Original article

Tuberculosis and Haptoglobin Phenotypes Association in Sudan

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Abstract

Tuberculosis (TB) is one of the world's deadliest communicable diseases. The infection by Pulmonary Tuberculosis (PTB) especially multidrug resistant pulmonary tuberculosis (MDR-PTB) in Sudan is increasing. The susceptibility of patients to infection and certain pathologies is associated to some host factor: age, gender, previous TB infection and haptoglobin (Hp) phenotype. The objectives of this study were to determine the association of these factors to PTB and MDR-PTB and to determine if the susceptibility to PTB and MDR-TB is related to specific Hp phenotype. In a case-control study 300 tuberculosis patients and 50 non-tuberculosis participants as healthy control were recruited, phenotyping of Hp was performed for 195 TB patient and 50 healthy control participant by polyacrylamide gel electrophoresis. In this study 140 (46.7%) TB cases were found MDR-PTB cases and 160 (53.3%) were sensitive to anti-TB drugs. Comparisons were made using chi2 test at α= 5%, the results showed that out of 140 MDR-PTB cases; males were more infected by MDR-PTB than females 97(69.3%) and 43(30.7%) respectively; there is no significant difference in getting the MDR-PTB infection (P=0.22), the age group 16-30 years had the highest frequency of MDR-PTB, the difference between age groups was insignificant (P=0.41), (32.9%) of MDR-PTB patients were new infection case and (67.1%) were retreatment cases (relapse, defaulter and failure cases), the retreatment was highly associated to MDR-PTB (P=0.00).The haptoglobin phenotype (2-1) was the dominant type in the healthy control group and in PTB patients; (58%) and (53.3%) respectively, Hp2-2 was (22%) in controls and (23.6%) in patients, Hp1-1 (20%) in controls and (21.5%) in patients, and Hp0-0 was detected only in patients group (1.5%), there was no association between Hp phenotype and PTB infection (P=0.9); also no association between Hp phenotype and getting the MDR strain of M.tb was detected (P=0.11), The study concluded there was no association between gender, age and Hp phenotype and TB infection, although (32.9%) of MDR-PTB patients were new infection; the strong association has been observed between anti-tuberculosis drugs resistance and previous treatment cases.

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Introduction

Tuberculosis (TB) is a major cause of ill health and one of the leading causes of death worldwide. It is a one of zoonotic disease. Until the corona virus (COVID-19) pandemic, Tuberculosis was the leading cause of death from a single infectious agent, ranking above HIV/AIDS. The disease affects the lungs (pulmonary TB) but can affect other sites such as bones, lymph node (Extra-pulmonary). Most people (about 90%) who develop the disease are adults and more cases among men than women. About a quarter of the world's population is infected with *M.tb*, the probability of developing TB disease is much higher among HIV patients, and among people affected by risk factors such as under nutrition, diabetes, smoking and alcohol consumption. Tuberculosis is curable and preventable; about 85% of people who develop TB disease can be successfully treated with a 6-month drug regimen (WHO, 2021).

Sudan harbors a high TB incidence among the WHO East Mediterranean Countries. According to WHO report, (2016a);

TB incidence rate in Sudan was 25-99 new TB cases per 100, 000 population per year, in 2017 the incidence rate was increased to 53-105 new cases per 100, 000 population per year (WHO, 2018a), the incidence rate was 50-99 new TB cases per 100, 000 population per year in 2020 (WHO, 2021). In the Fourth Global Report on anti-tuberculosis drug resistance the MDR rate amongst new TB cases in Sudan is estimated at 1.9% while the rate among previously treated cases is estimated at 9.8% (WHO, 2008). Total number of confirmed MDR TB cases by culture and DST is 90 (Sudan Federal Ministry, 2012). The pooled prevalence of TB cases with resistance to any anti-TB drugs (during 2002–2020) was 47.0% (95% CI: 35.5–58.6%). The overall prevalence of mono, multi, poly and extensive drug resistance were estimated to be 16.2% (95% CI: 9.0–23.4%), 22.8% (95% CI: 16.0–29.7%), 6.8% (95% CI: 0.5–13.0%) and 0.7% (95% CI: 0–2.1%), respectively. Considering any first-line anti-TB drugs, the resistance prevalence was highest for isoniazid (32.3%) and streptomycin (31.7%), followed by rifampicin (29.2%). In contrast, resistance against second-line drugs was reported for only two antibiotics, namely, ofloxacin (2.1%) and kanamycin (0.7%) (Hajissa *et al*., 2021).

