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Knowledge and awareness among college students about the importance of iron blockage in tuberculosis-A survey

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ABSTRACT

Tuberculosis threatens to destroy millions across the globe. There is an urgent need for new methods to prevent and treat the disease. Similar to most microorganisms, *Mycobacterium tuberculosis* - the causative agent of tuberculosis, requires iron for important metabolic pathways. Since iron is not readily accessible in the host, pathogens must compete aggressively to create an infection for this metal, but they must also carefully regulate the acquisition of iron, as excess free iron can be highly toxic. The aim of the study was to create awareness of iron blockage and stop tuberculosis among college students. A descriptive survey was conducted among various college students using questionnaires in google forms. The questionnaire consists of 16 questions were framed based on the knowledge and awareness of iron knowledge to stop tuberculosis and receive a response from 100 participants. In this survey, 67.53% were aware of *Mycobacterium tuberculosis* cause tuberculosis in the human body and 59.74% were aware that bacteria which causes tuberculosis to need iron to survive but 27.27% were not aware. Majority of participants were aware that iron was capable of restarting replication and refractive to antibodies and trigger the state of persistence bacteria. Nearly 57.14% of participants *Mycobacterium tuberculosis* has a remarkable ability to persist in the absence of Fe, but 19.48% stated it does not survive. By this current survey, it can be evident that the majority of participants are educated about knowledge of tuberculosis associated with causes, main role and physiological mechanisms involved in it.

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INTRODUCTION

The most devastating pathogens that live inside human cells are *Mycobacterium tuberculosis (M. tuberculosis)*, which causes tuberculosis which acquires iron from the host to cause infection, so it releases high-affinity iron-binding siderophores called exochelins (Gobin *et al.*, 1995). Mycobacterium tuberculosis, highly successful pathogen undergo two pathway (i.e.) At first, it stops the normal progression of phagosome into an acidic, hydrolytically active compartment and secondly, it avoids the development of localized, productive immune response which activates the host

cell (Russell, 2001). Mycobactin can be used as a short term storage molecule for iron transfer. For transport across the cell membrane, a reductase is used which converts Fe III mycobactin to Fe II form. The ferrous ion complexed with salicylic acid into various porphyrins and apoproteins for storage of iron within the bacterial cytoplasm, bacterioferritin (Ratledge, 2004; Schaible and Kaufmann, 2004; Abigail *et al.*, 2019). (David, 2019) Most recently Arnold and his co-workers have stated how iron mechanisms of transport occurs in bacteria which describes that when a human cell is infected, it reduces the iron concentration to a minimum and thereby tries to starve the invader (i.e.) bacteria, in turn, start to release small mycobactin, this would bind free iron extremely well and thus steel it from a host cell. The iron captured by mycobactin is then transported into the bacteria by a protein named Irt Ab. If it is absent or not functioning properly, *M.tuberculosis* can no longer reproduce inside the human cell (Arnold, 2020; Iyer *et al.*, 2019). *M.tuberculosis* containing phagosomes has early endosomal characteristics interacts with early endosomes and does not acidify below PH 6.3-6.5 (Mwandumba *et al.*, 2004; Schaible and Kaufmann, 2004). *M. tuberculosis* perceives the phagosome as a low-iron environment (Voskuil *et al.*, 2003; Appelberg, 2006). Hence it proves the importance of iron-acquisition strategies in the face of the host's iron-withholding mechanisms (Jones and Niederweis, 2011; Ilankizhai and Devi, 2016). Tuberculosis causes obstructive airway illness which manifests with chronic inflammation affecting the whole respiratory tract (Dave and Preetha, 2016). Lung function tests have been gradually used in evaluating the harshness of obstructive airway disease and provide an understanding of pulmonary physiology (Timothy *et al.*, 2019). Forced Expiratory Time can act as an indicator for upper airway obstruction, which is shown significantly in adenoid hypertrophy but insignificant among control and adenoidectomy (Devi and Sethu, 2018). Due to respiratory problems, snoring is more common, which may cause serious health problems such as obstructive sleep apnea (Shruthi and Preetha, 2018). Recently, tuberculosis disease was associated with an increased risk of acute myocardial infarction (AMI), ischemic stroke, and peripheral artery disease their treatment include thrombolytic therapy and coronary angioplasty (Renuka and Sethu, 2015).

