ORIGINAL RESEARCH ARTICLE

Zinc Supplementation Combats Tuberculosis by Reverting Back to Normal Compartmentalized State of Iron and Hence Increasing Blood Hemoglobin Concentration

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Abstract

Aim and background: To acquire iron (Fe), *Mycobacterium tuberculosis* (*Mtb*) expresses high-affinity Fe^{+3}-specific siderophores for scavenging Fe from host insoluble and protein-bound iron-like transferrin, lactoferrin, ferritin, and hemoglobin–haptoglobin. *Mycobacterium tuberculosis* by its specific membrane protein and Fe transporters can internalize Fe within cell cytoplasm. With infection by *Mtb*, activity of transferrin, the most dynamic Fe carrier gets setback with a decrease in its level due to infection and also by a decrease in its ability to leave out Fe in bone marrow cells through specific cell surface transferrin receptors. Thus, major decompartmentalization of Fe in host tissues sets in. Zinc (Zn), a redox-inert metal, acts as an antioxidant by stabilizing membrane structures, upregulating expression of metallothionein, protecting protein sulfhydryl group, and suppressing the formation of superoxides by competing with Fe and copper in the cell membrane and thiol group binding. The study interprets the effect of Zn supplementation on serum Fe and hemoglobin (Hb) percentage for tuberculosis (TB) patients.

Materials and methods: Serum Fe and blood Hb percentage were measured initially for TB patients. The same parameters were also assayed with continuation of anti-TB drugs for 1 month with or without Zn supplementation.

Results: Assertive and veritable increase in baseline serum Fe in TB patients had been recorded in this study. The same TB patients with anti-TB drugs for 1 month had recorded nonsignificant serum Fe and Hb percentage increase, whereas oral zinc supplementation with anti-TB drugs for 1 month had shown significant increase in serum Fe and Hb percentage.

Conclusion: Zinc hastens the process of normal compartmentalized state of Fe depriving *Mtb* to get Fe and superoxide required for dismutation reaction to get soluble oxygen for this obligate aerobe.

Keywords: Antioxidant, Decompartmentalization, Dismutation, Iron, Siderophore, Superoxide, Tuberculosis, Thiol group, Transferrin, Zinc.

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Introduction

Mycobacteria does require Fe as growth factor. In fact, as recorded in its genome, Fe is the cofactor of many enzymes meant for its metabolism like superoxide dismutase (SOD); enzymes involved with tricarboxylic acid (TCA) cycle, pyrimidine synthesis; 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, etc., numbering for at least forty.[1] To reduce Fe availability in mammalian tissue, almost all Fe is bound to proteins such as ferritin for storage, or to transferrin (present in circulating plasma) and lactoferrin (present in extracellular fluid) for transport or bound as a cofactor of haem in Hb or in Fe sulfur clusters.[2] To acquire Fe, *Mtb* expresses Fe^{+3}-specific small iron-binding siderophores (mycobactin and carboxymycobactin) having extremely high affinity for Fe, which can scavenge Fe from host-insoluble and host protein-bound Fe-like transferrin, lactoferrin and ferritin.[3–5] At the context of facing lower level of free Fe, *Mtb* through mycobacterial membrane proteins (Mmps) like MmpS4 and MmpS5 and along with associated MmpL4 and MmpL5 transport proteins releases desferricarboxymycobactin into the immediate environment and on release after chelating Fe^{+3} from insoluble or protein-bound Fe is converted to ferricarboxymycobactin.[6] *Mycobacterium tuberculosis* then by using Irt A/Irt B transporter present on cytoplasmic membrane reductively removes Fe (from Fe^{+3} to Fe^{+2}) by FAD-mediated reductase enzyme of Irt A protein and thus mediates internalization of Fe.[7] Fe^{+2} ion after being transported into the cytoplasm by an energy-dependent process can be utilized for various metabolic activities or excess Fe is stored as two *Mtb*-encoded storage proteins, Bfr A; a bacterioferritin; and Bfr B; a ferritin-like protein.[8] In the experiment where Fe was made available as a supplement for macrophage culture or in experimental animals infected with *Mtb*, there was enhanced multiplication of the pathogen.[9] Also, it was reported that with genetic disruption of siderophore expression, growth of *Mtb* could be impaired in mice and macrophages, demonstrating that virulence of mycobacteria is well dependent on its essential role of Fe acquisition by siderophores.[10] For mycobacteria, intracellular Fe level is regulated through Hup B, a positive regulator on the

