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ADAPTED HEIGHTS OF MARKERS FOR OXIDATIVE TRAUMA CAUSED BY LEAD TOXICITY ARE IMPERATIVE IN VERDICT AND FORECAST OF ANEMIA

***MUHAMMAD WAQAS[1], SHOAIB ASIF[1], HAROON HABIB[2], MAYAM MUNAWAR[1], RABBIA TARIQ KHAN**

1: School of Pharmaceutical Sciences, Johar Institute of Professional Studies (JIPS), Lahore. Pakistan

2: Avicena Medical College, Lahore, Pakistan.

***Corresponding Author: Muhammad Waqas (PhD): E Mail: waqaskhanjips@gmail.com**

Cell: +923213535005

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ABSTRACT

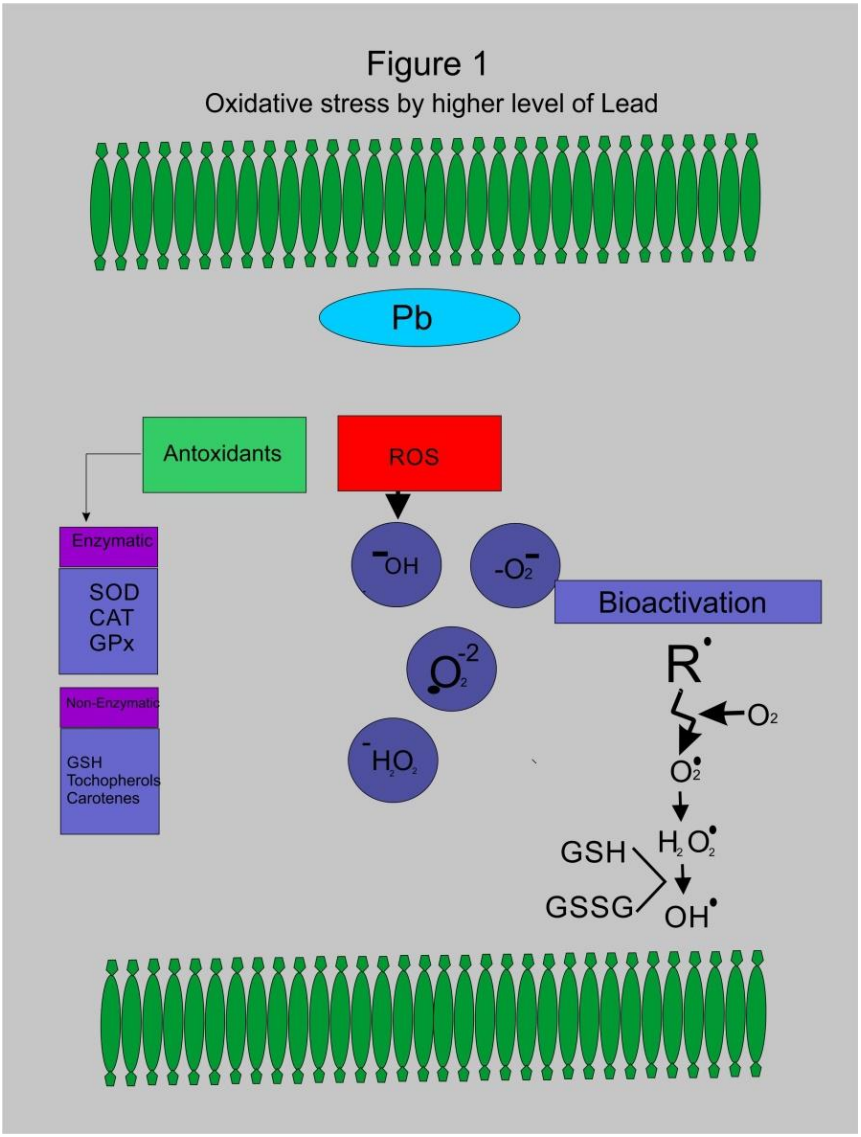
Pb is an early toxicant under the class of environmental pollutants; it has deleterious effects on human being in higher concentration and has no biological role exclusive for human being, it enters inside the human body through different motives but mainly through absorption through GIT, it interplay with variety of intracellular pathways and develop oxidative stress which subsequently plays a malicious role in damaging proteins lipid and DNA. Intracellular damaged me lead to development of carcinogenicity but its major role is to interaction with signaling pathway of heam synthesis, Pb is reported to inhibits all most all steps toward heam production n including this it also cause premature destruction of RBCs result in development of anemia, changed levels of biomarkers, particularly MDA and 4 HNE, of oxidative stress can be helpful in diagnosis and prognosis of anemia by oxidative stress.