This survey provides information on whether blockage of iron transport can stop the growth of pathogens in tuberculosis and know how the iron gets transported across cell membranes in pathogens. This also provides deep understanding which creates a better cure for tuberculosis by introducing new drugs by involving iron supplements effective. This survey aims to know about the knowledge and awareness of blocking iron transport among college students.

MATERIALS AND METHODS

The questionnaire-based study was carried out online through a google forms link. Individuality was ensured when the subjects filled up the survey. The 100 participants who undertook the survey are undergraduate students of various colleges selected through non-probability convenient sampling. A total of 16 questions were used to find if the subjects were aware of iron blockage to stop tuberculosis. The questions were mainly targeted at the physiological mechanism of iron transport in bacteria of tuberculosis. In light of reactions and answers from the subjects, the factual examination was performed and results were statistically analysed using SPSS software version 19 and descriptive statistics used to create the bar graph.

RESULTS AND DISCUSSION

Data were analyzed using SPSS and results showed that the majority of participants had known about physiological mechanisms of bacteria, which resulted in positive outcomes.

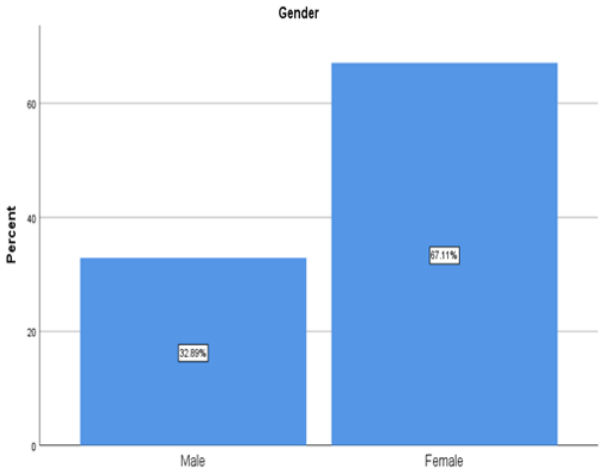

Figure 1: Gender distribution in which 87.11% of females and 32.89% of males undertook this survey

In this survey, Figure 1 shows that about 67.11% of female students undertook this survey, and 32.89% of male students did it. In Figure 2 about, 67.53% of participants were well aware *of Mycobacterium tuberculosis* bacteria causes tuberculosis in

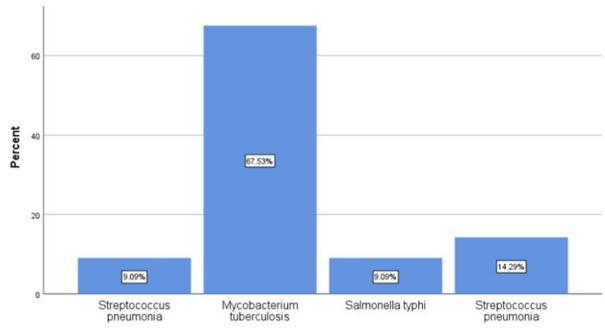

Figure 2: Bar graph showing responses to the question, "Name the bacteria which causes tuberculosis?"

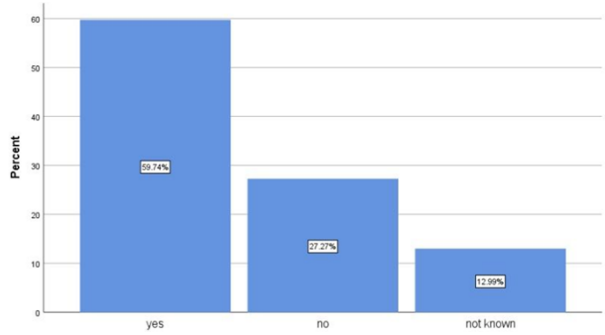

Figure 3: Bar graph showing responses to the questions, "Do you know the bacteria that causes tuberculosis to need iron to survive?.

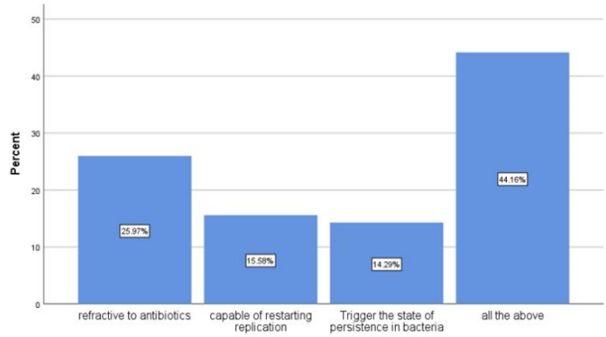

Figure 4: Bar graph showing the responses to the question, "What do you think that iron in mycobacteria has special characteristics?"

