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Study of changes in serum iron, albumin and calcium levels in smear positive pulmonary tuberculosis patients compared with healthy individuals before starting Directly Observed Treatment, Short course

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Abstract

Background: Tuberculosis one of the most important infectious diseases throughout the world. Bangladesh is a highly populated country and a significant amount of mortality and morbidity is caused by tuberculosis, especially pulmonary tuberculosis. **Objectives:** To evaluate the levels of serum iron, albumin and calcium in smear positive pulmonary tuberculosis (PTB) patients before starting Directly Observed Treatment, Short course (DOTS) regimen. **Methods:** This was a cross-sectional study. A total of 100 subjects were included in this study. Among them, 50 were smear positive PTB patients denoted as case group and 50 were apparently healthy individuals denoted as control group. Biochemical tests were carried out in the Department of Biochemistry, Mymensingh Medical College. All the statistical analyses were performed by SPSS version 21. Results were evaluated by using Student's t-test. **Results:** The study revealed that mean values of serum iron were 128.24±13.20 µg/dl and 86.13±24.56 µg/dl in control and case group respectively, and the difference between two groups were highly significant (p <0.001). The mean serum albumin level was lower in case group when compared to control group. The mean values of serum albumin were 4.1±0.33 gm/dl and 3.5±0.31 gm/dl in control and case group respectively, and the difference between two groups were highly significant (p <0.001). The mean values of serum calcium were 2.33±0.19 mmol/L and 2.5±0.16 mmol/L in control group and case group respectively. Serum calcium level was adjusted by serum albumin concentration. The analysis showed that, the difference in mean serum calcium levels between two groups were highly significant (p <0.001).

Keywords: Pulmonary tuberculosis, smear positive, serum iron, serum albumin, serum calcium.

Introduction

Tuberculosis is a chronic condition that causes both pulmonary and systemic diseases.[1] This is also considered as the second most common cause of death due to infectious disease.[2] WHO global report, 2016 showed that, Bangladesh is one of the countries with high burden of TB and approximately 73,000 people die annually due to TB.[3] Iron (Fe) is an important micronutrient that participates in a wide variety of metabolic processes including cellular respiration mechanism.[4] Iron has a consequential role on tuberculosis for both acquired and innate immune response.[5] Albumin is the major plasma protein with a high molecular weight.[6] Serum albumin plays important role as a biochemical marker of

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nutritional status of PTB patients and considerable changes take place in serum albumin level.[7] Calcium is another important macromineral with diverse function. PTB is a granulomatous disease where increase serum calcium level occurs.[8]

Methodology

Study design: This was a cross-sectional study carried out in the Department of Biochemistry, Mymensingh Medical College in cooperation with outpatient department of Mymensingh Medical College Hospital and DOTS centers of BRAC and DAMIEN foundation during the period of July 2016 to June 2017.

Sample size: A total of 100 subjects were included by purposive (non-random) sampling technique among which control group (group-I) consisted of 50 apparently healthy people from different age, sex and socioeconomic status where their age was from 18-65 years. The case group (group-II) consisted of 50 smears positive PTB patients (before starting DOTS regimen) of different age, sex and socioeconomic status where their age was from 18-65 years.

Data collection method: Ethical approval was taken from concerned authorities. All the subjects of this study were informed and included after their consent. Data were collected using preformed questionnaire.

Collection of samples: With all aseptic precaution, 5ml of venous blood was collected from median antecubital vein of the subjects. After clot formation and centrifugation serum was collected.

Laboratory investigations:

Estimation of serum Iron: Serum iron was measured by colorimetric method using the specific test kit[9].

Estimation of serum Albumin: Serum albumin was measured by using colorimetric method (Colorimetric method with Bromersol Green).[10]

Estimation of serum Calcium: Serum calcium was measured by using colorimetric method (Colorimetric method with o - Cresphtalein).[11]

Estimation of adjusted calcium for serum albumin concentration:

Adjusted calcium (mmol/L) = Total measured calcium in mmol/L + 0.02 (47 – albumin in gm/L).[12]

Analysis of different concentrations of standard solution of serum iron, albumin and calcium were performed to obtain a calibration chart. Control sera (normal and pathogenic), duplicate standard and duplicate test serum were run in every batch of the test procedure.

