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Gender and Age Proportion of *Mycobacterium tuberculosis* Detection Using Nested Real Time Polymerase Chain Reaction at Indramayu District General Hospital

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***Abstract*—Background: Tuberculosis (TB) is an infectious disease caused by Mycobacterium Tuberculosis (MTB) also the second cause of death among all infectious diseases in the world. World Health Organization (WHO) and lots studies have conclude that the risk of tuberculosis (TB) increases dramatically during young adult and males. Objective: To analyse the proportion of gender and age of MTB detected using hemi-nested RT PCR. Methods: This study used a descriptive method by taking 97 patients secondary data from the Indramayu General Hospital. Results: of 97 data showed 37 (38.1%) MTB positive patients. The proportion of rifampicin-sensitive (RS) positive MTB was (91.9%), in the age group 0-5 years (0%), 6-11 years (0%), 12-16 years (2.7%), 17-25 years (16.2%), 26-35 years (27%), 36-45 years (16.2%), 46-55 years (8.1%), 56-65 years (16.2%), >65 years (13.6%) and 54% in men. Rifampicin resistant (RR) as much as (5.4%) in the age group 56-65 years (2.7%) and age >65 years (2.7%) in men (5.4%). Rifampicin indeterminate (RI) (2.7%) was found at the age of 17-25 years in females (2.7%). Conclusion: The MTB detected proportion was mostly at the young adults of 26-35 years (27%), and based on gender, most were male (54%). Patients with RR were found at the age of >56 years and all were male.**

Keywords—***age; gender; Mycobacterium tuberculosis; proportion***

I. INTRODUCTION

Tuberculosis (TB) tropical infectious disease etiology is *Mycobacterium tuberculosis* from airbone transmission. [1, 2]. Globally, the reduction in the TB incidence rate from 2015 to 2022 was 8.7%, far from the WHO End TB Strategy milestone of a 50% reduction in the TB incidence rate between 2015 and 2025. In 2022, Indonesia is still the country with the third highest incidence of tuberculosis globally about 10% with estimated number of incidents cases of multidrug resistance MDR/RR-TB 2022: 31000 (increase from 27000 in 2022). There is also a need to increase the percentage of cases confirmed bacteriologically by scaling up the use of recommended diagnostics, in line with WHO guidelines [3]. Tuberculosis dominates in males than females. Research in South Africa states that sputum is a screening test for TB diagnosis and sex disparities in TB prevalence are widely reported, yet the underlying biological mechanisms underlying hormonal and genetic mechanisms that differentially modulate innate and adaptive immune responses in males and females, leading to sex differences in disease susceptibility [2, 4-5]. Tubercuosis can affect anyone, regardless of age or sex. The highest burden is in adult males (age ≥15 years), with an estimated 5.8 million cases in 2022, equivalent to 55% of the estimated total with estimated 3.5 million cases among adult

females (age ≥15 years), equivalent to 33% of the estimated total; and 1.3 million cases among children (aged 0–14 years), equivalent to 12% of the estimated total. The higher share of TB cases among males are consistent with evidence from national TB prevalence surveys, which show that TB disease affects males more than females, and that gaps in case detection and reporting are higher among male [2, 4-5].

The WHO encourages TB prevention and care strategies often overlook adolescents and young adult by grouping them with either children or adults and have particular physiologic, developmental, and social characteristics that require dedicated approaches comprise a uniquely important but understudied population in global efforts to end TB, the leading infectious cause of death by a single agent worldwide prior to the others. According to the Indonesian Ministry of Health, the adult age is 18-59 years and the elderly are ≥ 60 years, have social and developmental characteristics to induced TB transmission [6-8]. Theoretically, WHO guideline recommend for bacteriologically-confirmed pulmonary TB and practically provide by nested RT PCR [3-4].

Nested RT PCR *in vitro* for the detection of *Mycobacterium tuberculosis* complex DNA in raw sputum or concentrated sputum sediment or other body fluids also detects the RR-associated mutations of the *rpoB* gene. This MTB/RIF examination is able to qualitatively detect complex quickly and accurately. This device cannot be used as a follow-up examination (monitoring) in patients receiving treatment. The objective of this study is to describe the proportion of gender and age from *Mycobacterium tuberculosis* detection using nested real time polymerase chain reaction [2].