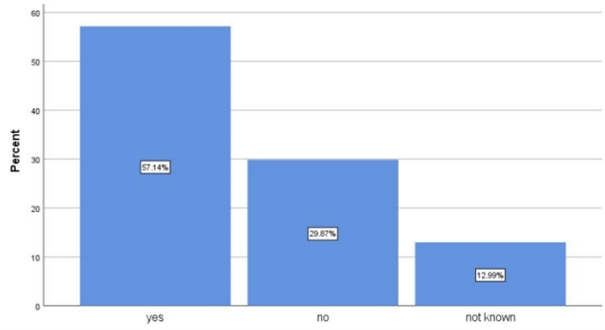

Figure 5: Bar Graph showing responses to the questions, "do you think that iron-restricted persistent bacteria can exploit for the design of new anti-tubercular drugs?"

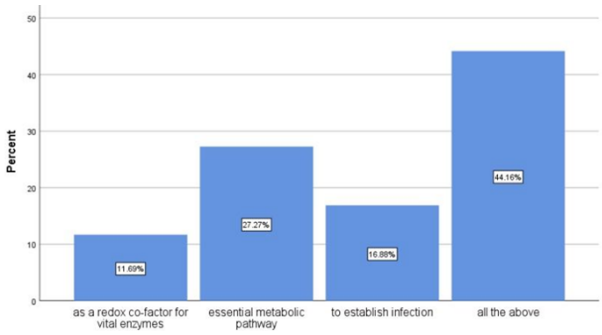

Figure 6: Bar graph showing responses to the questions, "Why do you think mycobacteria need iron?"

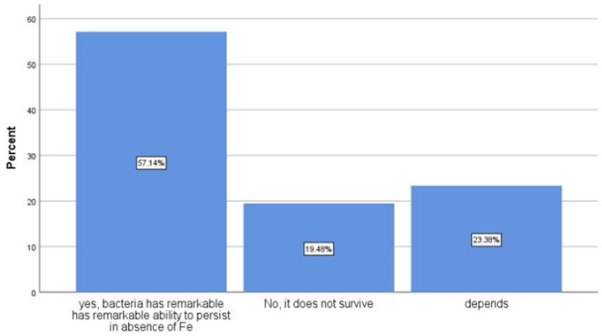

Figure 7: Bar graph showing responses to the questions, "do you think bacteria persists under Fe starvation?"

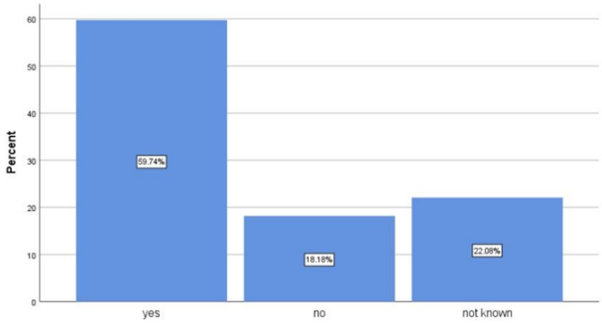

Figure 8: Bar graph showing the responses to the questions, "do you think M.tuberculosis manipulates phagosome (vacuoles in the cytoplasm of the cell) to gain access for incoming Fe?"

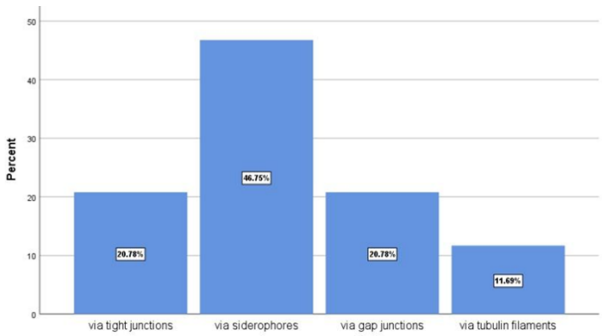

Figure 9: Bar graph showing responses to the questions, "how do you think it would occur?"