Keywords: Oxidative stress, Anemia, Lead Toxicity, MDA, 4 HNE

INRODUCTION

1. Oxidative stress

Living organisms are entirely reliant on aerobic circumstances, the main mechanism on which scientists differentiate between alive and non-livings are the practice of respiration **[1, 2]**. Respiration is an adaptive process given to human by nature. Respiration is the property of living one as unit or at cellular level. During the process of cellular respiration a lot of free radicals are produced, free radicals are oxygen caring molecules having only uneven number of oxygen, **[3, 4, 5],** which permit them to react easily with other molecules and may result in worsening the physiological procedures **[6, 7]**. To preserve it at normal position nature developed various anti-oxidant defense mechanisms in human beings to neutralize the un-usual free radical before they attack the nearby product **[8].** Oxidative stress is the phenomena when there is discrepancy between the production of these free radicals by the cell and their neutralization, by defense mechanisms. The imbalance may be due to over production of free radicals by the cell or may be due to failure in working of anti-oxidant shield system **[9, 10]**; these free radicals are also branded as reactive oxygen species (ROS). ROS are generated within the cell by different pathways, e.g. As byproducts of normal respiration or may be as second messenger in a variety of signal transduction processes, **[11]** they can also yield from exogenous springs**[12, 13]**. The ROS which produce endogenously are mainly finished through escape from electron transport in mitochondria **[14, 15]**. The exceeded quantity of ROS are unsafe for the cell itself in which it produced as well as the neighboring cell as it may attack the macromolecules (proteins, lipids and DNA) causes their oxidation, which may lead to tissue injury and subsequent inflammation which are the basis of many diseases. To set the quantity of ROS two types of defense systems are established with in the cell, one is enzymatic which includes SOD, CAT, and GPx while other non-enzymatic include GSH, tochopherols and beta carotenes, when the anti-oxidant system is defenseless the activation of ROS will be enhanced result in disruption of hemostasis and subsequently the compromised physiological functions of different amino acids, nucleic acids and lipids **[16]. Figure 1.**



1.1 Effect of Oxidative stress on Proteins within cell

Effect of oxidative stress on protein may cause division of amino acid chains, protein-protein cross linkage and outbreak on protein backbone, these smashed proteins affect intracellular pathways leading to serious diseases **[17],** several assays established to notice biomarkers of oxidative protein damage, Nitro-Tyrosine is a product of ROS mediated tyrosine damage, is a biomarker for sensing inflammatory responses **[12,18, 19, 20]**. Similarly protein carbonyl group are formed on proteins flank chain during oxidation **[21]**, higher levels witnessed in rheumatoid arthritis**[22, 23]** ROS cause fragmentation by setting cleavage of peptide bonds either by diamide or by alpha amidation pathways**[24].** ROS may also cause oxidation of side chain especially

sulfur containing amino-acids cysteineand methionine are sensitive, under insignificant conditions cytosine and methionine are converted to di-sulphide and sulfoxide residue respectively **[25].** Most biological system comprises reductases which can repair both cysteine and methionine in to their unmodified form by using enzymes di-sulphide reductase and methionine sulfoxide reductase, this is the only stresses oxidation which is reversible in biological system other than this oxidation by ROS produces deleterious effects on protein and may change the usual pathways leading to inflammation and tissue injury **[26, 27].** Oxidative attack on polypeptide backbone is also initiated by ROS the OH needed for this reaction is obtained by radiolysis of water or by metal catalyzed cleavage of H_2O_2 **[28, 29].**