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expression of mycobactin, and also through IdeR, a negative regulator and Fe-dependent transcription factor, acting and controlling gene-transcription involved in Fe uptake, transport, and storage.[11,12] Macrophages have the capacity for high Fe flux through specific cell surface receptors for transferrin, lactoferrin, and hemoglobin–haptoglobin, and thus recycle Fe from senescent erythrocytes, and thus they are important niches for *Mtb* for acquiring Fe.[13] Mature macrophages, the preferred abode of *Mtb*, have much less concentration of myeloperoxidase (MPO) in contrast to neutrophils and thus unable to catalyze production of hypochlorous acid (HOCl), highly toxic reactive oxygen species (ROS) from hydrogen peroxide in the presence of chloride ion (Cl^-), and thus mature macrophages are unable to kill intracellular *Mtb* by this microbicidal mechanism.[14]

This author in his previous published papers had demonstrated assay of three serum enzymes as early diagnostic for pulmonary tuberculosis (PTB) as well as extrapulmonary TB (EPTB) and also by serial assay of those enzymes during antituberculosis (A-TB) drug therapy; sensitivity toward drug treatment might be ascertained very early. The author had recorded the presence of L-methionine-S,R-sulfoximine (MSO)-sensitive serum glutamine synthetase (GS) would confirm diagnosis for TB.[15] The same author had endorsed highly elevated and sodium cyanide (NaCN)-resistant serum SOD as a diagnostic marker for TB.[16] The index author had also indoctrinated inhibited serum cholinesterase (ChE) activity as a reliable diagnostic aid for TB.[17] The presence of *Mtb* origin GS in serum, elaboration of *Mtb* origin Fe-cofactored serum SOD in enhanced concentration and inhibited host origin serum ChE activity increases specificity to diagnose TB.[17] *Mycobacterium tuberculosis* SOD having no leader peptide binds only with Fe and is exported extracellularly. Also, Fe^{+3} scavenged by siderophore secreted by *Mtb* might form acetylcholine-ferric hydroxamate complex binding more strongly with serum ChE resulting in inhibition of serum ChE activity in TB patients.[17] The ratio of serum SOD and whole-blood glutathione peroxidise had been also reported by this author as a diagnostic for TB.[18]

Zinc (Zn), more electropositive than Fe, exists as divalent cation and is redox neutral unlike Fe and copper and so not redox active under physiological conditions. It (Zn) performs multiple physiological roles in different biological processes. Being more electro-positive, Zn might replace Fe from the binding site with critical thiol groups. By this process, Zn not only inhibits formation of superoxide (O_2-) 2but also brings back intracellular decompartmentalized state of Fe to normal compartmentalized state of Fe.

Aim and Objective

Keeping in mind the unique role engineered by Zn to revert back decompartmentalized state of Fe to normal compartmentalized state of Fe, the present author has pondered a study to construe the effect of Zn supplementation in TB patients as reflected by serum Fe level in those subjects. With Zn supplementation, when intracellular decompartmentalized state of Fe reverts back to normal compartmentalized state of Fe in host tissues and Fe is egressed out of cells, this author has progressed to assay Hb before and after Zn supplementation in TB patients to mull and interpret whether the egressed Fe can be used up by host for Hb synthesis.

Materials and Methods

The cohort study was conducted at BS Medical College and Hospital, Bankura-722 102, West Bengal, India, from June 2004 to May 2009. Permission for research protocol along with a study on blood samples obtained from human subjects and also on zinc supplementation for TB patients was approved in writing by the Ethics Committee of BS Medical College and Hospital, Bankura (Vide Memo No. 3/BSMC/04 dt 14-05-2004).A total of 224 participants (aged 8–64 years), including normal control, lung disease control, PTB, and EPTB subjects, were enrolled for the project. The study was conducted in four phases with 56 participants being enrolled in each phase. The purpose of the study was explained to all participants, and before collection of blood, informed verbal consent was obtained from each of the subjects.

The following groups (Gp) of subjects were taken into consideration:

Gp I: (n = 56) *Normal control (NC)*: They were healthy relatives of TB patients having no clinical symptom, sign, or any finding suggestive of TB or any sort of disease. They were sputum-negative for acid-fast bacilli (AFB). Collection of blood was made from each subject for estimating serum Fe.

