in donor blood was also higher at 1.01 ± 1.45 μg/L compared to the US population test value of 0.81 μg/L. As explained by these researchers, blood donors have higher levels of heavy metals than the general population because patterns of heavy metal exposure in donor populations may vary according to their geographic location. Different correlations may relate to the type of exposure to heavy metals (e.g., contaminated soil as in Environmental Protection Agency Superfund Site Omaha, Nebraska.

Additionally, the concentrations of Pb in donor blood were variable and greater in some regions than the national average, with over 14% of measured packed red blood cell units exceeding the limit of concern for extremely LBW infants. White and co-workers opined that extra screening protocols for donor blood in extremely LBW infants and in children receiving recurrent or massive blood transfusions may be warranted. Their report was based on a previously proposed acceptable concentration of intravenous Pb exposure for extremely LBW infants of 1.84μg/dL.

In a prospective cohort study, 54 premature neonates were evaluated for the direct effects of packed red blood cells (PRBCs) transfusions on neonatal BLLs at the Cairo University Pediatric Hospital in Egypt [20]. The study showed that the median neonatal BLL after transfusion was significantly elevated relative to before transfusion, and multiple transfusions resulted in elevated values of post-transfusion Pb levels. In addition, post-transfusion BLLs demonstrated a positive, moderate and significant relationship with neonatal weight, Pb level in blood packs, gestational age, and blood creatinine level respectively, while the neonatal BLL% change showed negative, significant correlation with neonatal age and number of transfusion times. The researchers concluded that preterm neonates were at risk of being exposed to the hazardous effects of Pb as a result of receiving several red blood cells transfusions, denoting that red blood cells had a significant load of Pb and Egyptian donors are exposed to high Pb levels every day. The disproportionate risks of Pb poisoning to this vulnerable population are due to some factors, for example, kidney function of preterm neonates in the first week of life predisposes them to increased Pb reabsorption [23]. Also, in LBW infants, the majority of Pb is not excreted in urine at the same rates as in older children. Additionally, Pb absorption is inversely proportional to chronological age and there is the tendency of Pb to deposit in other tissues such as brain, lung, liver, kidneys, bone, and teeth; hence

children tend to retain more Pb in soft tissues than adults [20]. Even small exposure to Pb can significantly affect neonatal neuronal growth and may culminate in irreversible changes in the preterm brain [39]

In another study, the effect of smoking on the concentrations of Cd, Ni and Pb in donor blood was determined by comparing their levels between two groups made up of 65 smokers and 65 non-smokers [40]. The study reported a statistically significant correlation between Cd, Pb, and Ni concentrations in the blood of smokers and non-smokers. Blood Cd concentration for non-smokers was 0.02600 ppm, while that in smokers was higher (0.07400 ppm). For Pb the concentrations were 0.40100 ppm (non-smokers) and 1.21400 ppm (smokers); Ni, 0.00500 ppm (non-smokers) and 0.02600 (smokers). Although these concentrations were within the acceptable reference values, cigarette smoking will result in cumulative toxicity of these metals since smokers are eligible to donate blood. They recommended that clinicians should take special precautions by screening for heavy metals to avoid transfusion of blood and blood products to neonates, the elderly, and people with renal diseases, if the concentrations of these metals are in the toxic range. Tobacco smoke had been reported to contain not less than 3500 chemicals including heavy metals, carcinogens, mutagens, free radicals, and radioactive materials [41, 42]. Smoking has also been implicated in premature hemolysis, reducing the normal lifespan of 120 days of RBCs to 80–85 days [43].

In their study, Sundararajan et al. [102] collected samples from 100 donor blood units from the University Hospitals Cleveland Medical Center blood bank over a period of 2 weeks and the blood analyzed for 9 heavy metals namely, Aluminum (Al), As, Beryllium (Be), Cd, Manganese (Mn), Hg, Ni, Pb and Polonium (Po). Of the 100 units, 31 had at least one toxic metal concentration high enough to cause potential adverse health effects. Levels of Al, Mn, Ni and Pb exceeded the acceptable maximum limit in 5, 11, 4 and 26 units respectively. The estimated maximum limit of acceptable metal concentration in donor blood was calculated based on the assumptions of using a transfusion volume of 20 ml/kg and using either previously published acceptable intravenous doses or oral doses with an estimate of 95% gastrointestinal absorption for Hg and 10% for other metals. These researchers suggested that with over