1.2 Effect of Oxidative stress on Lipids within cell

Lipids are grouped as polar and a-polar, polar are organizational constituents of cell membrane and function as fence, while later one store in various cells exclusively in adipose tissue **[30, 31]**. Lipids also play a substantial role as signaling molecules. One of the concerns of uncontrolled production of ROS is lipid peroxidation, results in creation of a large no of adducts that hamper with normal signaling pathways results in alteration of signaling pathway, adaptation may be over excitation, slow progression stoppage or change of pathways. The most destructive ROS are (OH) and (OH_2). The (OH) is a short lived molecule produced from O_2 in cell metabolism under a variety of stress conditions **[32],** usually a cell produces 50 (OH) molecules every second **[5]** which may be neutralizes in normal physiological condition by anti-oxidant system but in stress it may attack proximate molecules disturbing innumerable signaling pathways**[3]**. The uncontrolled production of (OH) may cause cancer **[33, 34]** neurodegenerative disorder **[35, 36]** and cardiovascular diseases **[37]**. The overall process of lipid peroxidation is consisting of initiation propagation and termination steps **[38, 39, and 40]** Lipid peroxidation products which formed due to attack of ROS are known as primary lipid products. Primary products produced during propagation step **[39]**, this hydro peroxide moiety get attached to other lipid structure e.g. free fatty acids, triglycerols and sterols **[41, 42]** This conjugation may result in formation of secondary aldehyde product e.g. malondialdehyde (MDA), Hexanal, Propanal, 4-hydroxynonenal (4-HNE). Among these MDA is most mutagenic and 4-HNE is most toxic moiety **[43]**. MDA is most convenient biomarker for lipid peroxidation of omega 3

and omega 6 fatty acids **[43, 44]** MDA may produce enzymatically during synthesis of thromboxane A2 (metabolite of archidonic acid) **[45, 46],** used as inflammatory biomarker, and it may also produce none enzymatically from hydro peroxide product during lipid peroxidation in propagation step **[40, 44, 47, 48]** Once MDA is formed it causes tissue damage by disturbing protein or by attacking DNA. It has been proposed that MDA react with nucleosides such as guanosine and cytidine and altered their fate producing mutation within the cell **[49, 50]**. 4 HNE was discovered back in 1960 **[51]** while in 80s reported as cytotoxic Benedetti 1980. 4-HNE is generated by decomposition of archidonic acid and poly unsaturated fatty acids (PUFAs) having three functional groups is a highly reactive moiety **[39, 40]** 4-HNE is most extensively studied lipid peroxidation product and known for its cytotoxic effect, its effect may cause inhibition of gene expression by interfacing with translation step and development of many pathological states **[38].**

1.3 Effect of Oxidative stress on DNA within cell

Oxidative destruction by ROS is reason for DNA modification, single and double bond breaks and formation a-purinic or a-pyrimidinic lesions which may reveal the toxic and mutagenic properties **[52, 53]** therefore ROS not only implicated in disease development but the DNA impairment resulting from ROS outbreak may also liable for lethal biological consequences **[54, 55, 56]** 8-hydroxy guanine which is mutagenic is usually elevated in cancer cells, to control geno-toxic stress, cells have a number of mechanisms to repair or stand DNA damage **[57, 58].**

2. Environmental poisoning by heavy metals

Heavy metals are elements that have more density as compare to water **[59].** Heavy metals are kept in environmental pollutants class as of their toxic effect on plants animals and human being, heavy metal poisoning may outcome from mining, smelting, operation and agriculture happenings **[59, 60, 61]**, these activities may cause intense increase in the levels of heavy metals such as Cd, Cr, Co, Pb, and Ni in the environment**[62. 63, 64]**, heavy metals are persistent in nature so that they may get collect with soils plants and water and may destructive for aquatic life as well **[65].** In living system heavy metals are reported to be affect at cellular level **[66],** affect the organelles that involve in metabolism and damage repair, their deleterious effects may results in cell cycle disturbance, DNA damage signaling disturbance and apoptosis

[67, 68]. Several studies prove that heavy metals are responsible for production of ROS which may cause oxidative stress **[69, 70, 71, 72, 73, 74, 75, 76, 77, 78]**.