Data analysis: All the data were analyzed by SPSS version 21 for windows. Data were expressed in mean ± SD and statistical significance of difference among the groups were calculated by using Student's unpaired t-test. At the level of 95% confidence limit, $p < 0.05$ was considered as statistically significant and $p > 0.05$ as not significant.

Results

Serum iron:

The study revealed that mean serum iron level was lower in case group when compared to control group. The mean values of serum iron were 128.24 ±13.20 µg/dl and 86.13 ±24.56 µg/dl in control and case group respectively. The analysis showed that, the difference in mean serum iron levels between two groups were highly significant ($p < 0.001$). Analysis of mean serum iron levels is presented in Table 01.

Table 01: Comparison of mean serum iron levels between the study groups

Variable	Control (group-I) Mean±SD	Case (group-II) Mean±SD	p
Serum iron (µg/dl)	128.24±13.20	86.13±24.56	0.001

Serum albumin:

The study revealed that the mean serum albumin level was lower in case group when compared to

control group. The mean values of serum albumin were 4.1±0.33 gm/dl and 3.5±0.31 gm/dl in control and case group respectively. The analysis showed that, the difference in mean serum albumin levels between two groups were highly significant (p <0.001). Analysis of mean serum albumin levels is presented in Table 02.

Table 02: Comparison of mean serum albumin levels in the study population

Variable	Control (group-I) Mean±SD	Case (group-II) Mean±SD	p
Serum albumin (gm/dl)	4.1±0.33	3.5±0.31	0.001

Serum calcium:

The study revealed that serum calcium level was higher in case group when compared to control group. The mean values of serum calcium were 2.33±0.19 mmol/L and 2.5±0.16 mmol/L in control group and case group respectively. Serum calcium level was adjusted by serum albumin concentration. The analysis showed that, the difference in mean serum calcium levels between two groups were highly significant (p <0.001). Analysis of mean serum calcium levels is presented in Table 03.

Table 03: Comparison of mean serum calcium levels in the study population

Variable	Control (group-I) Mean±SD	Case (group-II) Mean±SD	p
Serum calcium (mmol/L)	2.33±0.19	2.5±0.16	0.001

Discussion

The present study was designed to observe the various changes of serum iron, albumin and calcium levels in smear positive PTB patients compared to apparently healthy people.

In the present study the mean values of serum iron were 128.24±13.20 µg/dl and 86.13±24.56 µg/dl. There was significant (p <0.001) decrease in serum iron in case group when compared with control group. This observation is similar with Kaminskaia & Abdullaev, Kassu et al. and Pourfallah et al.[13-15] The cause of decreased serum iron may be due to increased hemoptysis in PTB patients or body defense mechanism to limit the growth and expansion of mycobacterium tuberculosis. For growth of TB bacilli iron is important.[16] Tubercle bacilli also stimulate formation of exochelin- a form of iron that is transported from host cell to TB bacilli.[17] Free radicals produced by TB bacilli also affect post-transcriptional down regulation of regulatory protein for iron homeostasis.[18] Other studies by Karyadi et al. and Lettow et al. showed less association of TB bacilli with decreased serum iron.[19,20] It was mostly due to nutritional deficiency and helminthic infestation and malabsorbtion.[21]

In the present study serum albumin levels of case and control groups were estimated. The mean values of serum albumin were 4.1±0.33 gm/dl and 3.5±0.31 gm/dl in control and case groups respectively. There was significant (p <0.001) decrease in serum albumin level in case group when compared with control group. The results were in agreement with the result of Onwubalili from England, Saha & Rao from India and Tuskaguchi et al. from Japan.[22-24] WHO declared that TB prevalence is more in low socioeconomic condition where people are malnourished that favors the infection.[25] Pulmonary tuberculosis increases the production of IL-6, TNF-α that causes fever, decreased synthesis of serum albumin with shifts of nutrients causing malnutrition.[26] Other studies by Sasaki et al. and Yamanka et al. showed that decrease of serum albumin concentration occurs due to dehydration with immunological factors.[27,28]