Gp II: (n = 56) *Lung disease control (LDC)*: These patients were suffering from respiratory tract infection (n = 21) or chronic obstructive pulmonary disease (n = 14) or bronchial asthma (n = 11) or bronchiectasis (n = 8) or bronchogenic carcinoma (n = 2). The subjects were selected from patients attending Out Patient Department (OPD) of BS Medical College and Hospital, Bankura. For each subject of this group, blood sample was collected initially and also after 1 month with usual treatment. Serum Fe was estimated for each blood sample.

Gp III: TB patients: Patients suffering from tuberculosis (irrespective of age, sex, and socioeconomic status) attending OPD of BS Medical College and Hospital, Bankura, and also TB patients admitted in TB Ward of the same hospital were taken into account. Tuberculosis patients were diagnosed clinically by characteristic symptoms and signs as well as by other investigative procedures like radiological investigation, sputum for AFB, fine needle aspiration, and cytology (FNAC) whenever possible. ELISA technique for serodiagnosis (detection of myco-specific serum immunoglobulin level) was also adopted for TB diagnosis.[19] Multi-drug resistant TB (MDR-TB) patients were not considered for this study. It was an exclusion criterion regarding TB patients concerned. All the subjects in this study had normal liver function tests (LFT) and that was an important criterion in this research work as transferrin, a major iron-binding protein in human, is synthesized predominantly in the liver. Based on prior diagnosis, TB patients were categorized as follows:

Sub-group A (n = 56): It included patients suffering from pulmonary TB (PTB).

Sub-group B (n = 56): It included patients suffering from extra-pulmonary TB (EPTB), e.g., TB lymphadenitis (n = 12), TB pleural effusion (n = 23), TB meningitis (n = 9), and lupus vulgaris (n = 1); and also spinal (n = 1), hip joint (n = 6), intestinal (n = 1), urinary bladder (n = 1), and miliary TB (n = 2).

For all these TB patients, anti-TB (A-TB) drug therapy was started between 0 and 15 days. Blood samples were collected from each of

Table 1: Baseline serum Fe level and also after 30 days' additional A-TB drug treatment for TB subjects with and without oral zinc supplementation

			After 30 days					
	At baseline		*Without Zn supplementation*			*With Zn supplementation*		
Subjects	*No. of subjects (n)*	*Serum Fe (µg/dL)*	*No. of subjects (n)*	*Serum Fe (µg/dL)*	*p-value*	*No. of subjects (n)*	*Serum Fe (µg/dL)*	*p-value*
Normal control	$n = 56$	100.8 ± 10.6	–	–	–	–	–	–
Lung disease control	$n = 56$	117.4 ± 11.2	$n = 56$	123.8 ± 9.3	0.5	–	–	–
Tuberculosis patients Pulmonary (PTB)	$n = 56$	191.8 ± 22.6	$n = 28$	231.7 ± 18.8	0.15	$n = 28$	294.5 ± 14.7	0.004*
Extrapulmonary (EPTB)	$n = 56$	189.8 ± 20.3	$n = 28$	220.6 ± 16.4	0.15	$n = 28$	283.8 ± 10.6	0.004*

**p*-value significant

these TB patients initially for estimating serum Fe and whole-blood Hb. All TB patients were kept under directly observed treatment (DOT) program under Revised National Tuberculosis Control Program (RNTCP). Repeat blood collection was done after 30 days for estimating serum Fe and whole-blood Hb (wherever necessary). During additional 30 days' treatment, half of the patients under sub-groups A and B were administered oral supplementation of Zn daily in the form of zinc sulfate containing 25 mg of elemental Zn. Based on prior initial estimation of blood Hb, 18 PTB and 16 EPTB subjects were spotted having Hb percentages below 9 gm/dL. Of these TB patients, 9 PTB and 8 EPTB subjects were given oral supplementation of Zn for 30 days as already described. Repeat Hb estimation was made for these 18 PTB and 16 EPTB subjects exactly after 30 days from the initial collection.

Collection of blood: Morning fasting blood samples were collected by venipuncture. For estimation of serum Fe, blood samples were left to clot; serum so obtained was transferred in clean and sterile Eppendorf tubes and kept in a refrigerator at 2–4°C until assayed on the same day of collection. After the assay, the remaining serum was stored at −20°C if it might necessitate repeat assay procedure. For assaying whole-blood Hb percentage, heparinized blood [containing 200 µg of heparin per milliliter (ml) of blood] was used. Estimation of Hb percentage was done immediately after collection.