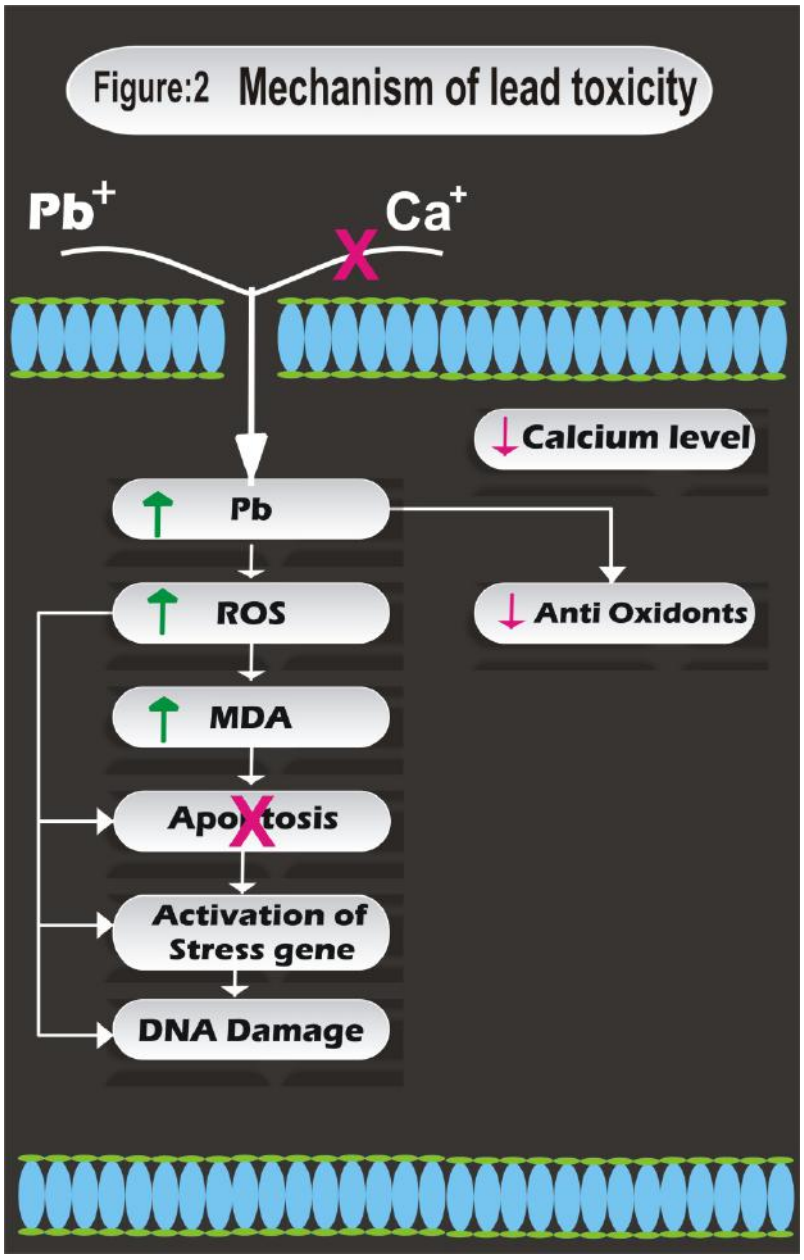
2.1 Lead as Environmental pollutant

Lead is a naturally accruing metal as part of earth crust in lesser amount, along with, this bluish grey metal **[79, 80]** may release due to anthropogenic activities in a large quantity, such as fossil fuel burning, mining, smelting and manufacturing **[81, 82]**. As it has wide range of industrial, agricultural and domestic application so produce in environment in a very high quantity, Pb environmental level is on regular increase as compare to previous era **[81]** Currently it is used in manufacturing of lead acid batteries [**83, 84]** and devices to shield X- Rays **[85],** In current years the industrial use of lead has been reduced meaningfully from paint and ceramics products **[86]**, despite this progress 25 % of the homes present in USA are still contaminated by lead through lead products and pay toward higher blood lead level. **[87, 88]** reported that this percentage is quite high in Asian and African countries. Lead reported at high level especially in children who play at bare contaminated soil **[89].** Today the largest source of lead toxicity is from dust and chips from deteriorating lead pain and interior surface, these children may contain plasma lead concentration about 20ug/dL or greater **[90]**. Exposure to lead mainly occurs via inhalation and ingestion **[91, 92]**, adult absorb 35% to 50% lead through drinking water while in children this rate is more than 50% **[93]**. Once it absorbed it must have its pathological role inside the body as it has no physiological role inside the body, it mainly damaged, kidney, hematopoietic system, liver and soft tissue, brain is the most defenseless target for its poisoning **[79]**. Lead has the ability to cross placental barrier so developing fetus is also under considerable risk due to repeated exposure of pregnant women to lead in daily routine life **[94]**.

2.2 Mechanism of lead toxicity and carcinogenicity

Lead applies its toxic influence by hindering the calcium ion and to interact with protein **[91]**, lead replaces calcium in minerals class of Skelton, binds to biological molecules and obstructs with functioning by number of mechanisms. Lead also races with other cations at receptor site inhibiting enzyme activity and altering the transport of other ions **[95]**. Recent studies suggest intoxication of lead at cellular level result in production of ROS. This higher amount of ROS results in elevated level of MDA and decreased level of Anti-oxidants **[96]**, as described in figure