In the present study mean values of serum adjusted calcium were 2.33±0.19 mmol/L and 2.5±0.16 mmol/L in control and case group respectively. There was significant (p <0.001) difference in case group when compared with

control group. The results were in agreement with the results of several other studies.[29-32] Hypercalcemia is very common in active PTB patients with a prevalence of about 28% in the United States and 16% in India.[33,34] PTB patients relatively show high frequency of hypoalbuminemia, so corrected calcium is important unless hypocalcemia remains undiagnosed.[35] Another study by Bhandari et al. showed 40% patients with low serum calcium level and rest of the patients with normal serum calcium level.[36] Decreased serum calcium level is due to malabsorption, malnutrition or other GIT diseases in PTB patients.[37]

Conclusion

PTB is an important cause of mortality and morbidity throughout the world including Bangladesh. In this study there were highly significant decrease of serum iron and albumin levels and highly significant rise of serum adjusted calcium level. So, every tuberculosis patient needs further evaluation of micronutrients status to find out the correlation with MTB.

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Conflict of interest: Nothing to declare.

References

1. Kumar V, Abbas A, Aster J. Robbins & Cotran Pathologic Basis of Disease (Robbins Pathology) 9th Edition. 9th ed. Philadelphia: Elsevier Saunder; 2015.
2. Walker B, Colledge N, Ralston S, Penmen I. Davidson's principle and practice of medicine. 22nd ed. London: Elsevier,; 2014.
3. World Tuberculosis Day 2017 [Internet]. Who.int. 2017 [cited 26 August 2021]. Available from: https://www.who.int/bangladesh/news/detail/23-03-2017 -world-tuberculosis-day-2017
4. Inocent G, Marceline N, Bertand M, Honore H. Iron status of malaria patients in douala Camer oon. Pakistan Journal of Nutrition. 2008;07(05):620-624.
5. Rodriguez G. Control of iron metabolism in Mycobacterium tuberculosis. Trends in Microbiology. 2006;14(7):320-327.
6. Satyanarayana U, Chakrapani U. Biochemistry. 4th ed. Kolkata: Elsevier a division of reed Elsevier India private limited; 2014.
7. Adebisi S, Oluboyo P, Oladipo O. The relative role of serum albumin and urinary creatinine as biochemical indices for Nigerians with pulmonary tuberculosis. African Journal of Clinical and Experimental Microbiology. 2003;4(1):72.
8. Fuss M, Pepersack T, Gillet C, Karmali R, Corvilain J. Calcium and vitamin D metabolism in granulomatous diseases. Clinical Rheumatology. 1992;11(1):28-36.
9. Bablok W, Passing H, Bender R, Schneider B. A General Regression Procedure for Method Transformation. Application of Linear Regression Procedures for Method Comparison Studies in Clinical Chemistry, Part III. Clinical Chemistry and Laboratory Medicine. 1988;26(11).
10. Webster D. Colorimetric determination of Albumin by Bromersol Green Method. Clin Chem Acta. 1974;53:109.
11. Barnett R, Skodon S, Goldberg M. Performance of "Kits" Used for Clinical Chemical Analysis of Calcium in Serum. American Journal of Clinical Pathology. 1973;59(6):836-845.
12. Gaw A, Cowan R, O'Reilly D, Stewart M, Shepherd J. Edinburgh: Churchill Livingstone; 1995.
13. Kaminskaia GO, Abdullaev R. Iron metabolism with different variants of pulmonary tuberculosis. Prol Tuberk. 2002;12:49-51.
14. Kassu A, Yabutani T, Mahmud Z, Mohammad A, Nguyen N, Huong B et al. Alterations in serum levels of trace elements in tuberculosis and HIV infections. European Journal of Clinical Nutrition. 2005;60(5):580-586.
15. Pourfallah, F., Javadian, S., Zamani, Z., Saghiri, R., Sadeghi, S., Zarea, B., Faiaz, S. h., Mirkhani, F., & Fatemi, N. (2009). Evaluation of serum levels of zinc, copper, iron, and zinc/copper ratio in cutaneous leishmaniasis. Iranian journal of arthropod-borne diseases, 3(2), 7–11.