Procedure Methodology

Estimation of Serum Fe (Normal Reference Value 60–170 µg/dL)

Serum protein was precipitated with a reagent containing hydrochloric acid (to dissociate Fe), thioglycolic acid (to reduce Fe), and trichloroacetic acid (to precipitate protein). Fe in ferrous state reacts with chromogen (sodium acetate in ferrozine) to develop pink-colored complex that is measured colorimetrically.[20] Kits from Span Diagnostics Pvt. Ltd. were used for the estimation.

Estimation of Blood Hb Percentage (Normal Reference Value 14–18 gm/dL for Men and 12–16 gm/dL for Women)

Hemoglobin is oxidized by potassium ferricyanide into met Hb that is converted into cyanomet Hb by potassium cyanide. Intensity of color so developed is proportional to Hb concentration. Absorbance of color is measured at 540 nm using a spectrophotometer (Baush & Lamb Co.) against standard quality control solution.[21] Kits from Bio Lab Diagnostics Pvt. Ltd. were used for the assay.

Statistical Analysis

Statistical analysis of the results was made using Statistical Software for Social Sciences (SPSS Version 21.0). The level of significance was assessed using Student's *t*-test. $p < 0.05$ was considered to be significant statistically.

Result and Discussion

Baseline serum Fe in PTB and EPTB patients had shown a significant increase when compared with those of NC ($p = 0.004$) and LDC ($p = 0.005$) subjects. While there was no noteworthy change in serum Fe level after 30 days treatment in LDC subjects ($p = 0.5$), there was a noteworthy but statistically insignificant increase in serum Fe after 30 days' additional A-TB drug treatment without Zn supplementation for both PTB and EPTB patients ($p = 0.15$). On other hand, for PTB and EPTB under Zn supplementation along with A-TB drugs for 1 month, there was a statistically significant increase in serum Fe level ($p = 0.004$) when compared with baseline serum Fe level in TB and EPTB patients (Table 1).

In the second part of this research study, it had been recorded that while both PTB and EPTB patients without Zn supplementation had shown increased but statistically insignificant increase in Hb concentration ($p = 0.2$), with Zn supplementation for 1 month, there was statistically significant increase in Hb percentage for PTB as well as EPTB patients ($p = 0.02$) (Table 2).

The increased serum Fe in TB patients is due to the presence of increased siderophore bound Fe^{+3} complexes in serum. In this study, it is evident that oral supplementation does increase significantly serum Fe level in TB patients, and also the increased serum Fe might be used up for Hb synthesis for them. So, the veritable question is what the mechanism behind it might be.

To begin with, it is worth mentioning that haem and Hb are well utilized by *Mtb* as Fe sources.[22] As already mentioned in the "Introduction", siderophores chelate Fe^{+3} from insoluble and protein-bound Fe. Again, several proteins like haem-binding protein, cell-surface protein, and RND efflux pumps had been implicated for *Mtb* to utilize haem and Hb as Fe sources.[23,24] Fe is

Table 2: Effect of zinc supplementation on Hb percentage of the TB patients having initial blood hemoglobin concentration below 9 gm/dL

		After 30 days' additional A-TB drug treatment					
At baseline		*Without Zn supplementation*			*With Zn supplementation*		
No. of subjects	*Hb percentage in gm/dL*	*No. of subjects*	*Hb in gm/dL*	*p-value*	*No. of subjects*	*Hb in gm/dL*	*p-value*
$n = 18$ (PTB)	7.8 ± 1.2	$n = 9$ (PTB)	8.1 ± 0.8	0.2	$n = 9$ (PTB)	11.4 ± 0.6	0.02*
$n = 16$ (EPTB)	7.6 ± 1.1	$n = 8$ (EPTB)	8.2 ± 0.7	0.2	$n = 8$ (EPTB)	11.1 ± 0.5	0.02*