31% of the donor blood having metal concentrations above the estimated tolerable level which may pose serious health risk to very low weight infants, neonatologists should consider the potential for adverse effects to infants during blood transfusion. Often times, premature infants are given dedicated units of blood from a single donor thereby leading to the administration of several transfusions from the same donor unit or pack. This procedure reduces the risk of exposing this population to blood-borne pathogens and other foreign antigens. On the contrary, premature infants who receive multiple blood transfusions from one donor unit are likely to be exposed to the cumulative effect of heavy metals from that blood unit. They further suggested several strategies for identification of such donor blood units like the universal screening of blood from blood banks for heavy metals, targeted screening of blood units sourced from communities with high risk for elevated metal blood concentration and the use of screening questionnaire for the purpose of identifying donors with high blood metal concentrations prior to blood donation.

Another study which was the first of its kind evaluated fetal exposure to two heavy metals (Hg and Pb) via intrauterine blood transfusions at the University of Maryland medical center [44]. Blood biomarkers for in utero quantification of exposure to Pb and Hg as well as their toxic threshold concentration to the developing brain in fetuses are yet to be established. Fetal exposure was estimated based on transfusion volume and metal concentration in donor red blood cells, with each intrauterine blood transfusions considered an intravenous dose and calculated from oral reference doses based on 10% and 95% gastrointestinal absorption of Pb and Hg respectively [23]. The oral reference dose of Pb used to compare doses of Pb given with each blood transfusion was obtained from the higher oral Pb intake of 1.9 μg/kg/day, defined by the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives to be of concern as a result of a population decrease of 3 IQ points [45], while that of Hg was obtained from oral reference dose of 0.1 μg/kg/day approved by the USEPA [46]. Based on the respective 10% and 95% oral absorption of Pb and Hg, their estimated intravenous reference doses in children would be 0.095 μg/kg/day for Hg and 0.19 μg/kg/day for Pb [23]. Determination of volume of blood for each intrauterine blood transfusion was predicated on the measured fetal hemoglobin/hematocrit, the target

hematocrit of the fetus, the hematocrit of the transfused red blood cells, and estimated fetal weight (EFW). Identification of fetuses at risk for moderate-to-severe anemia was obtained from both hematocrit and middle cerebral artery peak systolic velocity. The procedure for intrauterine blood transfusion was carried out in the operating room under intravenous sedation, with the position of the fetus, placenta, and the cord insertion site on the placenta identified by ultrasound, and a 21-gauge needle introduced into the fetal umbilical vein at the placental insertion site. Screened, irradiated, leukoreduced and washed packed red blood cells were transfused via the needle into the umbilical vein of the fetus. Fetal heart rate was intermittently monitored by ultrasound. On completion of transfusion, a post-transfusion hematocrit was established from another fetal blood sample [44]. From their results showing a total of three pregnant women that received a total of 8 single-donor intrauterine blood transfusions, (n= 5, 2, and 1 intrauterine blood transfusion per fetus) for treatment of fetal anemia secondary to immune hemolytic anemia, Pb and Hg were present in all 8 donor packed red blood cell units. Multiple intrauterine blood transfusions resulted in elevated concentrations of Pb and Hg that could result in harmful consequences to the fetus. One out of eight intrauterine blood transfusions resulted in five times the estimated intravenous reference dose for Hg; one fetus who received 5 single-donor intrauterine blood transfusions between 20–32 weeks gestation had median Pb dose of 3.4 μg/kg (range 0.5–7.9 μg/kg); one donor unit had 12.9 μg/dL of Pb that resulted in a fetal dose of 7.9 μg/kg which is 40 times the estimated intravenous reference dose at 20 weeks gestation. The fetus is very susceptible to neurotoxins during periods of rapid neuronal cell division and proliferation. Development of the central nervous system is not complete until well into and beyond infancy [47]. Neurotoxins such as Pb and Hg may affect neuronal differentiation, proliferation, migration and myelination that can result in neurodevelopmental impairment and permanent neuronal loss [44]. One more study aimed at determining the whole blood and serum levels of toxic metals namely, Cd and Pb, trace elements (Fe, Zn, and Cu) of 211 non-exposed Turkish male donors as well as the identification of individuals living under the risk of chronic metal toxicity. Results revealed the average levels of Cd and Pb in whole blood, and the average levels of Zn, Cu and Fe in serum to be 1.27±0.88 μg/L, 25.1±12.44 μg/L, 0.97±0.16 mg/L, 1.10±0.21 mg/L, and 476.53±42.41 mg/L, respectively and

statistically significant association was found between smoking and Cd levels ($p<0.01$) [2]. Authors stressed the necessity of determining toxic heavy metals in blood donations intended for specific populations like children and patients with thalassemia, and also recommended that toxicologists and clinical chemists should always ascertain whether people have trace element deficiencies or have been exposed to higher concentrations of heavy metals.

