Study of the Effect of Catechin of Green Tea Extract upon Sputum Smear Conversion Rate , Serum Malondialdehyde and Blood Iron Indices in Tuberculosis Patients

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ABSTRACT: Green tea with possessing iron chelating properties can be useful in TB treatment and management. We studied the effect of green tea consumption on iron status and improving process of pulmonary tuberculosis treatment (accelerating the negative sputum smear, reducing the level of oxidative stress). Following the approval by Ethics Committee for Human Studies of Golestan and Tehran Universities of Medical Sciences and also obtaining the written consent of patients , this double-blinded randomized clinical trial study, was conducted on patients with TB, who were assigned randomly to the intervention group (41 patients) receiving 500 mg catechin of green tea extract and the control group (39 subjects) receiving placebo for two months, since the beginning of concomitant anti-TB treatment . Sputum evaluation was carried out on three slides using the Ziehl Nelson method. At first, the demographic and dietary intake data were obtained. . After obtaining 10 ml of venous blood, Hemoglobin (Hb), Transferrin, Ferritin, Total iron binding capacity (TIBC), Iron and Serum malondialdehyde (MDA) were measured at the beginning and end of the study. Sputum samples were collected from the third week (every 10 days) and the reduction of microbial load was also tested until sputum smear became negative.Data were processed using independent and paired t-test, McNemar, Wilcoxon, Kaplan-Meier, Log-rank test and Cox regression model. P-value was taken significant as <0.05.Average daily energy intake of patients was 1518±431 kcal, distribution of which was as follow: carbohydrates (58%), protein (17%) and fat (22%).Vitamin D and Zinc intake of patients were less and iron intake was higher than the DRI. Weight changes in both groups of placebo and green tea had tendency of increase with a significant difference at two and six month follow ups (p0.0001). However, there were no significant changes due to intervention compared to placebo. Sputum conversion time (days) was 52.5± 24.5 (median= 53 days) and 40.6 ± 22.5 (median= 29 days) in placebo and catechin groups, respectively. The proportion of patients in the green tea group based on criterion of ; the short duration of being negative sputum smear; was significantly higher than the placebo group (p=0.032). To measure the mean of iron status after intervention, ANCOVA test showed mean difference level (Pvalue) in both groups for Hb, iron, TIBC, transferrin and ferritin as of: 0.004, 0.56, 0.65, 0.38 and 0.16, respectively which means that increase of hemoglobin in the green tea group was significant compared with the placebo group. There was just a 9.2 nmol/ml difference between the two groups for MDA at the beginning of study, which was not statistically significant (p=0.078) whereas, it was increased to 24.8 nmol/ml after the intervention, indicating a significant difference (p<0.001). The decline value was estimated -45.45 ± 14.69 nmol/ml for catechin group and -19.91 ± 18.38 nmol/ml for placebo group. In conclusion green tea can systematically reduce the inflammatory elements and oxidants (decrease of MDA as fatty acids oxidation indicator), and consequently, can improve the hematopoiesis and hemoglobin level. Therefore, localized inflammation and damage in the lung is reduced, and adjunct to antimicrobial therapy, accelerate sputum smear conversion, disease amelioration and treatment improvement. Finally, given the higher iron intake despite of lower micronutrients and macronutrients in diet of our patients, and considering the iron effect on mycobacterium survival and the incidence and exacerbation of inflammatory complications in patients, it seems that policy of mandatory flour fortification with iron, especially in provinces such as Golestan, must be viewed cautiously and its further implementation being revised meticulously. Email: segtesadi@gmail.comes..

Keywords: Sputum Smear, Weight, Blood Indices , Green Tea, Pulmonary Tuberculosis.

INTRODUCTION

Supportive studies about improving anti-tuberculosis treatment, indicate that special nutritional adjunct therapies, play an important role in treatment of TB or its side effects(1-15). High prevalence of TB in Golestan Province(near Caspean Sea) of Iran motivated us to use supplementation with nutritional elements such as green tea extract to promote healing and decrease secondary complications of this disease(16,19-22). Iron is basically essential for Mycobacterium tuberculosis replication within the host and its pathogenicity(24-25,28-29). Green tea with possessing iron chelating properties and its effect on gene expression of antioxidant proteins(Hepcidin and Transferrin) involved in regulating intracellular iron can be useful in TB treatment and management(30-36). Tea consumption is a part of food culture of Iranian people as a popular social entertainment drink,especially in the Golestan province. It may be useful to encourage families to drink green tea if its benefites get proven scientifically.