16. Gangaidzo I, Moyo V, Mvundura E, Aggrey G, Murphree N, Khumalo H et al. Association of Pulmonary Tuberculosis with Increased Dietary Iron. The Journal of Infectious Diseases. 2001;184(7):936-939.
17. Farhana A, Guidry L, Srivastava A, Singh A, Hondalus MK, Steyn AJ. Reductive stress in microbes: implications for understanding Mycobacterium tuberculosis disease and persistence. Adv Microb Physiol. 2010;57:43-117. doi:10.1016/B978-0-12- 381045-8.00002-3
18. Drapier J, Hirling H, Wietzerbin J, Klady P, Kuhn L. Biosynthesis of nitric oxidase activities iron regulatory factor in macrophage. EMBO J. 1993;12:3643-3649.
19. Karyadi E, Schultink W, Nelwan R, Gross R, Amin Z, Dolmans W et al. Poor Micronutrient Status of Active Pulmonary Tuberculosis Patients in Indonesia. The Journal of Nutrition. 2000;130(12):2953-2958.
20. van Lettow M, Harries A, Kumwenda J, Zijlstra E, Clark T, Taha T et al. Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. BMC Infectious Diseases. 2004;4(1).
21. Held M, Bungiro R, Harrison L, Hamza I, Cappello M. Dietary Iron Content Mediates Hookworm Pathogenesis In Vivo. Infection and Immunity. 2006;74(1):289-295.
22. Onwubali J. Malnutrition among tuberculosis patients in Harrow. Eur J Clin Nutr. 1988;42:363-366.
23. Saha K, Rao K. Undernutrition in lepromatous leprosy.V. Severe nutritional deficit in lepromatous patients co-infect with tuberculosis. Eur J Clin Ntur. 1989;43:117-128.
24. Tsukaguchi K, Yoneda T, Yoshikawa M, Narita N, Enoki Y, Miyazaki R et al. Interaction between interlukin-1 and TNF production by peripheral blood monocyte and nutritional disturbance in active pulmonary tuberculosis. Kekkaku. 1991;66:447-484.
25. Joint IUAT/WHO Study Group on Tuberculosis Control and World Health Organization and International Union against Tuberculosis. Tuberculosis control : report of a joint IUAT/WHO study group [meeting held in Geneva from 14 to 18 September 1981]. World Health Organization; 1982 p. kor published by: Seoul : Korean Institute of Tuberculosis, Korean National Tuberculosis Association.
26. Beisel WR. Metabolic response of the host to infections. Textbook of pediatric infectious disease. 1998.
27. Sasaki Y, Yamahishi F, Yasi T, Mizutani F. A case of pulmonary tuberculosis with pancytopenia accompanied to bone marrow gelatinous transformation. Kekkaku. 1999;74(04):361-364.
28. Yamanaka K, Sakai S, Nomura F, Akashi T, Usni T. A nutritional investigation of homeless patients with tuberculosis. Kekkaku. 2001;76(04):363-370.
29. ROUSSOS A, LAGOGIANNI I, GONIS A, ILIAS I, KAZI D, PATSOPOULOS D et al. Hypercalcaemia in Greek patients with tuberculosis before the initiation of anti-tuberculosis treatment. Respiratory Medicine. 2001;95(3):187-190.
30. Lind L, Ljunghall S. Hypercalcemia in Pulmonary Tuberculosis. Upsala Journal of Medical Sciences. 1990;95(2):157-160.
31. Pruitt B, Onarecker C, Coniglione T. Hypercalcaemia crisis in a patients with pulmonary tuberculosis. J Okla State Med Assoc. 2022;88(12):518-520.
32. Hourany J, Mehta J, Hourany V, Byrd R, Roy T. Chest Diseases. Southern Medical Journal. 1997;90(Supplement):S133.
33. Abbasi A, Chemplavil J, Farah S, Muller B, Arnestin A. Hypercalcaemia in active pulmonary tuberculosis. Ann Intern Med. 1979;90:324-328.
34. Sharma S. Serum calcium in pulmonary tuberculosis. Postgraduate Medical Journal. 1981;57(673):694-696.
35. Dosumu E, Momoh J. Hypercalcemia in Patients with Newly Diagnosed Tuberculosis in Abuja, Nigeria. Canadian Respiratory Journal. 2006;13(2):83-87.
36. Bhandari S, Gautam S, Parajuli A, Badade Z, Potdar P. Hypocalcaemia and Hypercalcaemia in Tuberculosis. UPBS. 2022;04(03):78-82.
37. Ijaz A, Mehmood T, Saeed W, Qureshi A, Dilawar M, Anawar M. Calcium abnormalities in pulmonary tuberculosis. Pak J of Med Res. 2004;43(04):177-180.