4. Discussion

According to the World Health Organization (WHO), the primary purpose of a blood transfusion service is the provision of a safe, sufficient, and timely supply of blood and blood products. The purpose of blood donor selection is to ensure patient safety by collecting blood only from donors whose donations, when transfused, will be safe for the recipients [11]. Universal safe blood transfusion as canvased by the WHO focuses on five significant areas namely: i) development of nationally coordinated blood transfusion services, ii) collection of blood exclusively from voluntary non-remunerated blood donors, iii) quality-assured donation testing, iv) reduction of inappropriate clinical use of blood, and v) implementation of quality systems and standards [48]. In many countries, the national systems for selection of blood donors are not well-developed, and donor selection criteria are neither clearly defined nor uniformly applied [11]. In several other countries, the criteria for selection of donors are still predicated on tradition and customary practice rather than on evidence. Criteria from one country are often adopted in other countries without commensurate regard to the profiles of the general and potential donor populations, the current epidemiology of infections and diseases, local culture, and availability of appropriate resources [49, 50]. Although some countries take serious precautions for the selection of donors for the safety of blood products, donors and patients, policies for donor selection should consider the need for a balance between the safety and sufficiency of the blood supply and available resources [50-52]. There is scarcity of high quality evidence on which to base decisions on selection of blood donors. In some circumstances, the principles of evidence-based medicine to transfusion medicine were applied [49, 50, 53, 54]. However, many long-established donor selection criteria are based on medical knowledge of the disease process and

human physiology, the haemodynamic effect of blood donation and the potential for harm to either the donor or the recipient. Generally, the criteria for blood acceptance are based on conditions where there are no or minimal risk to donor or recipient, based either on published evidence of safety from observational studies or on general medical principles [11].

In sub-Saharan Africa, although blood transfusion is very important in the treatment of various pathological conditions such as anemia, obstetric hemorrhage, and trauma [55-57], access to a safe and adequate blood supply is still a public health challenge [58]. Sub-Saharan Africa is affected by a high level of pollution, e.g. by heavy metals, due to several anthropogenic activities such as artisanal mining and illegal refining, among others [59]. Extreme poverty makes awareness of toxic metals and their mechanisms of action low, including the transfusion-induced Iron toxicity. These aspects are discussed in the following sections.

4.1 Mechanisms of heavy metal poisoning

Several mechanisms of toxicity of some heavy metals have been postulated. They include the induction of oxidative stress/oxidation of biological molecules, carcinogenesis, neurotoxicity, and ionic or biochemical mechanism of toxicity [60]. Redox-active metals, such as Fe, Cu and Cr, undergo redox cycling (which means accepting an electron from a reducing compound and passing it on to H_2O_2 known as the Fenton's reaction), whereas redox-inactive metals, such as Pb, Cd, Hg and others cause depletion of the cells major antioxidants, particularly thiol-containing antioxidants and enzymes. Either redox-active or redox-inactive metals may result in an increase in the production of reactive oxygen species (ROS) such as hydroxyl radical (HO.), superoxide radical (O2.-) or hydrogen peroxide (H_2O_2) [61, 62]. Heavy metals react with the sulphydryl (thiol) groups of proteins or enzymes to form stable and secure bonds. The ionic form of these heavy metals bind the thiol groups (-SH group of cysteine and -SCH3 group of methionine) and replace the hydrogen and the methyl groups, leading to inhibition of the protein or activity of the enzyme [60]. The generation of highly reactive free radicals by some heavy metals may result in oxidative stress and other cellular damage [63]. Occurrence of oxidative stress is due to an imbalance between free radical