II. METHOD

This was a retrospective observational study on gender and age from 97 suspected tuberculosis secondary data conducted in 2022-2023 using total sampling method. The data divided in three groups: RS, RR, and RI. The inclusion criteria were suspected tuberculosis who examined the molecular detection of MTB to Clinical Pathology Laboratory Indramayu regional general hospital. Exclusion criteria was incomplete data.

The data source is secondary from the results of MTB examination with nested RT PCR in 2022-2023 than grouped by male, female, and age groups at RS, RR, and RI. The author than calculated the proportion in 100%. The results are reported, analyzed, discussed, and concluded by the authors, not forgetting to convey the limitations of this study, also conveyed ideas for the next research theme related to the conclusion of this present paper.

III. RESULTS AND DISCUSSION

The proportion of positive MTB result between year 2022 and 2023 showed males more than females in RS and RR, but females more than males in RI. Most age group were adults (26-35) in RS, adolescents (17-25) in RI, and elder aged in RR ≥56 years aged.

TABLE I. GENDER AND AGE PROPORTION OF POSITIVE MTB

Variables	RS		RR		RI	
	n=34	%	n=2	%	n=1	
Gender						
Male	20	**54.0**	2	**5.4**	0	0
Female	14	37.9	0	0	1	**2.7**
Age (years)						
0-5	0	0	0	0	0	0
6-11	0	0	0	0	0	0
12-16	1	2.7	0	0	0	0
17-25	5	13.5	0	0	1	**2.7**
26-35	10	**27**	0	0	0	0
36-45	6	16.3	0	0	0	0
46-55	3	8.1	0	0	0	0
56-65	5	13.5	1	**2.7**	0	0
>65	4	10.8	1	**2.7**	0	0

We found in this study that males may represent an epidemiological influence resulting from various geographical, cultural, and socioeconomic factors. Females have limited access to healthcare and the incidence of suspected tuberculosis infection is not recorded [6]. Sex differences in TB are likely to be multifactorial and elucidating the underlying mechanisms will require a thorough understanding of the social, behavioral, hormonal, and genetic factors that mediate differing immune responses in males and females. Female steroid estrogen hormones consist of three isoforms, namely estrone (E1), estradiol (E2), and estriol (E3). Level of E2 in female during menstrual cycle is 100–600 pg/mL while E1 in postmenopausal female more than E2. Level of E2 in elderly male is 5–20 pg/mL. E1 isoforms in menopausal women are more than E2 (E2 <5 pg/mL) [6]. The amount of E2 and progesterone hormones in reproductive-age women with TB infection is lower than in healthy female. Estrogen levels in postmenopausal female with TB infection are more than in healthy female [6]. This cause the result of this study with young age group dominates than the other age groups. These sex steroids induced immune responses positively or negatively, most prominently in the reproductive years (young ages) of females. This sex steroids hormone modulate the proliferation and immune function of natural killer (NK) cells, macrophages, monocytes, dendritic cells (DC), neutrophils, B, and T cells of human. Estrogen has been deemed to promote immunoenhancing effect on the immune mechanism in human studies, while testosterone, and progesterone are suprresed the immune [6]. (Figure 1).