Polyphenols fight inflammation through inhibition of the synthesis and function of inflammatory mediators like eicosanoids and cytokines . The increase in the level of inflammatory cytokines of the plasma may be associated with malnutrition in active pulmonary TB . Oxidative stress may suppress the immune system(16-17). In patients with TB, consumption of antioxidant supplements may tackle oxidative stress(26). Green tea can protect the immune system against oxidant compounds and free radicals through controlling oxidative stress and boost its function as well . It seems that green tea may help to accelerate the process of recovery and weight gain in TB patients through decreasing oxidative stress(30-36).

Conversion of the sputum smear after two months of intensive antibiotic therapy in sputum smear positive patients is an important indicator of treatment(18) . Acceleration in sputum smear conversion helps with faster improvement and decreased probability of the transfer of TB in smear-positive patients with tuberculosis(26-27). Green tea catechin is an important potential immunotherapeutic agent against respiratory infections and negatively affects the survival of M. tuberculosis through inhibition of the NF-B pathway in vitro (13,19,37-40). For this reason, consumption of green tea or supplementation with concentrated polyphenols derived from green tea can be regarded as an adjuvant therapy in patients receiving antibiotics(41-44) .

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Considering the lack of such studies in Iran, we decided to perform this study to evaluate the effect of green tea as an adjuvant therapy on shortening the sputum smear conversion time , weight and its effect upon the malondialdehyde and blood iron indices in patients with smear positive pulmonary tuberculosis. The primary outcome of the study was the sputum smear conversion time and the secondary outcome was changes in weight and BMI within two months.

Materials and Methods:

This double-blinded randomized clinical trial study, was conducted on patients with TB, who were assigned randomly to the green tea group (41 patients) receiving 500 mg catechin of green tea extract and the control group (39 subjects) receiving placebo for two months since the beginning of concomitant anti-TB treatment(Figure1)(20-21).This study included patients with smear positive pulmonary TB, who were diagnosed with this disease, based on clinical manifestations and sputum smear postivity according to the guidelines of the Iranian Ministery of Health. The patients received Isoniazide, Rifampin, Pyrazinamide , and Ethambutol according to the DOTS(Directly Observed Treatment, Short Course)strategy.In this protocol the patients receive a 4-drug regimen at the first 2month, with a two-drug regimen in the following four month of the treatment. Sputum smear evaluation was performed at two and six month post-treatment to assess improvement. Random allocation and allocation concealment was observed. Height and weight were measured at first and two and six months thereafter. From day 20 onward, sputum smears were obtained every 10 days and the reduction of microbial load was also tested until sputum smear became negative.Sputum evaluations were performed on three slides using the Ziehl Nelsen method, and sputum smear conversion was defined as two negative slides for M.tuberculosis.Survival time(in months) was calculated from the date of diagnosis to the date of death or last follow-up. Failure was defined as death by any cause during the follow-up period, and the sputum smear conversion time of more than two months was censored in the patients. The duration of intervention was considered from the commencement of the therapy to the date of sputum conversion in the second month of the treatment(41-44).

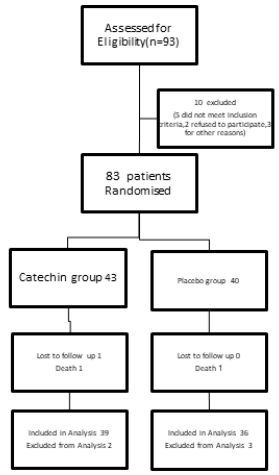

Figure 1) *Flow diagram of the study screening and selection process.*

Demographic , food frequency questionnaires and 24-hour dietary recalls of three non-consecutive days were completed. After obtaining 10 ml of venous blood, Hemoglobin (Hb), Transferrin, Ferritin, Total iron binding capacity (TIBC), Iron and Serum malondialdehyde (MDA) were measured at the beginning and the end of the study. Data were processed using independent and paired t-test, McNemar, Wilcoxon, Kaplan-Meier, Log-rank test , Cox regression model and nutrition4 software. P values < 0.05 were considered significant.