Haptoglobin (Hp) is a glycoprotein with antioxidant and immune modulatory properties in humans (Mark and David, 2016). It is a plasma alpha-2 sialoglycoprotein synthesized by the liver (Langlois and Delanghe, 1996), is made up of two dissimilar constituent α and β polypeptide chains (Smithies, 1955). The β chains are identical in all individuals, while α chains are polymorphic, with two predominant alleles, *Hp*1 and *Hp*2, having incomplete dominance. The three predominant phenotypes of haptoglobin are Hp 1-1, Hp 2-1 and Hp 2-2 (Smithies *et al.,* 1962). Several additional haptoglobin phenotypes have also been described (Langlois and Delanghe, 1996), each of which has a functional specificity that can have a different impact on the prognosis of certain pathologies (Mark and David, 2016). A genetically conditioned absence of haptoglobin, Hp 0-0, is present in one in a thousand Caucasians (Delanghe and Langlois, 1998a) and may be more frequent in Blacks (Raymond and Fletcher, 1983). The major function of the haptoglobin molecule is to bind plasma haemoglobin, thereby preventing its loss and that of iron by glomerular filtration (Langlois and Delanghe, 1996). Hp binds with hemoglobin (Hb) and form an Hp-Hb complex that plays an important role in the defense of the organism; while iron is necessary for bacterial growth, it's binding by the Hp-Hb complex reduces its extra-erythrocyte availability and slows down the bacterial growth (Mark and David, 2016). This binding is phenotype- dependent, with Hp 1-1 being more efficient than Hp 2-2. The Hp 2-2 phenotype is a weaker antioxidant than the other phenotypes (Langlois and Delanghe, 1996). Healthy individuals expressing the Hp 2-2 phenotype show some degree of iron accumulation, as indicated by greater iron concentrations in monocytes and macrophages and higher levels of serum ferritin and transferrin saturation (Delanghe and Langlois *et al.,* 1998b).. Haptoglobin polymorphism has been considered a candidate factor in the pathophysiology of tuberculosis (Fedoseeva *et al.,* 1993). In one study of 74 patients with recurrent pulmonary tuberculosis, the Hp 2-2 variant was over represented in comparison with other phenotypes (Eisaev, 1995), while in another study, patients with pulmonary tuberculosis typed as Hp 2-2 exhibited large cavities due to tissue destruction, advanced dissemination and poor prognosis (Fedoseeva *et al.,* 1993). There are no reports regarding a possible association between Tuberculosis and haptoglobin polymorphism in Sudan. Studying and analysis of factors that concern to the host susceptibility to TB infection and the effect of non-adherence to the treatment regimen is very important which may reveal more potential strategies for TB and MDR-TB control.

Materials and Methods:

A case-control study was performed to study the association between MDR-PTB and gender, age and treatment adherence and compare the distribution and characteristics of Hp phenotypes in tuberculosis and healthy subjects.

The study population:

The study population was TB patients receiving treatment at Omdurman Educational Hospital for chest diseases (Abu anja) during the period November 2019 - November 2022 and voluntary blood donors from the Tropical Medicine Research Institute (TMRI). The study was conducted with the authorization from the Human Ethical Committee of Tropical Medicine Research Institute (TMRI). Written informed consent was obtained from all participants. 300TB patients and 50 healthy donors were recruited. For each of the patients included, the consent form and questionnaire were filled, followed by a venous blood sampling on a plain container. Phenotyping of Hp were carried out at the Tropical Medicine Research Institute (TMRI).

Sample collection

The biological specimen was whole blood obtained through venipuncture on a plain blood container. The serum obtained after centrifugation was used for the haptoglobin phenotyping.