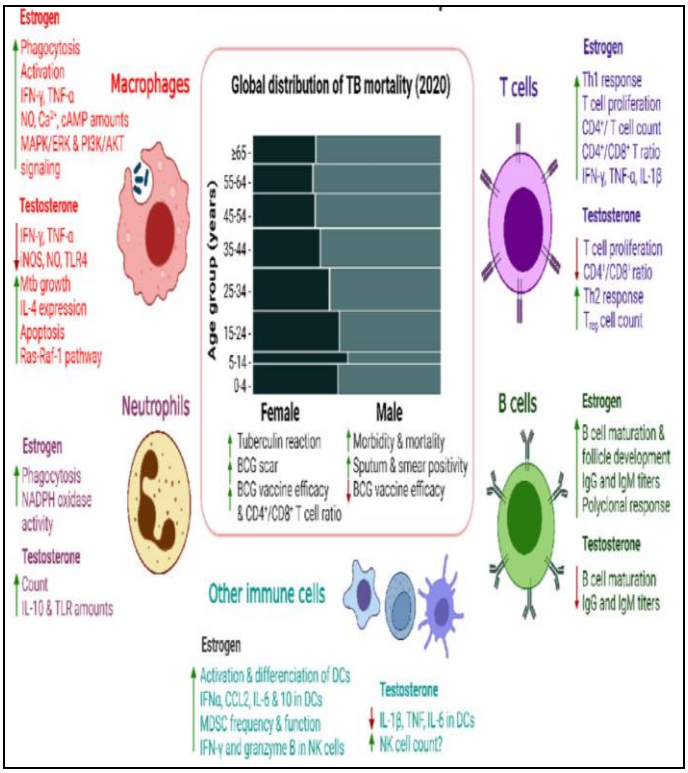

Figure 1. Effect of Sex Hormones of Immune Responses

Sex-dependent expression of autosomal genes suggested regulation either via sex hormones and their receptors or stemming from sex dependent epigenetic changes influencing transcription. Accordingly, the promoters of a large number of sex-biased genes harboring hormone receptor elements (HREs) were recognized by transcription factors such as the estrogen receptor-a (ERa), glucocorticoid receptor (GR), as well as two X-linked transcription factors (TFs) - the androgen receptor (AR) and ELK1. Androgen response elements (AREs) and estrogen response elements (EREs) are present in the promoters of several innate immunity genes [7].

Factors that influence the proportion of adolescent and young adult (AYA) aged groups dominates RS and RI are: social and community, health system, and individual. Additional factors influencing: migrant youth, youth living in human immunodeficiency virus (HIV), and experiencing homelessness, substance use, or incarceration. Migrant AYA, including refugees and other displaced AYA, have heterogeneous ex-periences that are poorly captured in surveillance data, complicating efforts to measure their TB risk and outcomes. However, displaced people of all ages often face a disproportionately high risk for TB due to a combination of overcrowding, malnutrition, poverty, psychological stressors, and healthcare system disruptions that lead to delayed diagnosis and treatment. Birth in a country with high TB prevalence consistently predicts an increased risk for TB infection. A screening program for immunocompromised cases such as HIV needs to be carried out because it can cause treatment failure, resulting in transmission of active TB through contact with other individuals. Though the incidence of new HIV infections in AYA has declined overall, progress lags behind global targets and remains uneven, with AYA from key populations and in some regions experiencing more limited progress. In 2019, an estimated 3.4 million people aged 15–24 years were living with HIV worldwide. AYA living with HIV (ALHIV) experience more complex barriers and remain more underserved in many areas of HIV care compared to other age groups. Data on TB in ALHIV remain limited, but growing evidence demonstrates that ALHIV also experience excess TB risks. Early initiation of ART reduces the risk of TB disease in ALHIV [7-8].