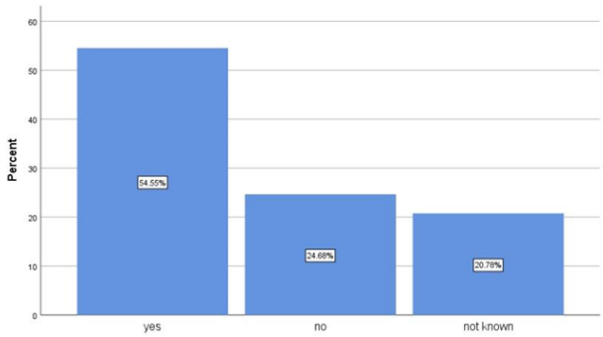

Figure 10: Bar graph showing responses to the question, "Do you know M.tuberculosis encodes two iron storage proteins?"

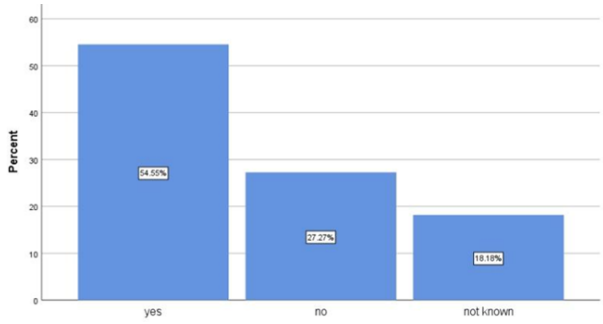

Figure 11: Bar graph showing responses to the question, "Do you know that BfrB seems to be housekeeping Fe storage proteins and shows hypersensitivity to oxidative stress and antibiotics?"

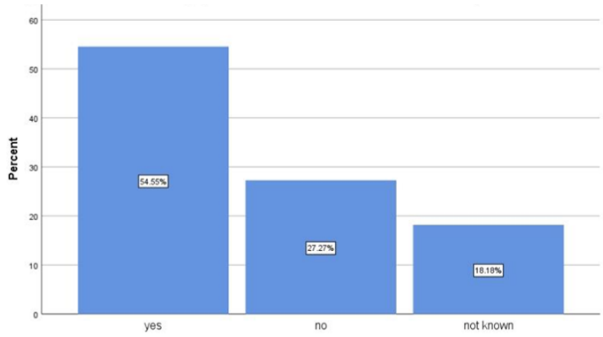

Figure 12: Bar graph showing responses to the question, "Do you think the size of the Fe storage pool would determine whether the bacteria can adjust to Fe starvation?"

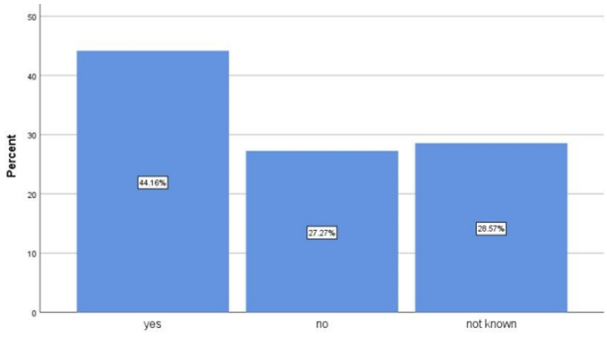

Figure 13: Bar graph showing responses to the question, "Does M.tuberculosis universally profit from granuloma formation?"

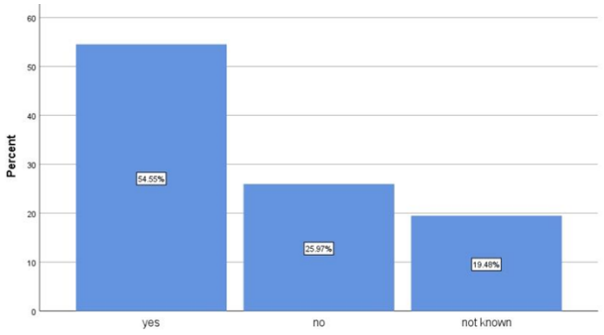

Figure 14: Bar graph showing responses to the question, "Do you know that absence of granuloma formation has 2 protection for bacteria?"