2, Recent studies also suggest that lead intoxication may several altered molecular processes including inhibition of apoptosis **[97, 98]**, transcriptional activation of stress gene **[71, 72]** molecular alteration at DNA, DNA damage and mutation **[99, 100]**. It starts with the beginning of oxidative stress, **[101, 102, 103]**, which produced in two ways, first is generation of ROS and second is depletion of anti-oxidants reserves in the cell **[104, 105]**, anti-oxidant system is responsible to neutralize the ROS production, the most important member of anti-oxidant defense system is GSH**[106]**, which is a triple bond containing moiety serve to destroy ROS **[105]**, it is in two forms GSH oxidized GSH dismutase GSSH, **[107, 108],** In reduced form, GSH donates reducing equivalent (H+ + e-) from its thio group in cystine residue to ROS**[109]** thus making them balanced **[110]**. After donating its electron, it then readily combines with another molecule of its own to form GSH disulfide (GSSG) in the presence of GPx. **[111, 112]** The reverse reaction generates GSH GSSG in the presence of GSH reductase (GR). Normally, only about 10% of GSH exists in the oxidized form (GSSG), the rest exist in reduced form as GSH. However, GSSG concentration is much higher than that of GSH during oxidative stress **[113]**. Lead shows a strong electron sharing property which aids in the formation of covalent bonds. These covalent bonds are formed between the lead moiety and the sulfhydryl groups in the antioxidant enzymes, making the enzymes most susceptible targets for lead, eventually rendering them inactive **[110, 114].** On the other hand, lead inactivates GSH by binding with its sulfhydryl. This process gives rise to the synthesis of GSH from cysteine via the γ-glutamyl cycle, but would normally not be effective enough to replenish the supply of GSH **[115, 116]**. Furthermore, in addition, lead inactivates δ-ALAD, GR, GPx, and GSH-S-transferase enzymes, and further depresses the levels of GSH. Others are; SOD and CAT **[117]**. Decreased concentration of SOD reduces the clearance of superoxide radical, while reduced CAT impairs the superoxide radical (O2 – •) scavenging. Lead is also able to replace the zinc ions which serve as important co-factors for these antioxidant enzymes in trying to inactivate the enzymes, this is apart from targeting their sulfhydryl groups **[118]**.