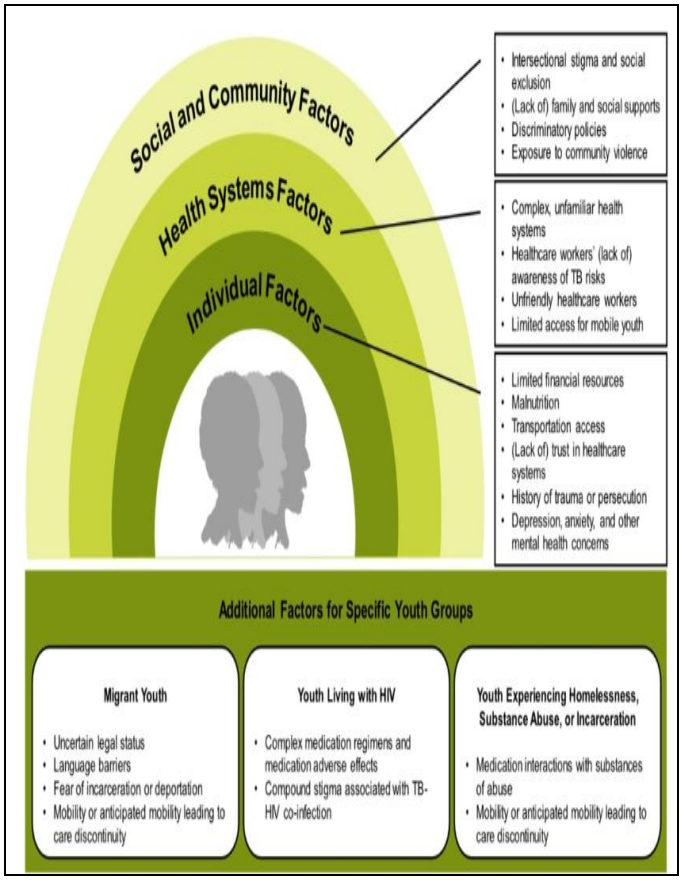

FIGURE 2. BARRIER TO TB CARE FOR MARGINALIZED ADOLESCENTS AND YOUNG ADULTS

Adults who experience homelessness, harmful alcohol or drug use, or incarceration have documented excess risks for acquiring TB and for unfavorable TB outcomes, but very little is known about AYA with the same vulnerabilities. The same immunologic, socioeconomic, and health systems factors that impair the health of these adults likely also give vulnerable AYA excess TB risks. AYA are highly mobile, with more social contacts each day than either young children or older adults. While a new TB diagnosis in a child <5 years generally signals recent TB transmission within the household, the

complex social networks of AYA, and the potentially longer interval between TB infection and onset of TB disease, complicate identification of the exact settings for TB transmission to AYA. Also highlight that for AYA, increasing mobility provides increasing opportunities for cumulative exposures outside the home [6-8]

Outside their homes, AYA tend to spend the majority of their time in school settings. Schools gather groups of AYA in repeat, prolonged close contact in buildings that may have poor ventilation, all conditions that favor TB transmission. AYA with pulmonary TB disease often have highly transmissible disease, schools also remain important sites of potential TB transmission in communities with low TB prevalence. Public transit has emerged as an important contributor to TB transmission, particularly in urban high-prevalence communities. A case-cohort study conducted in Lima, Peru, found that commuting via minibus was an independent risk factor for TB disease. Exposures on public transit held similar importance for TB transmission to AYA. As with school-based exposures, the degree of exposure on transit appears to affect the risk of TB transmission, with prolonged close contact associated with higher risk of TB infection than more brief contact. Transit drivers had an annual TB infection risk 10 times that of transit riders, a result of longer time spent on poorly ventilated buses [6-8].

Elderly patients have been repeatedly reported to have a lower treatment completion rate and are less health aware than younger patients, which means the diagnosis and treatment in elderly TB cannot be reached due to limited mobility. immune system would be weakened while susceptibility to chronic disease would increase with aging. may be caused by limited drug penetration into cavities, the suitable environment provided by cavities for bacilli, patients' immunity. These may be the reasons that the DR-TB pattern changed. The change of the DR-TB pattern will bring us a huge challenge to control the DR-TB in the elderly. Elderly age with immunocompromised state makes it easy to infected by active tuberculosis [9-10].

Future studies in humans and in animal model systems evaluating the influence of sex hormone and identification of sex-linked biomarkers at different stages of TB infection will be valuable in understanding male sex bias in TB and the development of specific therapeutics. However, it remains to be determined whether the male bias in these different infections has a common biologic basis or whether disease-specific mechanisms apply (see outstanding questions). A better understanding of sex-based differences in these disparate infections offers greater insight into infectious disease pathogenesis, mechanisms of immunity, and may enable development of new and sex-specific interventions and therapies. sex-based differences in these disparate infections offers greater insight into infectious disease pathogenesis, mechanisms of immunity, and may enable development of new and sex-specific interventions and therapies [9-12]