2.3. Haptoglobin phenotyping:

Discontinuous Polyacrylamide gel electrophoresis (non-reducing) according to Davis and Ornestein (1968) method which was modified by Linke, (1984) was applied using the Mini- V 8.10 (BRL, Life Technologies Inc, Gaithersburg, USA) to detect the haptoglobin phenotypes briefly; after preaparing the resolving gel and stacking gel into the gel cassette ten micro-liter of serum sample was added to a mixture of 4 µl of the Haemolysate and 5 µl of the loading buffer. Then 10 µl from each prepared mixture was added to each well. The loaded gels were then placed in the electrophoresis tank which was filled with running buffer, then a voltage of 80 V was applied and the run was continued till the dye front reach the resolving gel level. The voltage then was elevated to 100 V and the run was continued till the blue line pass out of the gel. Very faint yellow bands remain on the gel and the run continued till it reaches 1cm from the bottom of the gel. Then the electrodes were disconnected, the cover of the tank was removed, the gels were transferred carefully to clean container and covered with electrophoresis buffer to prevent drying. After that the gels were carefully handled and kept in benzidine stain for 15 -30 minutes, till the bands appear and then the stain replaced with distilled water and then was photographed.

Statistical Analysis:

The data collected was entered using SPSS Software v20.0. The qualitative data such as age group, Gender and patient treatment status were tabulated as percentage. The proportions of Hp phenotypes obtained were expressed as percentage and compared between the two study populations: tuberculosis patients and healthy subjects. The statistical analysis was carried out using the same software and the comparisons were made by the Chi –Square test.

Results

Demographics Characteristics

In this study, 350 individual were recruited, comprising from 50 non-tuberculosis as healthy control subjects and 300 tuberculosis patients. The 50 controls included male (40%) and female (60%), age range was 23 and 50 years. The tuberculosis patients also were male (72.7%) and female (27.3%); their age was ranged from 12 to 80 years old with different PTB infection case (Drug Susceptible (160 patient) and Multi Drug Resistant (140 patient).

Prevalence of MDR-PTB according to the gender:

The study showed that males were more infected by MDR-PTB than females (69.3%) and (30.7%) respectively, no significant difference between male and female in getting the MDR-PTB infection (*P=0.22*), Figure (1).

Prevalence of MDR-PTB according to the age group:

The study showed that the age group 16-30 years old has a highest frequency of MDR- PTB infection, there was no significant difference between age groups in infection by MDR-PTB (*P=0.41*), figure (2).

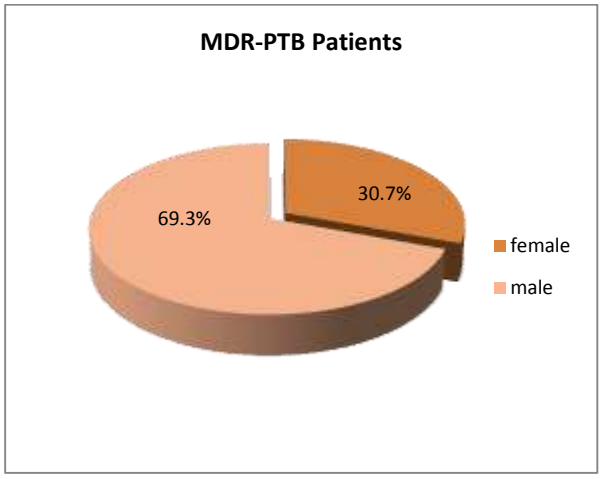

Figure (1): The prevalence of MDR-PTB according to gender

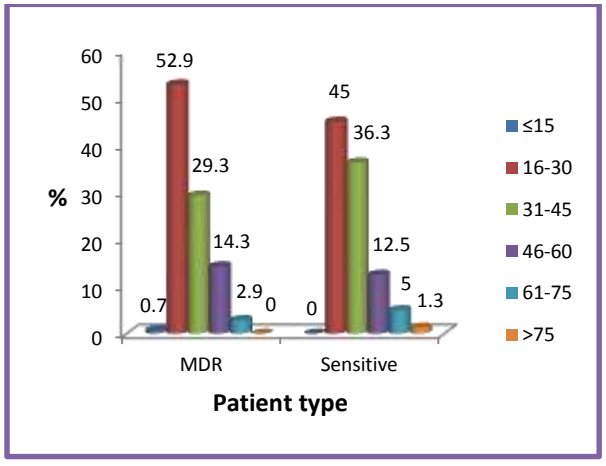

Figure (2): The prevalence of MDR-PTB according to age group

The prevalence of MR-PTB according to TB patient type:

In this study, 46(32.9%) of MDR-PTB patients were new infection case and 94(67.1%) were retreatment cases (relapse, defaulter and failure cases), Out of the total MDR-PTB (140 case) 37(26.4%) were relapsed, 29(20.7%) were defaulter of the treatment, 28(20%) failure cases and 46 (32.9%) were new infection case. There was highly significant association between the retreatment case and getting the MDR-PTB infection (*P=0.00*), figure (3).