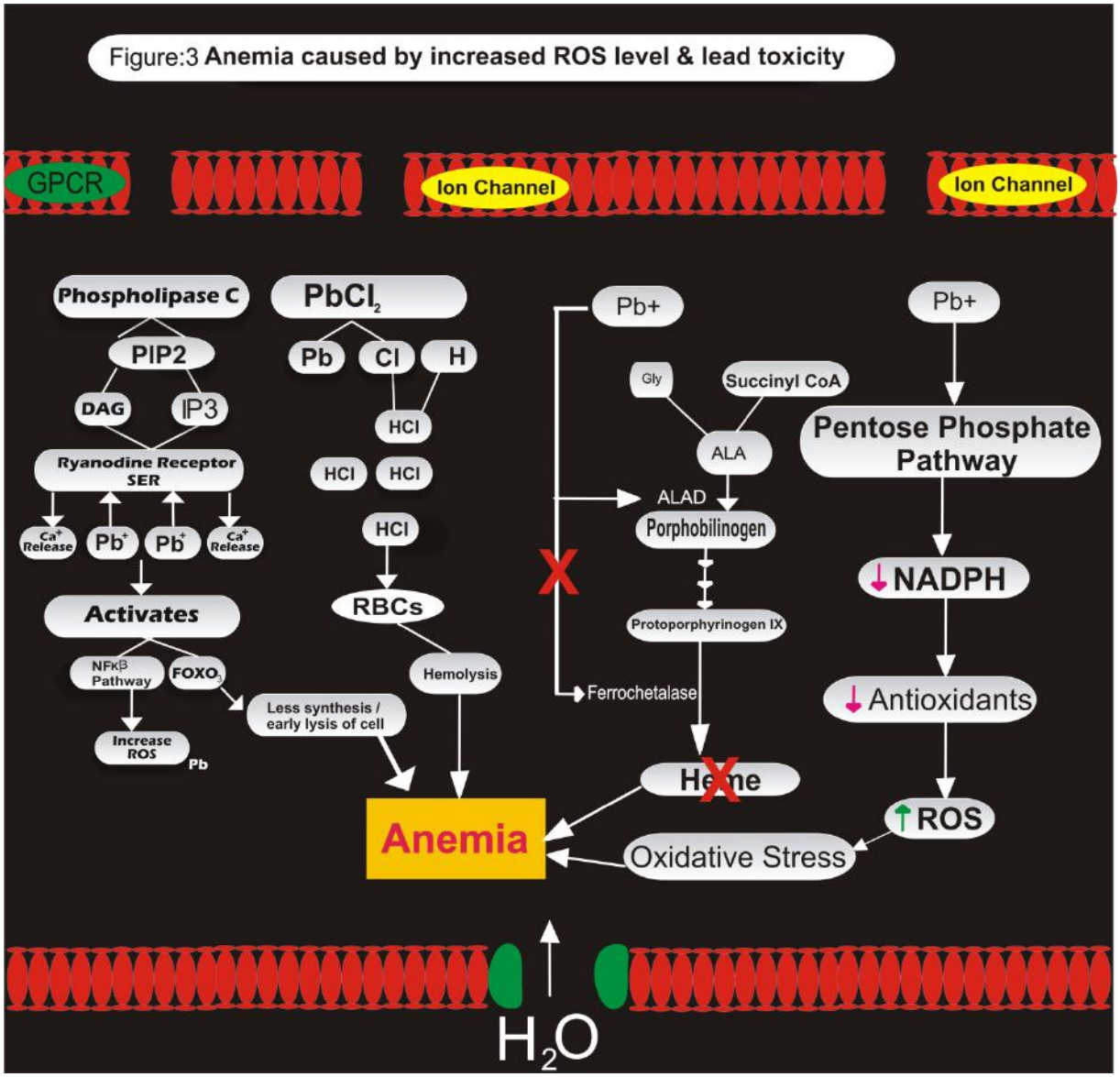
3 Anemia

According to World health organization about 30% of world population is affected directly or in directly by anemia, anemia ismainly caused by iron deficiency but other factors also play their role. Anemia is mainly defined as decrease in red blood cell mass; the usual role of RBC is to transport oxygen from the lungs to tissue and carbon dioxide from tissue to lungs by using hemoglobin as transporter **[119].** A healthy adult produces about 2 million RBCs/sec through a process

named as hematopoietic stem and progenitor cells (HSPCs), to maintain normal level of Hb hematostatic machinery sense the condition and requirement of Hb and amend it accordingly. This hematostatic mechanism get disturbed by one of three factors, decreased production of RBCs, increase hemolysis or blood loss. Less production or high hemolysis associated with inflammation, the basis of which is oxidative stress, so unevenness in ROS production may be adjusted cause of anemia by modifying several signaling pathways **[119, 120].**

