# A Study to Find the Correlation between Blood Groups and Sputum Submission Protocol in the Diagnosis of Pulmonary Tuberculosis among School Going Children

## Rajesh Potti[1], Gunta Raja Sekharam[2]

[1]Associate Professor, Department of Physiology, Alluri Setharama Raju Academy of Medical Sciences (ASRAMS), Eluru.
[2]Assistant Professor, Department of Pulmonary Medicine, Government Medical College, Srikakulam.

**Abstract**

**Introduction:** Several studies have been conducted to find the correlation between tuberculosis (TB) and blood groups. A study was conducted with an aim to find the correlation between the blood groups and sputum submission in the school going children.

**Methods:** It was a prospective research conducted in ASRAMS, Eluru. Study protocol was approved by the institution committees. Informed consent was taken from the parents of all the study members. School going children from class 6 to 10 with signs and symptoms of TB were included in the research. Importance of submission of sputum, difference between sputum and saliva was shown practically. After clarifying all the doubts, the participants were explained how to produce good quality sputum sample. Simultaneously it was demonstrated practically. Then the participants were provided with sterile, plastic containers and they were asked to collect the sample and stained as per the guidelines. Simultaneously blood groping was done for all the study members by slide agglutination test. Chi square test was used to find the statistical analysis. P <0.05 was considered to be statistically significant.

**Results:** The mean age of the study child was 14. 3 years. Among the study members, 61 satisfied the sputum submission protocol; gender wise there was no significant difference. Out of the 11 (100%) TB cases, maximum was AB+ve (4) followed by A+ve (3), O+ve (3) and A-ve (1); statistically there was no significant difference.

**Conclusion:** More number of girl children satisfied the sputum submission protocol. Maximum TB cases were detected in AB+ve group and minimum in A-ve; statistically there was no significant difference. Long term studies with big sample size are recommended.

**Keywords:** Tuberculosis, Blood Group, Child, Group.

## Introduction

Mycobacterium tuberculosis (MTB) complex is the causative agent of tuberculosis (TB). This white plague causes significant financial burden to the globe. India is one of the high TB burden countries. [1] In 2019, 10 million people were infected with TB; 95% in this was from low and middle income countries. [2] To answer this issue, World Health Organization has developed Directly Observed Treatment Short-course (DOTS), cost effective TB control strategy. This consists of combination of antimicrobials

such as isoniazid, rifampicin, pyrazinamide and ethambutol to treat drug susceptible *MTB.*

Sputum Smear microscopy (SSM) is a simple, rapid and inexpensive technique with its high specificity. [3] In very high TB burden areas, this technique is widely applicable in various populations with different socioeconomic levels. Hence, it has been an integral part of the global strategy for TB control and RNTCP. At this point, sputum submission protocol is the most important influencing factor of the SSM results.

Several studies have been conducted to find the correlation between TB and blood groups. [4] School going children are in a stage to listen as well as things with their high grasping capacity. Hence a study was conducted to find the correlation between the blood groups and sputum submission in the school going children.

## Methods

It was a prospective research conducted in ASRAMS, Eluru. Study was conducted for 3 months, August to October 2021. Study protocol was approved by the institution committees. Informed consent was taken from the parents of all the study members.

School going children from class 6 to 10 with signs and symptoms of TB were included in the research. Adults and non-cooperative children were not considered in this research. Initially the study was explained in local language. The importance of submission of sputum sample, the difference between sputum and saliva was shown practically. After clarifying all the doubts, the participants were explained how to produce good quality sputum sample. Simultaneously it was demonstrated practically. Then the participants were provided with sterile, plastic containers and they were asked to collect the sample.

**Collection of Sputum sample:** Sputum samples were collected in an open place by coughing. Initially, patient should inhale deeply two to three times with mouth open, cough out deeply from chest, and sputum sample was spitted out directly in the sterile, new, leak-proof sample container. Then, the container was closed tightly.