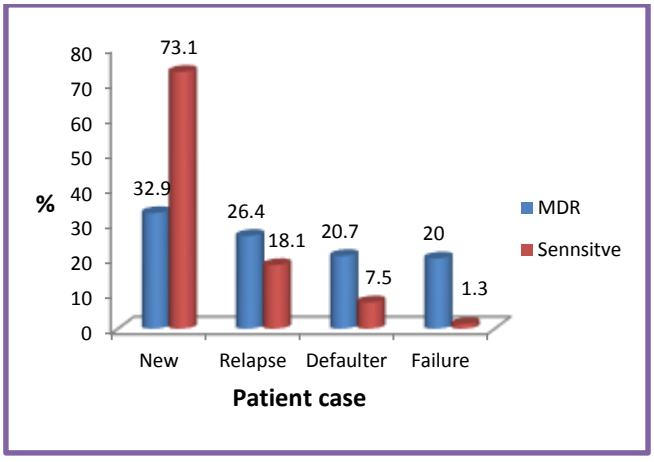

Figure (3): The prevalence of MDR-PTB according to TB patient case

Haptoglobin Phenotyping:

In this study the four type of haptoglobin were found figure (4). The haptoglobin phenotype (2-1) was the dominant phenotype among both TB cases and controls (53.3%) and (58%) respectively, followed by Hp2-2 with frequency of (23.6%) in TB cases and (22%) in controls, Hp1-1 was (21.5%) in cases and (20%) in controls. Hp0-0 was detected in four patients only with (1.5%), figure (5), there was no association between Hp phenotype and infection by PTB (*P=0.9*), also there was no association between Hp phenotype and getting the MDR strain of *M.tb* (*P=0.11*), figure (6).