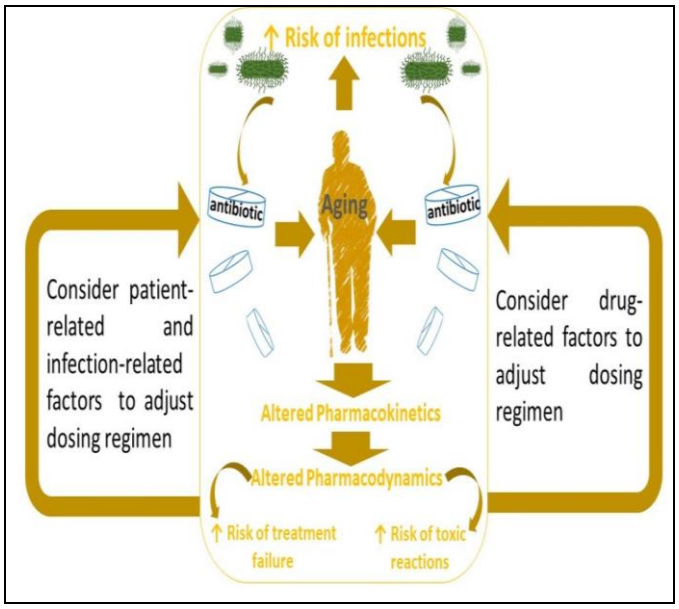

FIGURE 3. INCREASED RISK OF INFECTION IN ELDER AGE

Age-related physiological and pathological changes, poor functional status, poor nutrition, and comorbidities predispose older adults to infections and their complications. The incidence and severity of infections increase with advancing age. Compared to younger age groups, elderly patients are more prone to pneumonia, skin and soft tissue infections, urinary tract infections and septicemia. An additional problem is a substantial risk of antibiotic (AB) resistance; its typical risk factors in the elderly include frequent contact with the healthcare system, frequent AB exposure, depressed immune system, frailty, and comorbidity. Elderly patients are considered the high-risk group for the development of healthcare-associated infections caused by MDR bacteria. The elderly population has longer hospital stays compared to younger adults and a significantly higher mortality rate (25%) compared to the general population (10%). Infections aggravate the course of concomitant chronic diseases, including cardiovascular and cognitive disorders, and contribute to the emergence of a new comorbidity [6, 13-15]

High infectious morbidity leads to high consumption of antimicrobial agents by the elderly. ABs are among the most frequently prescribed medicines to seniors and their use is accompanied by a significant rate of side effects and clinically relevant drug-drug interactions compared to younger counterparts. Adverse drug reactions (ADRs) are an important cause of morbidity and mortality in the elderly and their risk is significantly increased in the presence of comorbidity and polypharmacy [14-17]

Recurrence was a significant source of RR-TB among elderly patients, so it's important determine the risk factors for recurrence by comparing patients with recurrent TB with those with non-recurrent TB. Recurrence usually occurs if there is universal incorrect usage of rifampicin to treat primary TB,

according to the WHO guidelines. Furthermore, HIV positivity also had an important effect on RR-TB recurrence [15-17].

IV. CONCLUSIONS

The conclusion of this paper figure the predisposition factors of ages group and gender prevalence of tuberculosis infection. the research and suggestions for future studies. Tuberculosis (TB) keep remain one of the world's most important medical challenges with a significant male bias. Elucidating the underlying mechanisms that mediate differences in immune responses to *Mycobacterium tuberculosis* in males and females will not only enlighten our understanding the role of TB immune patomechanism but also aid in eradicating, surveilance, preventing, treatment evaluating, and also developing novel sex-specific vaccines especially in develop countries. Strategies to better prevent and treat TB among AYA are critical to halting TB transmission within communities. develop more effective approaches to identify, treat, and prevent TB among AYA Especially, developing countries or those that have not yet achieved optimal TB eradication rates towards improving the quality of human life. The limitation of this paper is that it does not analyze on male rifampicin resistance which is greater than female and why it is dominated by older age. It is hoped that future studies will be able to analyze the mechanism of rifampicin resistance in men so preventing bias of the result of study.

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