Results and Discussion:

At the beginning of the survey, two groups were similar according to age, gender and clinical status (p>0.05)(Table1).Average daily energy intake of patients was 1518± 434 kcal, distribution of which was as follow: carbohydrates (58%), protein (17%) and fat (22%).Vitamin D and Zinc intake of patients were less and iron intake was higher than the DRI

TABLE 1

Comparison of some selected nutrient intakes between Placebo and Catechin groups of TB subjects

Nutrients	Placebo	Catechin	P value
Energy(Kcal/day)	1499±15	1531±453	0.751
Carbohydrate(g/day)	221±74	219±80	0.903
Protein(g/day)	62.8±18.4	67.7±23.3	0.312
Fat(g/day)	42±22	43±18	0.723
Vitamin D(µg/day)	1.6±2.3	1.5±1.8	0.935
VitaminA(µg/day)	842±1364	852±2044	0.972
VitaminE(µg/day)	3.5±5	2.6±4.2	0.427
Fe(mg/day)	17.3±9.8	9.5±4.1	0.322
Zn(mg/day)	5.72.5	6.7±3.2	0.151

Weight changes in both groups of placebo and green tea had tendency of increase with a significant difference at two and six month follow ups (p0.0001). However, there were no significant changes due to intervention compared to placebo(Figure2)

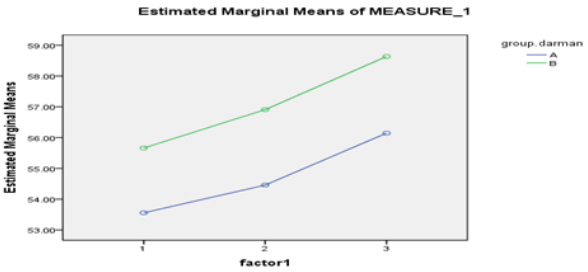

Figure 2) *Patient mean weights at Beginning, two month and the end of treatment..(A=Placebo, B= Catechin)*

Sputum conversion time (days) was 52.5± 24.5 (median= 53 days) and 40.6 ± 22.5 (median= 29 days) in placebo and catechin groups, respectively. The proportion of patients in the green tea group based on criterion of ; the short duration of being negative sputum smear; was significantly higher than the placebo group, Log Rank(Mantel-cox)=4.61, (p=0.032). (Figure3)

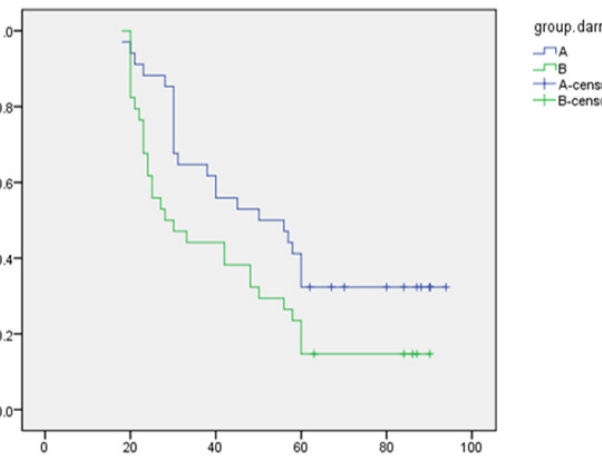

Figure 3) *Sputum smear conversion of patients (A= placebo group. B=Catechin group)*

Based on the Cox regression model, the hazard ratio of the relative risk of delay in sputum smear conversion was 3.7 (CI 1.7-8.6, p=0.002) in the higher microbial load group compared to the placebo group and 0.54 (CI 0.31-0.94) in the intervention group compared to the placebo group(Figure4)

Considering the status of the sputum microbial load, the time to sputum smear conversion is expected to be shorter in the scanty and +1 group versus the +3 group. The two groups of GT and placebo were compared for days to sputum smear conversion considering the microbial load of the sputum at the beginning of the study. (Figure4).Therefore ,it was found that the sputum smear conversion time was significantly shorter in the GT group than the placebo group (p=0.031) (Figure3),and the risk of delay in sputum conversion augmented with the increase in the microbial load and decreased with the consumption of green tea(Figure4).