4 Anemia caused by higher level of ROS and lead toxicity

Lead causes oxidative stress and subsequent anemia by a variety of pathways, lead is a competitive agonist of Iron; it replaces iron at receptor site and entered in RBCs instead. Pb is inhibitor of ALAD and ferrochelatase, these enzymes are responsible for heam synthesis, and inhibition may result in compromised synthesis of Hb **[121]**. Pb enters in the form of $PBCl_2$, chloride ion dissociated from complex and grab hydrogen from phospholipid bilayer and cause production of HCL, responsible for very early hemolysis of RBCs, entrance of Pb also change the osmolarity of the cell, as its appearance is also responsible for exit of K ion from the cell, to balance this higher salt concentration al lot of water get in to the cell result in cell burst, all these factors contribute to less synthesis and early lysis of cell and subsequent anemia. Lead developed oxidative stress by a variety of pathways; it entered inside the cell by G protein coupling receptor. Phospholipase C cause destruction of PIP_2 and production of DAG and IP_3, this IP_3 knock and ryanodine receptor settled at the gate of sarcoplasmic reticulum, result in release of calcium from its entrapper source, this calcium is replaced by Pb and by activation of FOXO3 and NFkB pathways developed inflammation and cause increase in ROS, on the other hand Pb also interfere with pentose path way by inhibiting its enzyme result in less production of NADPH, which result in decrease in antioxidants within the cell, increase production of ROS and decreased production of antioxidants result in development of oxidative stress, which in turn a known cause for development of anemia **[122]**. **Figure 3**.



5. Importance of Transformed level in diagnosis and prognosis

There are sum of biomarkers, which are supportive in diagnosis and prognosis of anemia, as verdict is of much status in order to give precise treatment, Anemia may cause by a variety of reason, but when it is due to oxidative stress the adducts levels need to be checked being formed in response to oxidative stress, as studies prove that if the level of lead is more than 20 ug/dl in an anemic patient, then it should be assumed that there may be some failure of antioxidant defense system, which result in very high concentration of ROS and subsequent lipid peroxidation, the adducts formed in response to lipid peroxidation are responsible for inhibition of various enzyme dependent

reactions in the synthesis of Hb. ALAS, ALAD and ferrochelatase are key enzyme in the synthesis of Hb, lead toxicity cause inhibition of later two enzymes while increase serum level of ALA is evidence that ALAS is not inhibited in lead toxicity. Increase level of 4-HNE and MDA is also show higher peaks in oxidative stress patient result in development of inflammation, tissue response in turn cause inflammation. In patients with higher lead level lower level of antioxidants especially SOD has been reported which is proposal on development of oxidative stress. Similarly higher level of serine and histamine are also indicators of inflammation caused by oxidative stress due to failure of anti-oxidant defense system in patient suffering from anemia due to higher lead level. Increased level of MMP 1,2,3,8, 9, 13 is also a signal of inflammation. Strongly inhibited level of G6PD is an evidence and adduct of lipid peroxidation are responsible for inhibition of this enzyme. This enzyme play a key role in development of substrates e.g. serine, glycine and erythrose 4 Phosphate needed for synthesis of RBCs and Hb.**[122]**.

6. CONCLUSION

Anemia may cause for a variety of motives, oxidative stress is one of the salient aspects, among other heavy metals, toxicological profile of lead comprises inception of oxidative stress. Lead roots the liberation of ROS that results in uncontrolled damage to several biomolecules, which concurrently damages the antioxidant defense system. Prognosis and diagnosis of anemia may improve by comparative analysis of biomarkers involved in oxidative damage.

7. Conflict of Interest

The authors state that they do not have any conflict of interests

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