**Smear preparation:** New unscratched slides were selected for smear preparation. Smear was prepared with sterile loop. A good smear is spread evenly, over a size of $2 \times 3$ cm and is neither too thick nor too thin. This was allowed to air dry for 15–30 min and fixed by passing it over a blue flame 3–4 times. [5]

**ZN Staining:** Smears were flooded with filtered 1% Carbol Fuchsin (CF) and heated until they were steamed and left to steam for 5 min. After rinsing the slides with a gentle stream of water, 25% sulphuric acid ($H_2SO_4$) was used to decolorize the smears for 2 to 4 min, and if necessary, the decolorization step was repeated for another 1–3 min. The slides were rinsed as mentioned earlier and counterstained with 0.1% methylene blue (MB) for 30 s. The slides were then washed, air dried, and examined under oil immersion. The reagents for ZN staining were prepared as per the RNTCP guidelines (1998). [5] Simultaneously blood groping was done for all the study members by slide agglutination test. [6]

**Statistical analysis:**

The data were analysed using SPSS version 21. Chi square test was used to find the statistical analysis. $P < 0.05$ was considered to be statistically significant.

**Results**

Total 83 children were included 8 were dropped. The data were analysed with 75 (100%). The male female ratio was 29 and 46 and the mean age of the study child was 14. 3 years. Among the study members, 61 satisfied the sputum submission protocol; gender wise it was 26 and 35 respectively

in male and female; statistically there was no significant difference (Table 1).

In this research, 11 were found to be smear positive (SP). Among the SP cases, 8 were male and 3 were female; the male female ratio was 2.7. Out of the 11 (100%) SP cases, maximum were AB+ve (4) followed by A+ve (3), O+ve (3) and A-ve (1); statistically there was no significant difference.

**Table 1: Gender wise sputum submission protocol among the study members; n (%)**

| Gender | Satisfied | Not satisfied | Total |
|---|---|---|---|
| Boys | 21 | 8 | 29 |
| Girls | 40 | 6 | 46 |
| Total | 61 | 14 | 75 |
| Statistical analysis | Chi square: 2.4777; P = 0.1157; Statistically not significant | | |

## Discussion

Total 83 children were recruited but 10% (8) were dropped. Patient dropout (PD) is very common in health care sector. As per Desalegn DM et al. [7] there were 5.3% PD among the SP cases and it was 6.2% in another report. [8] Both the studies were from western world, developed countries compared to India. Inspite of the development, significant number of PDs were reported. Whereas it was reported to be 5.3% in one of the Indian report. [9] Financial issue, loss of working days are the common reasons for the patient PD. In this research school going children were included so we feel there was no financial issue. But the cause was found to be the same. When it was enquired we found that the children were not ready to miss the afternoon food provided by the government in the school under midday meal program. With this, it is very clear that PD is the common scenario in the medical field.

Gender wise, more number of girl child satisfied the sputum submission protocol (Table 1); statistically there was no significant difference. [10] Though statistically there was no significant difference, we should consider this point because the sputum submission protocol is the most important influencing factor of SSM in the diagnosis of TB. [11] Because if the quality of sample is good automatically it leads the false negative result. If not diagnosed, each individual with active TB case spread disease 10 – 12 per annum which can increase significant financial burden on the program. [12] In this research, ZN staining was used for the diagnosis of TB. The specificity of the technique is high, and care was taken to get the correct sputum sample as per the study protocol. Moreover, it was research report hence there were no involvement of technical members. So, there is no missing of the cases because care was taken in each step of the study in the entire study period.

Out of the 11 (100%) SP cases, maximum was AB+ve (4) followed by A+ve (3), O+ve (3) and A-ve (1); statistically there was no significant difference. Chen H et al. [13] mentioned that AB blood group is at risk in developing TB; the exact reason for high prevalence of TB in AB blood group system were not clear. Various reports are available in the literature on correlation between different blood groups and various infectious diseases. However limited literature is only available on this point. Hence further research is required for long time with high sample load to find the correlation between the blood groups and TB infection. [14]

## Conclusion

More number of girl children satisfied the sputum submission protocol. Out of the 11 SP cases, maximum was AB+ve (4) followed by A+ve (3), O+ve (3) and A-ve (1); statistically there was no significant

difference. Long term studies with big sample size are recommended.

**Author's contribution:**

Rajesh Potti: Literature review, article writing, bench work.

Gunta Raja Sekharam: Statistical analysis, article writing.

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