generation and the production of antioxidants to detoxify the reactive species or mitigate cellular damage. The mechanism of heavy metal toxicity is illustrated in Figure 2. For metal iron (Fe), this mechanism of toxicity involves the formation of hydroxyl radicals from superoxide or hydrogen peroxide (Fenton's reaction) which affects carbohydrate, protein and DNA [64] as well as the formation of lipid peroxides, the ROS with a longer half-life than hydroxyl radicals and higher capacity for chronic cell toxicity and DNA damage [65 The generation of free radicals by Fe is also postulated to be one of the leading causes of cancer [60]. Lead metal causes toxicity through the induction of oxidative stress by increasing the level of the ROS, while decreasing that of antioxidants and through lipid peroxidation [66, 67], and this may cause damages to proteins, cells, nucleic acid and membrane phospholipids [68]. Lead also exhibits its toxicity via an ionic mechanism which involves its replacement of divalent cations like Zn2+, Ca2+, Mg2+, Fe2+ and monovalent cations like Na+, thereby upsetting cellular metabolisms such as enzyme regulation, neurotransmitter release, cell adhesion, intra- and inter-cellular signaling, protein folding, maturation, apoptosis and ionic transport [66]. Generation of ROS by Pb, which may cause DNA damage, disruption of DNA repair system, and cellular tumor regulatory genes, are reported as possible mechanisms of Pb-induced carcinogenesis [69]. Lead impairment of memory and learning occurs through its inhibition of N-methyl-d-aspartate receptor (NMDAR), inhibition of neurotransmitter release, blocking of neuronal voltage-gated calcium (Ca2+) channels and reduction in the expression of brain-derived neurotrophic factor (BDNF) [60].

Mercury has been shown to induce malignant growth through the generation of free radicals as well as disruption of DNA molecular structure [70]. It binds to sulfhydryl groups and inhibits critical enzymes involved in cellular stress response, protein repair, and prevention of oxidative damage [71]. Methylmercury inactivates sodium-potassium adenosine triphosphatase (Na+/K+-ATPase), leading to membrane depolarization, calcium (Ca) entry, and eventually, cell death [72] and also inhibits the muscarinic cholinergic systems in the brainstem and occipital cortices [73]. Mercury induction of red blood cells adhesion to endothelial cells may be the potential mechanism for the initiation and propagation of Hg-associated cardiovascular disease and thrombosis [74, 75]. Several cell surface and

membrane changes may contribute to this adhesion, for example, an enhanced phosphatidylserine exposure on the outer surface of the red blood cell membrane has been linked to the abnormal red blood cell adhesion to endothelial cells *in vitro* in the presence of Hg toxicity [74]. Sulfatides on the red blood cell membrane bind to a sub-endothelial matrix protein, laminin, with high specificity and affinity and have been reported to enhance red blood cell adhesion in sickle cell disease [75].

Arsenic has been shown to 'generate free radicals like superoxide (O2•–), singlet oxygen (1O2), nitric oxide (NO•), hydrogen peroxide (H2O2), the peroxyl radical (ROO•) [76], dimethylarsinicperoxyl radicals ((CH3)2AsOO•) as well as the dimethylarsinic radical (CH3)2As•)' [77]. Its carcinogenesis mechanisms include 'epigenetic alterations, damage to the dynamic DNA maintenance system, and generation of ROS' [78, 79]. Its malignancy has also been attributed to alterations targeting DNA methylation, histones, and miRNA [79]. The risk of As-induced carcinogenesis has also been attributed to its ability to bind DNA-binding proteins and cause disruption in DNA repair processes [60, 80].

Cadmium, another heavy metal, has been postulated to exhibit its toxicity by causing apoptosis, oxidative stress, DNA methylation, and DNA damage [60]. Another mechanism of cadmium toxicity is its ability to replace other ions such as zinc (Zn) and Ca from Zn-finger proteins and metalloproteins [60, 81, 82], for example, the replacement of Zn by Cd in certain dehydrogenating enzymes can render them inactive. Like Pb, Hg, and As, Cd has been reported to disrupt the refolding of proteins, thus preventing the misfolded protein from being resuscitated by reduced glutathione or EDTA chelator [60, 83]. Other metals like Copper (Cu) [84], Chromium (Cr) [85], Cobalt (Co) [86], Vanadium (V) [87], and Nickel (Ni) [88], have been reported to exhibit their mechanism of toxicity via the formation of free radicals which contribute to carcinogenesis.