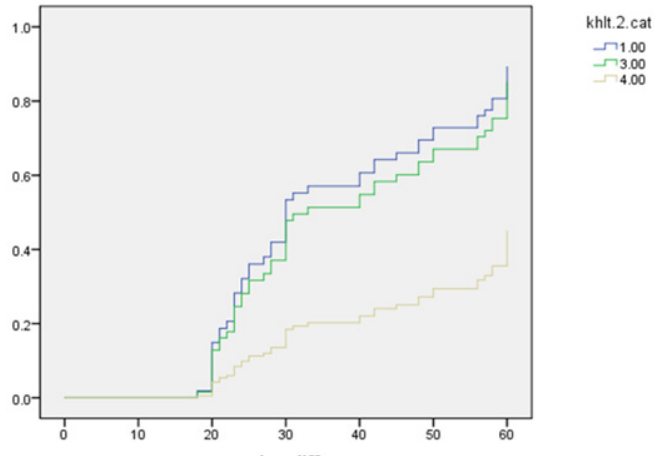

Figure 4) *Sputum smear conversion of patients according to smear grading.(1= Scanty and grade 1. 3= grade 2. 4= grade 3.)*

To measure the mean of iron status after intervention,ANCOVA test showed mean difference level (P value) in both groups for Hb, iron, TIBC, transferrin and ferritin as of: 0.004, 0.56, 0.65, 0.38 and 0.16, respectively which means that increase of hemoglobin in the green tea group was significant.

compared to the placebo group. There was just a 9.2 nmol/ml difference between the two groups for MDA at the beginning of the study, which was not statistically significant (p=0.078) whereas, it was increased to 24.8 nmol/ml after the intervention, indicating a significant difference (p<0.001). The decline value was estimated -45.45 ± 14.69 nmol/ml for catechin group and -19.91 ± 18.38 nmol/ml for placebo group.

To our khowledge ,our study might be the first to be conducted on the use of green tea extract as a supplement in the treatment of tuberculosis in Iran.The present survey showed the Green tea can systematically reduce the inflammatory elements and oxidants (decrease of MDA as fatty acids oxidation indicator), and consequently, can improve the hematopoiesis and hemoglobin level. Therefore, localized inflammation and damage in the lung is reduced, and adjunct to antimicrobial therapy, accelerate sputum smear conversion, disease amelioration and treatment improvement. If researchers implement catechin as an adjuvant with antibiotic therapy as well as

nutritional modification especially effective micronutrients on the immune status of patients, more improvement of disease treatment might be achieved.

Our study well documented the effect of catechin on sputum smear conversion. The microbial load of the sputum decreased faster in patients who received catechin compared to the control group.In fact high grade of smear positivity was associated with failure in sputum smear conversion, due to the higher initial bacterial load that required a longer time for clearance in the early stages of treatment.In our study a higher microbial load was correlated with delay in sputum smear conversion.It seems that the contribution of green tea to decreasing the inflammatory status of the patients has a little effect on improving the nutritional status of the patients when compared to the effect of antibacterial therapy. Some studies have evaluated the status of the inflammatory indices. For example, Kim et al reported that the levels of TNF-α and IL-6 were higher in malnourished TB patients than the control group and well-nourished TB patients (31) and the level of C-reactive protein (CRP) and the proportion of CRP to albumin was lower in patients not afflicted with TB than TB patients 30 and 60 days after treatment (32).

Numerous studies have highlighted the role of green tea, especially its catechins, in regulating inflammatory reactions (21, 33-35). Green tea entraps reactive oxygen species (ROS) and is protective against oxidative stress. Agarwal et al showed the effect of green tea extract on the reduction of oxidative stress in patients receiving anti TB treatment (23).

In short,in our study green tea had a little effect on weight gain but because its catechins contents are natural compounds with relatively safe profiles, the use of green tea as an adjuvant therapy in TB patients may be a new method for faster rehabilitation of the patients through shortening the time to sputum smear conversion.

The main limitation of our study was the lack of sputum culture, which is considered the gold stabndard of TB diagnosis. In addition, difficulty obtaining sputum spcimens during treatment, due to the clearance of the patients'lungs,was another limitation of the sudy. Finally , considering the DOTS protocol,few patients were asked to take X-ray to follow the treatment.

It is important to mention that given the higher iron intake despite of lower micronutrients and macronutrients in diet of our patients, and considering the iron effect on mycobacterium survival and the incidence and exacerbation of inflammatory complications in patients, it seems that policy of mandatory flour fortification with iron,especially in provinces such as Golestan, must be viewed cautiously and its further implementation being revised meticulously

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