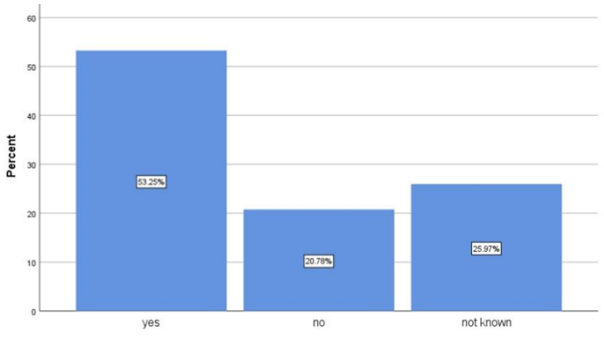

Figure 15: Bar graph showing responses to the question, "Do you know that HIV infected individuals have poor control of mycobacterial growth?"

human, but 14.29% of participants were stated as *Yersinia pestis*, 9.09% as *Streptococcus pneumonia* and 9.90% of participants as *Salmonella typhi*. In Figure 3, 59.74% of participants were aware that bacteria which cause tuberculosis need iron to survive but 27.27% did not agree and 12.99% were not known about that. Similarly, a previous study has proved that the causative agent of tuberculosis requires iron for essential metabolic pathways (Rodriguez, 2006). Due to deficiency of iodine, swelling of the thyroid gland causes endemic goitre which is very common in the people living in regions away from the shore (Samuel and Devi, 2015). In Figure 4, majority of participants 44.16% were reported that iron has special characteristics of refractive to antibodies and capable of restarting replication and also trigger the state of persistence in bacteria when Fe is restored. Similarly, a study expands the idea and proves that host responses to infection due to iron might trigger quiescence of *M.tuberculosis* and provides to iden-

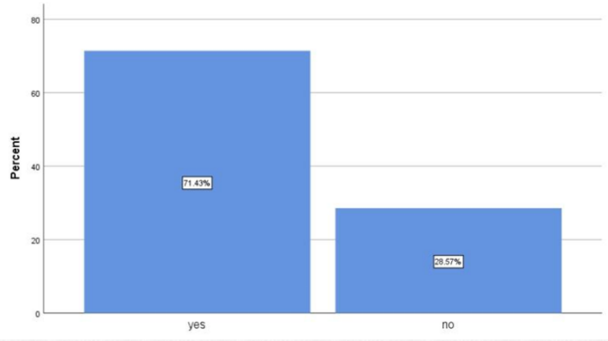

Figure 16: Bar graph showing the responses to the question, "Is this survey helpful for you to k2w more about tuberculosis?"

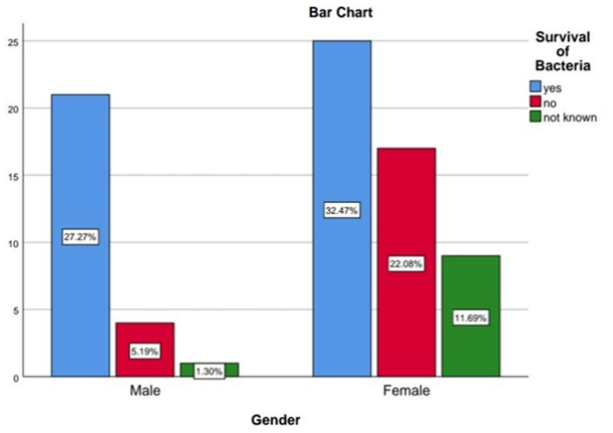

Figure 17: Bar graph represents the association between gender and awareness about Iron for the survival of bacteria among the study population

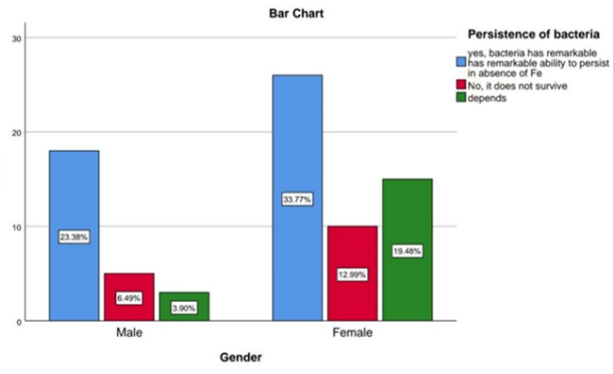

Figure 18: Bar graph correlates with gender and awareness of persistence in bacteria under Fe starvation.