*p-value significant

readily accessible in aqueous medium to oxidative states ferrous (Fe^{+2}) and ferric (Fe^{+3}) and thus determines participation of a large variety of biochemical reactions concerned with electron transport, activation of molecular oxygen and nitrogen, and also binding of oxygen with Hb and myoglobin. In ferric state, Fe is liable to be hydrolyzed forming polynuclear hydroxide complexes, biologically unavailable molecules. So, redox reaction to release Fe as relatively water-soluble Fe^{+2} might be essential combating the kinetic barrier. In view of the redox equilibrium of Fe^{+2}–Fe^{-3} couple involving transfer of one electron in oxidation–reduction in biological system, it is very logical to infer that free radicals are likely to be involved in the process. Therefore, in Fe overload in biological systems, free radical intermediates may account for toxicity of Fe. Important factors for mobilization of Fe are ligand complexes of Fe^{+3}, macromolecules for Fe storage and transport, and also the presence of lower-molecular-weight chelating molecules. Transferrin, the most dynamic Fe carrier in human, after binding to specific cell surface transferrin receptor (Tfr), e.g., erythroid precursors in bone marrow, leaves out Fe in endocytosed vesicles within the cell and comes back as apotransferrin. Nature has made provision that vital sites sensitive to Fe-catalyzed oxidation are buried in macromolecular structure preferably in hydrophobic milieu or by being bound to catabolically inert Zn ion (Zn^{+2}) and thus well-protected from interaction with Fe. So, compartmentalization of Fe in the human system is very important as Fe-catalyzed oxidation of thiol groups results in formation of highly reactive and damaging free radicals.

With infection caused by *Mtb*, there is decompartmentalization of Fe in host tissue by binding of Fe^{+3} to siderophores secreted by *Mtb*, which are stronger binding sites as well as diffusible ones. Siderophores can chelate Fe^{+3} from insoluble and protein–bound Fe as already mentioned. It had been reported that two *Mtb*-encoded storage proteins Bfr A and Bfr B aggregate to form macromolecules having 24 subunits holding 600–2400 Fe atoms per molecule.[12] After Irt AB-mediated reduction of Fe^{+3} in internalized Fe^{+3}-siderophore complex into Fe^{+2} and release, Fe-free siderophore (desferricarboxymycobactin) is recycled through Mmps bound to transporter complex through inner membrane.[6] Recycling of desferricarboxymycobactin, however, is an important mechanism for *Mtb* to acquire Fe at lower metabolic cost.[25] Jones et al. reported that Fe salts could not be utilized by *Mtb* in the absence of siderophores and genetic disruption of siderophore export, and recycling had reduced the capacity of *Mtb* to take up Fe and had caused siderophore-mediated self-poisoning.[25] Siderophore-mediated Fe uptake is essential for survival of *Mtb* in macrophages as knockout mutants, defective in siderophore synthesis and uptake, had inhibited growth in macrophages.[10] It is interesting to note that three protons (H^+) released per molecule of apo-transferrin being oxidized by molecular oxygen to Fe^{+3}–transferrin–carbonate complex, are well-utilized by *Mtb* for dismutation reaction catalyzed by Fe-cofactored SOD secreted by it, thus helping to provide soluble oxygen for survival of the aerobe. It is also reported that within phagosomes, *Mtb* interacts early endosomes and does not acidify below pH 6.3–6.5.[26] But, reduction of pH of vesicles to 5.5 by hydrogen-proton pump (H^+ATPase) is required for dissociation of Fe-bound transferrin to release its Fe.[27] Thus, *Mtb* by preventing phagosome acidification and lysosomal fusion is able to acquire Fe from host endosomal holotransferrin.

Therefore, in prevailing situation of infection by *Mtb*, there is a major decompartmentalized state of Fe in host tissues. In TB, it is then logical that siderophores having more affinity toward Fe^{+3} become major transporter of Fe in serum instead of transferrin. So, the major role of transferrin to play a pivotal role in innate immune system chelating free toxic Fe and thus acting as protective scavenger, gets a setback. Also, with increased degree of inflammation in TB, and hence decreased serum transferrin, major decompartmentalization of Fe prevails in host tissues. In that situation, there is generation of remarkable quantity of highly reactive superoxide (O_2-) by Fe-catalyzed oxidation of thiol groups. Interestingly, *Mtb* utilizes this O_2- for dismutation reaction to generate soluble oxygen for its survival. Also, *Mtb* utilizes O_2-produced by phagosome with the help of its membrane-bound NADPH oxidase system to generate soluble oxygen by dismutation reaction.[28]