4.2 Health effects of exposure to some heavy metals

In developing countries, the adverse effects of heavy metal exposure from both occupational and environmental settings are of immense public health importance [59]. In South Africa, maternal and umbilical cord blood from occupants of selected geographical areas (coastal, rural, urban and industrial sites) showed very high concentrations of Pb, Hg, selenium (Se), and Cd [89]. Pollution by

heavy metals and other chemicals is widespread in sub-Saharan Africa [90]. Human exposure to toxic metals occurs via food, water, air, or industrial settings [91]. High concentrations of Pb, Cd, and Ni in some fruits and vegetables have been reported in Nigeria [92]. High concentrations of heavy metals, namely Hg, Antimony (Sb), and Tin (Sn), were also reported in some herbal remedies sold in Nigeria [93]. Natural and anthropogenic sources such as the use of leaded petrol, improper waste disposal, artisanal mining, illegal refining, burning of toxic waste as well as industrialization and urbanization form their major points of entry into the environment [9, 94, 95]. Due to the high level of pollution in several African countries, the current concentrations of different metals in food, water, soils, fish, and vegetables are above international limits [96]. Long-term exposure of humans to some heavy metals may cause cancer [97]. Several elements such as Pb, Ni, Cd, and Cr are known for their systemic toxicity, causing multiple organ damage, even at minimum exposure levels [98] and are equally classified as either proven or potential carcinogens to humans according to the US Environmental Protection Agency (USEPA) and the International Agency for Research on Cancer (IARC) [99, 100]. In South Africa, exposure to dust from gold mines was associated with lung, liver, esophageal, and lymphatic system cancers in miners [101, 102]. Nickel mining was also associated with the risk of lung cancer in Zimbabwe [103]. Several heavy metals are known to be multi-organ/tissue toxicants. Lead poisoning may cause nephrotoxicity and neurotoxicity and affect heme synthesis [104]. About 95% Pb is accumulated in red blood cells [105]. Young children are particularly at higher risk of Pb because they have the tendency of taking particles from the ground to their mouths, a condition known as *pica* and due to their ability to absorb a higher amount of ingested Pb than adults [106]. Even blood lead levels (BLLs) less than 5 ug/dL have been reported to cause developmental problems such as impaired cognition, behavioral difficulties, impaired hearing, and growth retardation in infants and children [106]. Epidemiologic studies have shown that in children below five years of age, low-level Pb exposure (5–25 μg/dL in the blood) resulted in intellectual impairment manifesting as loss of intelligence quotient points [107].

'Exposure to high levels of inorganic, organic and metallic Hg can damage the kidney, brain and developing fetus, while methylmercury is highly carcinogenic' [60, 108]. Mercury has been reported to

cause enzyme denaturation [109], and red blood cell adhesion to endothelium [74]. In pregnant women, exposure to Hg can affect the fetus by causing 'mental retardation, cerebellar symptoms, and retention of primitive reflexes, malformation, and other abnormalities' [110]. Mercury may cause neurotoxicity and adversely affect the immune and digestive systems [111]. Cadmium may cause lung cancer, renal tubular dysfunction, bone diseases, diabetes, breast cancer and prostate cancer [112-114]. Chromium exposure has been reported to result in dermatitis and allergic skin reactions [115]. Nickel was reported to cause asthma, carcinogenesis, allergic contact dermatitis, oral cancer, as well as reproductive toxicity [116, 117]. Long-term exposure to As can result in 'skin lesions, pulmonary disease, some cancers (e.g. of the bladder, lung, skin, liver), neurological problems, peripheral vascular disease, diabetes mellitus, hypertension, and cardiovascular disease' [118].

4.3 Transfusion-induced Iron toxicity

Many patients receive multiple blood transfusions, particularly those with sickle cell disease, leukemia, thalassemia major, myelodysplastic syndrome, aplastic anemia, hemolytic anemia and refractory sideroblastic anaemias, resulting in the accumulation of excess Fe in various tissues which may cause mortality and morbidity [119, 120]. Patients receiving multiple red blood cell transfusions inadvertently develop cumulative Fe overload and are at risk of developing Fe toxicity [121, 122]. Transfusion of red blood cells delivers 1 mg per mL of additional elemental Fe. Multiple blood transfusions of 20-units of a 220 mL per unit RBCs/year are associated with a significant Fe overload of 4400 mg exogenous Fe/year [121, 123]. Iron toxicity is caused by the accumulation of non-transferrin bound iron (NTBI) as free Fe in tissues due to Fe overload, resulting in organ dysfunction [121, 124]. Diagnoses of Fe overload are usually predicated on the results of liver or myocardial biopsy, use of a superconducting quantum interference device, serum ferritin, transferrin saturation, the measurement of urinary Fe 24 hours after Fe-chelating therapy, magnetic resonance imaging, and/or major organ function [125]. Non availability of early symptoms or non-specific early symptoms of Fe toxicity namely, fatigue and abdominal discomfort may cause delay in diagnosis until clinical manifestation of serious organ damage or dysfunction [27].