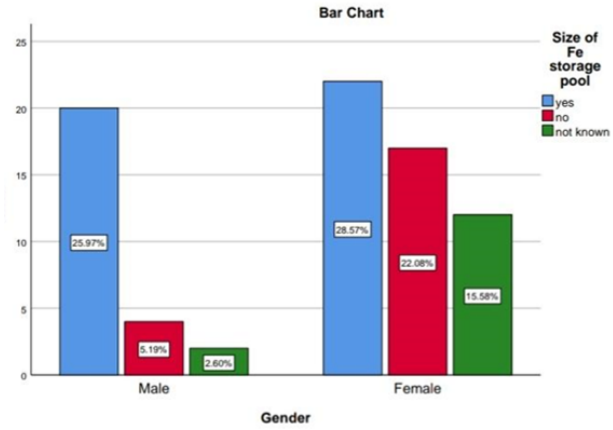

Figure 19: Bar graph represents the association between gender in the x-axis and the percentage of awareness of determining bacterial adjustment by the size of the Fe storage pool in the y-axis.

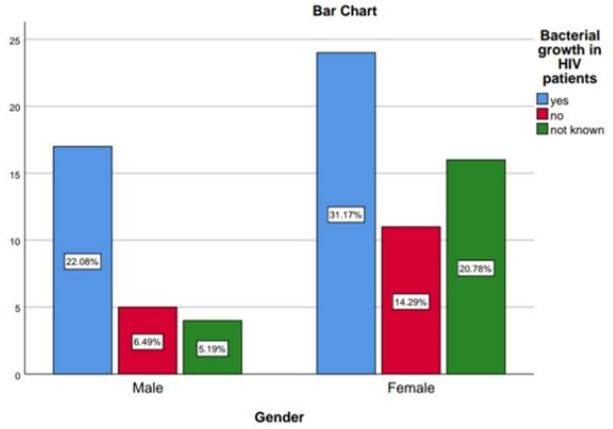

Figure 20: Bar graph represents the association between gender and awareness about bacterial growth in HIV patients using a chi-square test.

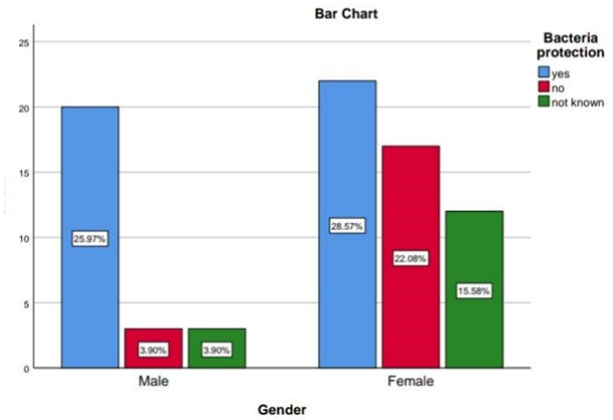

Figure 21: Bar graph represents the association between gender and awareness about bacterial protection associated with granuloma formation.

tify metabolic vulnerabilities of persistent bacteria (Kurthkoti *et al.*, 2017; Swathy and Sethu, 2015). In Figure 5, majority of participants about 57.14% were aware that iron-restricted persistent bacteria could exploit for the design of new anti-tubercular drugs and 29.87% were not aware and 12.99% of participants were not known. A previous study also proved the new view of persistent tuberculosis bacilli that can provide opportunities for new host and pathogen - directed therapies (Kurthkoti *et al.*, 2017). In Figure 6, about 44.16% of participants reported that mycobacterium needs iron as a redox co factor for vital enzymes; essential metabolic pathway and to establish infection. This proves by several previous studies that causative agents of tuberculosis require iron as co factors for enzymes that are involved in redox reactions and other essential functions (Weinberg, 1984). Figure 7 shows that about 57.14% of participants were aware that mycobacteria have remarkable ability to persist in the absence of Fe but 19.48% told that it does not survive and 23.36% of participants answered it depends. Relevant to this, a study has proved that the iron has the ability to persist in Fe starvation conditions which could be implemented by the human immune defence in necrotic granulomas and by employing genetic, genomic and metabolic approaches, it can identify determinants of survival (Kurthkoti *et al.*, 2017) **.** Preoperative diagnosis of tuberculosis as the cause of neonatal jaundice due to bilirubin level of more than 5 mg/dL (Harsha, 2015). Non-alcoholic fatty liver disease promises to be the leading cause of liver disease in industrial countries (Choudhari and Jothipriya, 2016).