Notwithstanding, physical decompartmentalization of Fe in TB is by complexing of Fe with high-affinity siderophores that are diffusible ones, decreasing and damaging normal Fe-binding sites, and altering the barriers. These facts have really corroborated with the present study of this author. Definite increase in baseline serum Fe in TB patients is due to abundant extracellular release of high-affinity Fe^{+3} chelator siderophores that can scavenge Fe not only from insoluble and protein-bound Fe but also from mineral phases (Fe oxide and hydroxide) by formation of soluble Fe^{+3} complexes that can be taken up by active transport mechanism.[29] Also, nonsignificant increase of serum Fe ($p = 0.15$) has been reported in this study for TB patients after 30 days' additional A-TB drug therapy (without Zn supplementation). This increase can be acclaimed that with A-TB drugs, there is decrease in mycobacterial load, resulting in decrease in siderophores and with decrease in the degree of inflammation, there is recovery of serum transferrin activity and thus; decompartmentalized state of Fe in host tissue begins to revert back to normal compartmentalized state of Fe. On other hand, highly significant increase in serum Fe ($p = 0.004$) with oral Zn supplementation along with A-TB drugs entails that Zn destabilizes decompartmentalized state of Fe in host tissues and hastens tremendously the process of reverting back to normal compartmentalized state of Fe and hence significant increase in serum Fe level.

Zn belonging to group 12 of periodic table is stable only as divalent cation (Zn^{+2}) and due to its filled *d*-shell, it cannot directly take part in redox reactions, whereas two other bioactive metals Fe and copper (Cu) can stay in two different ionic states, e.g., Fe

as ferrous (Fe^{+2}) and ferric (Fe^{+3}); and Cu as cuprous (Cu^{+}) and cupric (Cu^{+2}), and hence they are redox-active metals. Searle and Tomasi had demonstrated decrease in spin-trapped OH from Fe and cysteine in the presence of Zn. They inferred that competition between Fe (transition-active metal in biological system) and Zn for thiol amino acid had interfered with transfer of electron to O_2-.[30] So, Zn is able to antagonize Fe-mediated transport of electron and thus oxidation under normal and pathological conditions. Analogically, Zn might replace Fe bound to thiol groups. Hence, generation of O_2- is inhibited by Zn. With inhibition on generation of O_2-. *Mycobacterium tuberculosis* gets a jolt with remarkable decrease in formation of soluble oxygen by dismutation reaction, required badly for survival of this obligate aerobe. With hastening process of normal compartmentalization of Fe, supply of Fe for *Mtb* is curtailed, thus activity of Fe-cofactored SOD is diminished. In fact, as per co-ordination chemistry similarities, Zn can compete with Cu and Fe for certain types of binding, thus suppressing their ability in certain particular environment to transfer electron might suppress O_2- generation. Also, competition of Zn^{+2} with Fe and Cu can lead to inhibition of NADPH-oxidase enzyme and thus can curtail O_2- production and thus can limit generation of soluble oxygen by dismutation reaction and thereby accelerate killing of *Mtb* in host tissue. It is recently reported that Zn^{+}-limited *Mtb* is not only more resistant to oxidative stresses but also has increased replication *in vivo* and thus has increased survival as evidenced by formation of severe pulmonary granuloma in mice.[31]

In the second part of this work, TB patients with Hb% less than 9 gm/dL at baseline were spotted to observe the result on Hb% with Zn supplementation, which might be well-precipitating and well-marked keeping into view the veritable acceleration of decompartmentalized Fe to revert back to normal compartmentalized state of Fe in host tissues. It is interesting and unequivocal to note that TB patients with A-TB drugs for 1 month but without any Zn supplementation had shown nonsignificant increase in Hb%, however, TB patients with A-TB drugs and oral Zn supplementation for 1 month had shown significant increase in Hb% ($p = 0.02$). This entails that A-TB drugs can bring decompartmentalized state of Fe reverted back to normal compartmentalized state of Fe at low pace by decreasing mycobacterial load and hence Fe^{+3}-chelator siderophores along with mycobacterial membrane protein and Irt A/Irt B proteins in host tissue. On other hand, with oral Zn supplementation and A-TB drugs, process of decompartmentalized state of Fe reversing to normal compartmentalized state of Fe gets accelerated to new height by curtailing superoxide formation and by other mechanisms as already elaborated and thus accelerating killing of *Mtb* in host tissue. With remarkable recovery from infection and hence inflammation serum transferrin level increases and with well-marked decrease in siderophore level caused by enhanced killing of *Mtb*; transferrin might get bound in greater number to specific cell surface transferrin receptors like erythroid precursors in bone marrow and leaving out Fe in endocytosed vesicles within the cells. This Fe can again be used up for Hb synthesis, which has been reflected by statistically significant increase in Hb% with oral Zn supplementation. Thus, oral Zn supplementation (25 mg elemental Zn daily) along with A-TB drug treatment might act as a great bactericidal against *Mtb* causing accelerated killing of *Mtb*, making an onslaught against virulence of *Mtb*, and thus also accelerating the general well-being of the patient as reflected in this study by significant increase in Hb%.