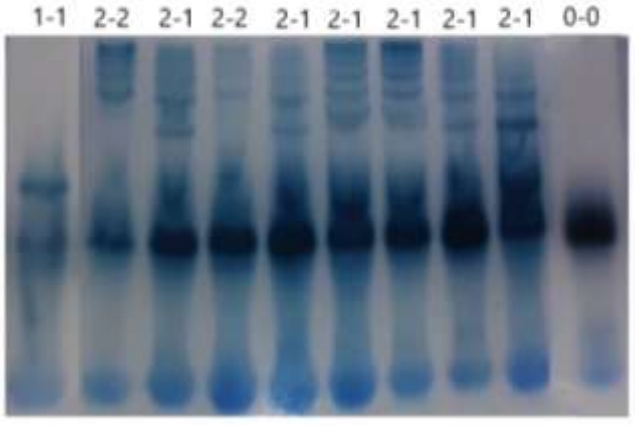

Figure (4): Haptoglobin phenotypes of the study groups

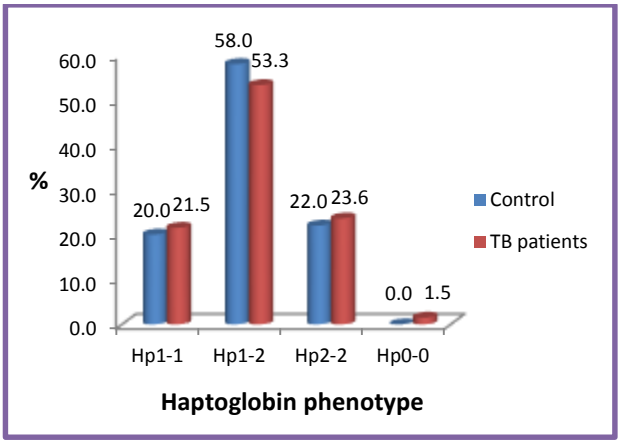

Figure (5): Distribution of Haptoglobin Phenotype in control and PTB patients

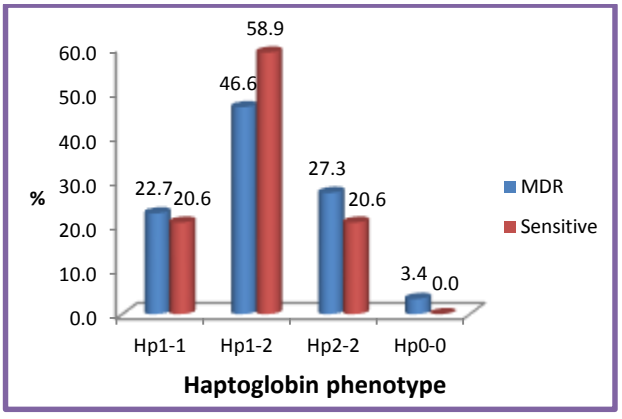

Figure (6): Distribution of Haptoglobin Phenotype in PTB patients

Discussion:

MDR-TB has become a serious problem in TB treatment and control worldwide because of delayed diagnosis and its resistance to many first-line drugs. Urgent efforts are needed to improve the coverage and quality of diagnosis, treatment, susceptibility factors and immunity of people with TB or drug-resistant TB, (WHO, 2018).

This study showed that males were predominant TB patients than females; 218 (72.7%) and 82 (27.3%) respectively and were recorded the highest percentage of MDR-PTB than females 97(69.3%) and 43(30.7%). This finding is in agreement with the previous studies conducted in Sudanese; Elsony, (2003), Elmutasim, (2005), Elhassan, (2009), Khalid, (2009), Eldirdery *et al*., (2017) and varied population; Telzak *et al.*, (1995), Yew *et al.*, (2000), Bellamy, (2000). Datta *et al.*, (2010) in India reported that an X- chromosome susceptibility gene may contribute to excess of males with tuberculosis observed in many populations, these results suggest that many genes may be involved in determining host susceptibility to tuberculosis (male have only one copy of X chromosome while diploid female are subject to X chromosome inactivation in addition, the X chromosome codes for many immune-related gene; this support the hypothesis of X-linked genes). This result may also had been due to social behavior, females fear of social stigma beside the treatment seeking behavior, when females don't decide by themselves to seek medical assistance and don't go to investigation and treatment and reject the infection these behaviors may hide the true number of infected females. However, although males excess of several risk factors that are associated with MDR/RR-TB, such as non-adherence to complete the treatment, alcohol consumption and smoking, whereas these factors are more common in males, hence male TB patients may have a higher relative risk of MDR/RR-TB than female TB patients but based on Chi-square statistic test derived value P=0.22 > (α=0.05); results can be concluded that there is no significant relationship between gender and MDR-PTB infection in tuberculosis patients. This result supported by other research which showed that there is no significant relationship between MDR-TB incidence and gender (Elmi *et al*., 2015) and also similar to McQuaid *et al*., (2020) study findings as it found that the risk of MDR/RR-TB, among those with TB, is the same for males as for females and it conclude that while males are at greater risk than females of developing TB, males with TB are at no greater risk of MDR/RR-TB than females with TB. The study results are also consistent with previous global analyses suggesting that males with TB are no more at risk of MDR/RR-TB than females (WHO, 2010; Pradipta *et al*., 2018).

In this study all age groups were infected by PTB with varying frequencies and the most infected ages was young adults

ranged 16-30 and 31- 45 years old. The age group 16-30 years recorded the highest frequency of PTB infection 143 (48.7%), followed by 31-45 99 (33.1%) and also had the highest frequency of MDR- PTB infection 74 (52.9%), 41 (29.3%) respectively, however, the statistical analysis showed that there was no significant difference between age groups in infection by MDR-PTB. Murray *et al*., (1990) documented that 80% of the TB populations in developing countries were under the age of 50 years, where as in developed countries most patients were elder. In accordance to this finding, previous studies conducted in Sudan by Elmutasim, (2005), Elhassan, (2009) and Khalid, (2009), in India by Sinha *et al*., (2017) and Shivikar *et al*., (2020) and in Amhara region by Workicho *et al*., (2017) and Getahun and Gebiyaw, (2021) all stated that all age groups were infected but the highest frequency of infection was among the **PRODUCTIVE AGE GROUP** (26-45years) which adversely affect the income and the quality of life of the families and their ability to get the proper drug or to afford its long term-need expenses.