Following Fe overload from chronic transfusion, the free Fe accumulates in macrophages when Fe storage proteins are saturated. Subsequently, the free Fe is deposited in parenchymal cells of the heart, liver, pancreas, and endocrine tissues which have greater capacity for safe storage of Fe than macrophages [27, 126]. This overload from regular blood transfusion sometimes cause damages to the heart, liver, pancreas, thyroid, and other endocrine glands [26]. In the liver which is the major organ for storage of Fe, the excess free Fe is deposited in Kupffer cells and hepatocytes causing swelling and rupturing of the mitochondrial membranes and eventually, cell death, fibrosis/cirrhosis and hepatocellular carcinoma; in the heart, the Fe permeates cell membranes of cardiomyocytes thereby circumventing normal homeostatic mechanisms [27, 127, 128, 129], with resultant congestive cardiomyopathy [130]. Commonly affected endocrine structures include 'pancreas (diabetes mellitus), anterior pituitary (growth hormone deficiency with short stature), testes/ovaries (hypogonadism with delayed puberty and infertility), thyroid (hypothyroidism), parathyroids (hypo-parathyroidism), and adrenals (adrenal insufficiency)' [131, 132]. In addition, Fe overload cause impairment in the proliferation of erythroid progenitor cells, which may aggravate the already poor hematopoietic function [132]. In sickle cell disease, transfusion is a commonly employed therapy, particularly in the treatment of stroke, preoperative prophylaxis as well as treatment and prophylaxis of acute chest syndrome [123, 134, 135]. Knowledge of Fe toxicity in sickle cell disease is of great importance because patients who are over loaded with Fe due to multiple transfusions have significantly higher mortality than those who are less transfused without Fe overload, in addition to age and race-matched normal controls [123, 135, 136]. In order to avoid complications associated with Fe overload, unnecessary transfusions should be avoided or minimized in patients with sickle cell disease [123]. Organ damage due to Fe overload in these patients is under recognized, partly because the impairment is often attributed to the disease itself and because of the lack of familiarity with Fe overload by some caregivers [137].

In a retrospective study, Gao et al. [125] evaluated the clinical outcome of transfusion-related Fe overload in 13 patients who had received more than 50 units of red blood cells for over one year. They aimed at determining the degree of Fe overload and the efficacy of Fe-chelating therapy.

Their result showed that all patients had high serum ferritin levels ranging from 1,830 ng/mL to 5,740 ng/mL and ten patients had abnormal liver function. Eleven patients had significant increase in CT Hounsfield units in the liver. Nine patients had increased skin pigmentation, liver and endocrine dysfunctions with serum ferritin >3,500 ng/mL, eight out of these patients have since died. However, following Fe-chelating therapy, there was no significant decrease in nine of the transfusion-dependent patients. They concluded that patients who depend on multiple blood transfusions may progress to secondary Fe overload that may result in organ impairment with fatalities in those who are heavily overloaded with Fe. In another observational cohort study of 139 children with acute leukemia, the prevalence of transfusion-related Fe overload, and its impact on end-organ function were evaluated. Measurement of ferritin in 68% of the 139 patients in their study showed that 23% of this measured population had elevated ferritin levels (>1000 mcg/L). Endocrinopathies were the most common end-organ abnormality and liver dysfunction was significantly higher in patients with ≥10 red blood cell units transfused compared with those with <10 units [120]. The authors recommend routine screening for transfusion-related Fe overload in children with leukemia, potentially at risk of multiple transfusion burdens.

Iron chelation therapy is used for treatment of Fe toxicity and to prevent harmful accumulation of Fe through matching of Fe intake from blood transfusion with Fe excretion via chelation [124]. It may however take several months or years to reduce serum ferritin to a safe range [138]. Liver and cardiac transplantations 'should be considered in patients with end-stage disease' [119]. While Fe that is bound to the liver can be chelated to a certain degree, the Fe bound to other organs such as the heart is not readily chelated, hence, cardiac failure is known to be the major cause of death in patients who undergo long-term blood transfusions [139]. The computed tomography (CT) Hounsfield unit is a sensitive biomarker of Fe overload in the liver. Iron chelation therapy should be started when serum ferritin is >1,000 ng/mL and therapy continued until it is <1,000 ng/mL in patients overloaded with Fe following blood transfusion [125]. Deferoxamine is commonly used for Fe chelation and is administered via subcutaneous or intravenous routes due to its short half-life and poor oral bioavailability, leading to low level of

compliance [140]. Deferiprone and deferasirox are two examples of orally administered Fe chelators used in the treatment of Fe overload [119].