Conclusion

It is great solemnity to promulgate that oral Zn supplementation (25 mg elemental Zn daily) may be added to drug regime to treat TB for enhancing *Mtb* killing pertaining to general well-being of host as reflected by significant increase in Hb%.

References

1. Cole ST, Brosch R, Parkhill J, et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 1998;393(6685):537–544. DOI: 10.1038/31159.
2. Anderson NC. Iron homeostasis: Insights from genetics and animal models. Nat Rev Genet 2000;1(3):208–217. DOI: 10.1038/35042073.
3. Rodriguez GM. Control of iron metabolism in *Mycobacterium tuberculosis*. Trends in Microbiol 2006;14(7):320–327. DOI: 10.1016/j.tim.2006.05.006.
4. Ratledge C. Iron, mycobacteria and tuberculosis. Tuberculosis 2004;84(1–2):110–130. DOI: 10.1016/j.tube.2003.08.012.
5. Gobin J, Horwitz MA. Exochelins of *Mycobacterium tuberculosis* remove iron from human iron-binding proteins and donate iron to mycobactins in the *M. tuberculosis* cell wall. J Exp Med 1996;183(4):1527–1532. DOI: 10.1084/jem.183.4.1527.
6. Wells RM, Jones CM, Xi Z, et al. Discovery of a siderophore export system essential for virulence of *Mycobacterium tuberculosis*. PLoS Pathog 2013;9(1):e1003120. DOI: 10.1371/journal.ppat.1003120.
7. Ryndak MB, Wang S, Smith I, et al. The *Mycobacterium tuberculosis* high-affinity iron importer, Irt A, contains an FAD-binding domain. J Bacteriol 2010;192(3):861–869. DOI: 10.1128/JB.00223-09.
8. Reddy PV, Puri RV, Khera A, et al. Iron storage proteins are essential for the survival and pathogenesis of *Mycobacterium tuberculosis* in THP-1 macrophages and the guinea pig model of infection. J Bacteriol 2012;194(3):567–575. DOI: 10.1128/JB.05553-11.
9. Cronje L, Edmondson N, Eisenach KD, et al. Iron and iron chelating agents modulate *Mycobacterium tuberculosis* growth and monocyte-macrophage viability and effector functions. FEMS Immunol Med Microbiol 2005;45(2):103–112. DOI: 10.1016/j.femsim.2005.02.007.
10. De Voss JJ, Rutter K, Schroeder BG, et al. The salicylate-derived mycobactin siderophores of *Mycobacterium tuberculosis* are essential for growth in macrophages. Proc Natl Acad USA 2000;97(3):1252–1257. DOI: 10.1073/pnas.97.3.1252.
11. Rodriguez GM, Voskuil MI, Gold B, et al. ideR, an essential gene in *Mycobacterium tuberculosis*: Role of IdeR in iron-dependent gene expression, iron metabolism and oxidative stress response. Infect Immun 2002;70(7):3371–3381. DOI: 10.1128/IAI.70.7.3371-3381.2002.
12. Sritharan M. Iron homeostasis in *Mycobacterium tuberculosis*: Mechanistic insights into siderophore-mediated iron uptake. J Bacteriol 2016;198(18):2399–2409. DOI: 10.1128/JB.00359-16.
13. Hamilton TA, Gray PW, Adams DO. Expression of transferrin receptor on murine peritoneal macrophages is modulated by *in vitro* treatment with interferon gamma. Cell Immunol 1984;89(2):478–488. DOI: 10.1016/0008-8749(84)90348-4.
14. Mendoza-Aguilar MD, Arce-Paredes P, Aquino-Vega M, et al. Fate of *Mycobacterium tuberculosis* in peroxidise-loaded resting murine macrophages. Int J Mycobacteriol 2013;2(1):3–13. DOI: 10.1016/j.ijmyco.2012.11.002.
15. Chattopadhyay DK. Serum glutamine synthetase activity as biomarker for tuberculosis diagnosis and monitoring anti-tubercular drug therapy success. Indian J Biochem Biophys 2019;56(6):427–432. DOI: 10.56042/ijbb.v56i6.29214.
16. Chattopadhyay DK. Superoxide dismutase: A biomarker for early diagnosis of tuberculosis. J Clin Diagn Res 2019;13(7):BC01–BC03. DOI: 10.7860/JCDR/2019/35298.12968.
17. Chattopadhyay DK. Decreased serum cholinesterase activity – A reliable diagnostic aid for tuberculosis. J Clin Diagn Res 2021;15(3):BC16–BC19. DOI: 10.7860/JCDR/2021/46501.14657.
18. Chattopadhyay DK. Ratio of serum superoxide dismutase and whole blood glutathione peroxidase: A noteworthy parameter for