The acquired drug resistance of *M. tuberculosis* to anti-TB drugs can occur when there is a history of incomplete or inappropriate TB treatment regimens, this may be because prior inadequate anti-TB treatment only suppresses the growth of susceptible bacilli and does not affect other resistant strains, leading to suitable conditions for the dominant multiplication of pre-existing drug-resistant mutants, which is a rise and fall phenomenon. In this study the occurrence of MDR-TB was strongly associated with previous treatment with anti-TB drugs, when the patients were classified according to their PTB case status they were found 163 (54.3%) case were new infection and 137(45.7%) cases were retreatment cases, out of the retreatment cases 66(48.2%) were relapsed, 41(30%) were defaulter of the treatment and 30(21.8%) failure cases. 46 (32.9%) of MDR-PTB patients were new infection case and 94(67.1%) were retreatment cases; (37(39.4%) relapse, 29(30.9%) defaulter and 28(29.7%) failure cases)), there was highly significant association between the retreatment of TB and getting the MDR-PTB infection (*P=0.00*). Globally this result is consistent with previous findings stated that significant association between history of treatment with resistance of anti-tuberculosis drug in TB patient in different population ((Faustini, 2006), (Gunther *et al*., 2015), (Sinha *et al*., 2017), (Sapriadi and Syahridha, 2018) and (Shivekar *et al*., 2020)).This finding was consistent also with several previous studies conducted in Sudan by Zaki and Ibrahim, (2004) and WHO, (2010).

Out of total MDR-PTB (140 case) 37(26.4%) were relapsed, 29(20.7%) were defaulter of the treatment, 28(20%) failure cases and 46 (32.9%) of MDR-PTB patients were new infection case. From this result; the appearance of new MDR cases with high percentage comparing to the remain MDR patient case categories is serious and dangerous sign because this result may be due to the high spread of MDR PTB stains in community either from patient whom were MDR patients and they were under treatment but move freely in community or from unknown MDR cases whom were not discover yet as some people had symptoms but they don't went to investigation.

In this study all the Hp phenotypes were detected with the highest frequency of Hp2-1 among both tuberculosis patients and control group. No association was observed between Hp phenotype and infection by PTB (*P=0.9*), also no association between Hp phenotype and getting the MDR strain of *M.tb* (*P=0.11*) was detected in the examined subjects, the present results showed that the common haptoglobin phenotype among all TB patients was Hp2-1. Since this phenotype was similar to the healthy controls, therefore the risk of TB infection is higher among people with Hp2-1. This result is comparable to that obtained by Kaminskaia *et al.,* (1997) in Russian subjects and Kasvosve *et al.,* (2000) in Zimbabwean subjects with pulmonary tuberculosis there study concluded that Hp 2-1 is the commonest phenotype in healthy control and TB patients and there was no association between the Hp phenotypes and susceptibility to TB infection. The haptoglobin phenotype frequencies in the patients with MDR tuberculosis were similar to those in the sensitive, suggesting

that haptoglobin phenotype was not a factor modulating susceptibility to clinical pulmonary tuberculosis. The study result is disagreed with study finding of Fatoumata *et al*., (2018); there was an association between the phenotype of Hp and the frequency of TB infection and the presence of allele Hp2 seemed to be associated with a high rate on mortality of TB.

Conclusion

This study concluded male and middle-aged patients in the study population were more probably infected by *M.tb* strains. However, many factors related to patients characteristics such as age and gender were not statistically associated with MDR-TB in this study. The absence of a statistical association between these factors and MDR-TB in this study does not mean that they are not important factors affecting the occurrence of MDR-TB. These findings may be due to possible variations in the epidemiology of the disease and people`s life style and attitudes such as malnutrition, immune and health status, weaning period of females, seeking treatment or fear of social stigma.The strong association of resistance with previous treatment observed and the high percentage on MDR-PTB in new cases patients suggests that improved monitoring of treatment and direct observation of therapy should be implemented to limit the emergence of drug resistant disease.

Four phenotypes of Hp were found in Khartoum- Sudan: Hp1-1, Hp2-1, Hp2-2 and Hp0-0. There was no association between the phenotype of Hp and the TB infection, also there was no association between Hp phenotype and getting the MDR strain of *M.tb*, the present results showed that the common haptoglobin phenotype among TB patients (DS and MDR) was Hp2-1.

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