5. Conclusion

In conclusion, from reasonable evidences obtained from scientific studies discussed in this review, blood transfusion has been revealed as a source of heavy metal poisoning from donors with high blood concentrations of these heavy metals. The risk of toxicity is especially high in vulnerable populations like children (neonates, preterm infants), pregnant women, and other individuals with conditions requiring multiple blood transfusions. This is made worse in developing countries where inhabitants engage in rudimentary, illegal refining and artisanal mining activities due to extreme poverty and low level of awareness of the adverse health implication of toxic metals. Additionally, poor healthcare system and regulatory lapses have exacerbated the existing precarious effects of metal poisoning in sub-Saharan Africa and other developing nations. In order to reduce toxic metal exposure in vulnerable populations and given the widely acclaimed no safe blood levels of some of these metals, blood meant for transfusion in children should be routinely screened for heavy metal concentrations especially in high risk donors who can be identified from administration of pre-blood donation questionnaires. Patients receiving multiple blood transfusions should also be routinely screened and monitored for Fe overload and its attendant toxicities.

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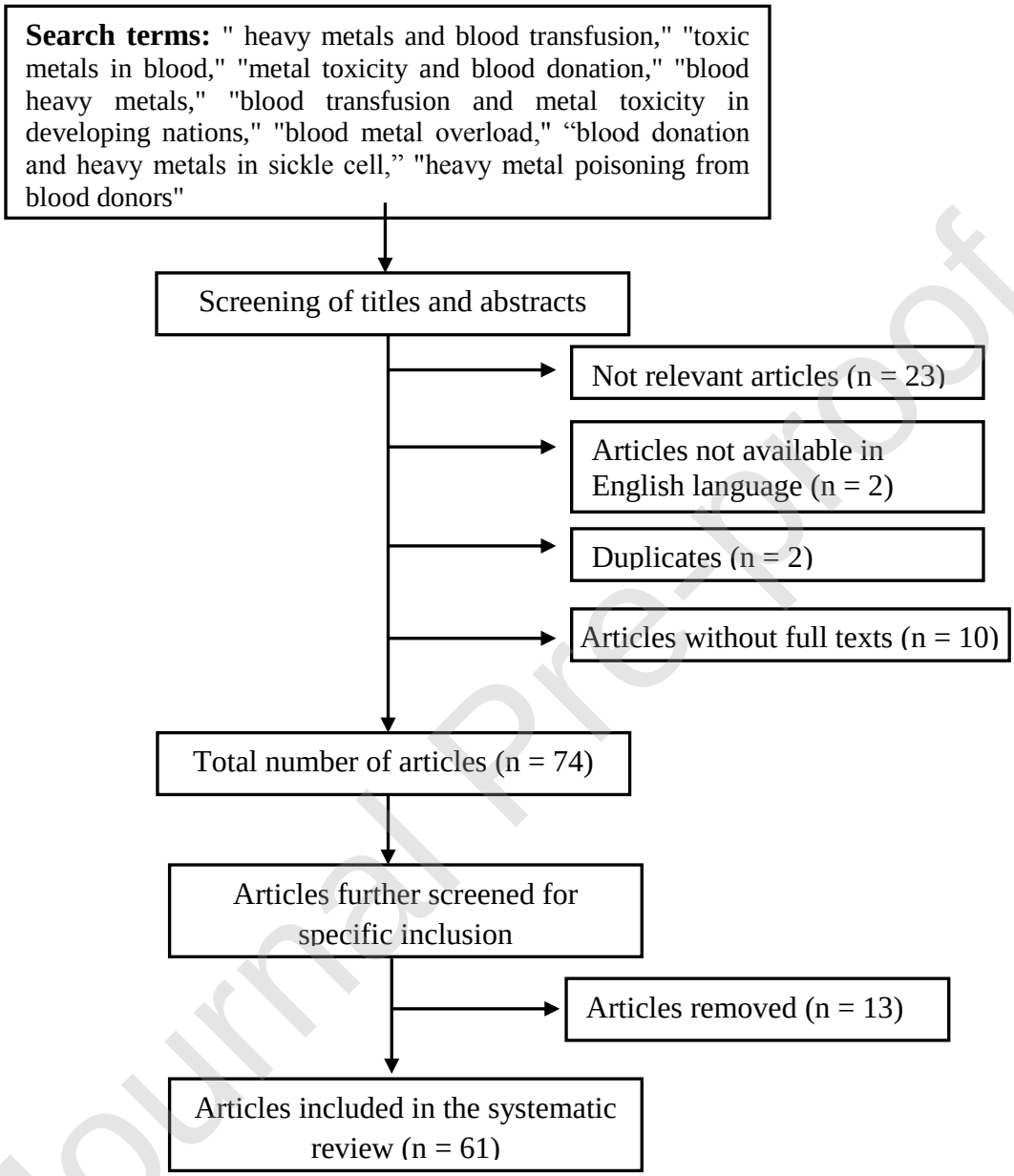