In Figure 8, about 59.74% of participants reported that *M.tuberculosis* manipulates the phagosome to gain access for incoming Fe, but 18.18% were answered 'NO' and 22.08% had no idea about that. Russell (2001) concluded that highly successful pathogen (i.e.) mycobacterium parasitizes macrophages of its host so its success can be attributed directly to its ability to manipulate the phagosome. Figure 9 shows that 46.75% of participants were aware that bacteria gain access for Fe incoming via siderophores, 20.78% via the tight junction, 20.78% via gap junction 11.69% of participants answered via tubulin filaments which conclude that they were not well aware of it. A Previous study, Sritharan (2016) explained that pathogen expresses Fe3+ through specific siderophores called mycobactin and carboxymycobactin to chelate the metal ion from insoluble iron and also concluded that siderophore mediates uptake is essential for the survival of mycobacterium. Figure 10 shows that about 54.65% of participants were well aware that *mycobacterium tuberculosis* encodes two iron storage proteins but 24.68% were not aware of it and 20.78% of participants had no idea about that. A previous study Murray *et al.* (1978) reveals that mycobacterium encodes by two iron storage proteins (i.e.) heme-containing bacterioferritin (BfrA) and ferritin like protein (BfrB). Similarly, Reddy *et al.* (2012) convey that two iron storage proteins are crucial for storage and supply of iron for the growth to withstand oxidative stress. The person who is obese affects the quality of life like depression, disability, physical discomfort, sexual problems, shame and social isolation (Baheerati and Devi, 2018). These obese people have changes in thyroid function and show a statistically significant difference between TSH levels among obese and non-obese people (Fathima and Preetha, 2016; Swathy and Sethu, 2015).

Figure 11 shows that about 54.55% of participants were aware that BfrB seems to be housekeeping Fe storage proteins and shows hypersensitivity to oxidative stress and antibiotics and 27.27% of participants were not being aware of it and also 18.18% had no idea about it which proved with a previous study (Reddy *et al.*, 2012). In Figure 12 , about 54.55% were aware that the size of the Fe storage pool would determine whether the bacteria can adjust to Fe starvation, but 27.27% were not aware of if. Figure 13 shows that about 44,16% of participants were well aware that *M.tuberculosis* universally profits from granuloma formation, but 27.27% were not aware and 28.57% had no idea about it. Figure 14 depicts that about 54.55% of participants were aware that the absence of granuloma formation has no protection for bacteria, but 25.97% were not aware of it. Previous study Ehlers and Schaible (2013) highlights that granuloma is primarily or uniquely relevant for the protection of hosts. In Figure 15, about 53.25% of participants were aware that HIV infected individuals have poor control of mycobacterial growth but 20.78% were not aware and 25.97% not known about it. Lawn *et al.* (2002) proved that HIV infected individuals exhibit poor granulomes inflammation and poor control of mycobacterial growth. In Figure 16, the majority of participants about 71.43% showed positive results about this survey and 28.51% showed contrasting opinions about it. Figure 17 explains that the bar graph correlates between gender and awareness about iron for the survival of bacteria using chi-square analysis which depicts p-value as 0.031 statistically significant, proving males have better awareness than females. In Figure 18, Bar graph correlates with gender and persistence of bacteria under Fe starvation using chi-square test,

which shows p-value as 0.222, which is not statistically significant. Figure 19 describes a bar graph that correlates with gender and awareness of determining the adjustment of bacteria by the size of Fe storage pool which depicts the p-value as 0.025 proves to be statistically significant proving males have better awareness than females. In Figure 20, Bar graph associates gender with Bacterial growth in HIV patients using a chi-square test which shows p-value as 0.295 proves to be statistically not significant. In Figure 21, Bar graph correlates with gender and awareness about bacterial protection which shows p-value significantly as 0.025 using the chi-square test. proving males have better awareness than females

In this survey, certain limitations were applied that awareness was restricted on a particular population with limited age among college students, and they randomly selected 100 participants. For targeting new drug developments for tuberculosis, exact mechanisms of bacteria could be useful. So understanding the position of infected vacuoles by iron transport on human cells due to mycobacteria should help us to develop improved drug delivery.

In future for the better cure for tuberculosis, new drugs should be introduced involving iron supplements and also provides new vaccine development in iron acquisition and iron metabolism for treating tuberculosis.

CONCLUSION

By this current survey, it can be evident that the majority of participants are educated about knowledge of tuberculosis associated with causes, main role and physiological mechanisms involved in it. More research might be required to edify tuberculosis among general populations for the well being of the society by the impact of iron's role in the human body.

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The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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