tuberculosis diagnosis. Indian J Med Biochem 2021;25(3):100–104. DOI: 10.5005/jp-journals-10054-0193.

19. Chattopadhyay DK, Maity CR, Nag D. Effect of zinc supplementation on mycospecific immunoglobulins in tuberculosis patients. J Indian Med Assoc 2010;108(2):92–93. PMID: 20839565.
20. International Committee for Standardization in Haematology (Expert Panel on Iron). Revised recommendation for the measurement of serum iron in human blood. Br J Haematol 1990;75(4):615–616. DOI: 10.1111/j.1365-2141.1990.tb07808.x.
21. Balasubramanian P, Malathi A. Comparative study of haemoglobin estimated by Drabkin's and Sahli's methods. J Postgraduate Med 1992;38(1):8–9. PMID: 1512732.
22. Jones CM, Niederweis M. *Mycobacterium tuberculosis* can utilize heme as an iron source. J Bacteriol 2011;193(7):1767–1770. DOI: 10.1128/JB.01312-10.
23. Tullius MV, Nava S, Horwitz MA. PPE 37 is essential for *Mycobacterium tuberculosis* heme-iron acquisition (HIA) and a defective PPE 37 in *Mycobacterium bovis* BCG prevents HIA. Infect Immun 2019;87(2): e00540-18. DOI: 10.1128/IAI.00540-18.
24. Mitra A, Speer A, Lin K, et al. PPE surface proteins are required for heme utilization for *Mycobacterium tuberculosis*. mBio 2017;8(1):e01720. DOI: 10.1128/mBio.01720-16.
25. Jones CM, Wells RM, Madduri AY, et al. Self-poisoning of *Mycobacterium tuberculosis* by interrupting siderophore recycling. Proc Natl Acad Sci USA 2014;111(5):1945–1950. DOI: 10.1073/pnas.1311402111.
26. Mwandumba HC, Russel DG, Nyirenda MH, et al. *Mycobacterium tuberculosis* resides in nonacidified vacuoles in endocytically competent alveolar macrophages from patients with tuberculosis and HIV Infection. J Immunol 2004;172(7):4592–4598. DOI: 10.4049/jimmunol.172.7.4592.
27. MacGillivary RT, Moore SA, Chen J, et al. Two high-resolution crystal structures of the recombinant N-lobe of human transferrin reveal a structural change implicated in iron release. Biochemistry 1998;37(22):7919–7928. DOI: 10.1021/bi980355j.
28. Chattopadhyay DK, Nag D. Efficacy of zinc supplementation as an adjunct to anti-tubercular drug therapy. Ind Med Gaz 2014; CXLVIII:21–24. https://pesquisa.bvsalud.org/portal/resource/pt/sea-157577.
29. Kraemer SM. Iron oxide dissolution and solubility in the presence of siderophores. Aquat Sci 2004;66:3–18. DOI: 10.1007/s00027-003-0690-5.
30. Searle AJF, Tomsai A. Hydroxyl free radical production in iron-cysteine solutions and protection by zinc. J Inorg Biochem 1982;17(2):161–166. DOI: 10.1016/S0162-0134(00)80085-9.
31. Dow A, Sule P, O'Donnell TJ. Zinc limitation triggers anticipatory adaptations in *Mycobacterium tuberculosis*. PLoS Pathog 2021; 17(5):e1009570. DOI: 10.1371/journal.ppat.1009570.