Figure 1. Flow diagram of study selection

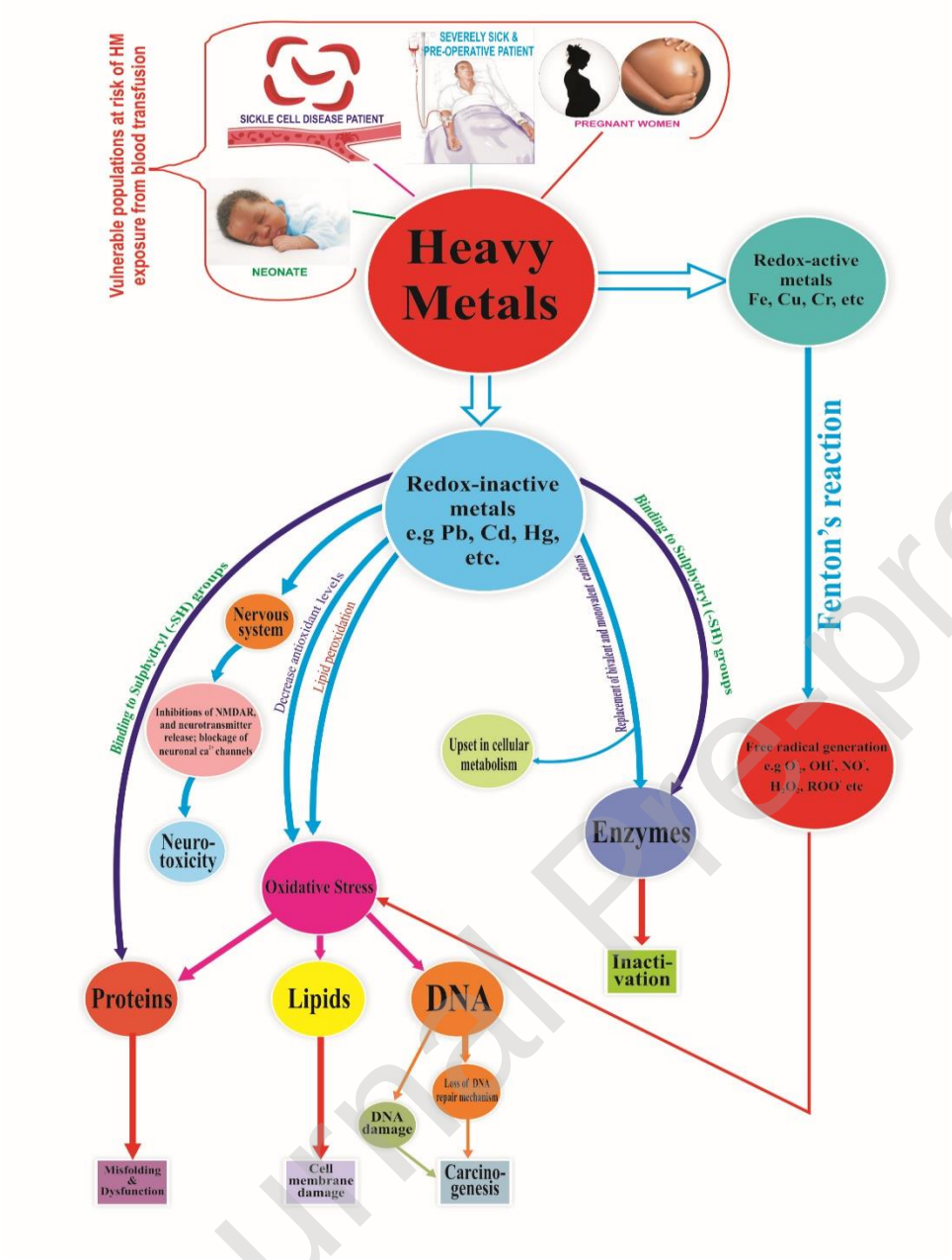

Figure 2. Mechanisms of heavy metal toxicity.