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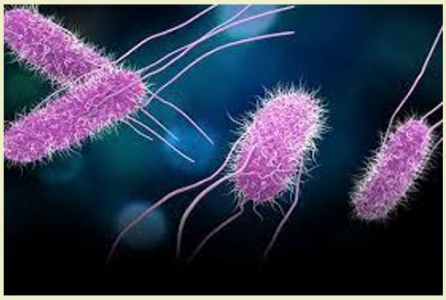

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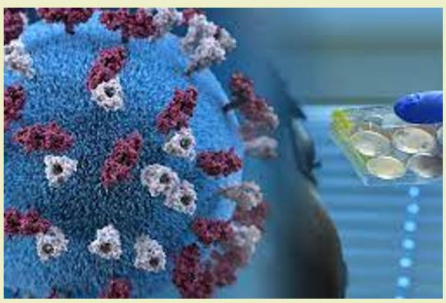

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ORIGINAL ARTICLE

Antimicrobial Profile and Prevalence of *Salmonella Species* from Blood Culture in A Tertiary Care Hospital

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ABSTRACT

Introduction: Enteric fever is a major public health concern around the world and endemic in low- and middle-income countries like, India. Typically, it spreads through contaminated food or water. *Salmonella Typhi* proliferate and spread throughout the bloodstream seeding multiple organs in the body. Incidence data of culture confirmed Typhoid cases is approximately 377per 100000 population with an approximate case fatality rate of 1% in India. The management of cases are hampered due to emerging the drug resistance of isolates because of rampant and misuse of antibiotics. This study investigates to analyse the current pattern of antibiotic susceptibility among Salmonella isolates from cases of enteric fever seen at ESIC Medical College & Hospital, Faridabad.
Methods: This is a retrospective, cross-sectional study. Blood cultures from patients with suspected enteric fever from Jan 2017 to Dec 2019 were included. The blood cultures were processed using the BacT/Alert automated blood culture system.
Results: During the study period, a total of 4064 blood culture specimens were received from the patients suspected for fever. Out of a total of 2717 culture positive samples, 373 (13.73%) were found positive for the growth of *Salmonella enterica* [*Salmonella typhi 87.13% (325/373), Salmonella paratyphi A (12.86%)]*, confirming the enteric fever.
Conclusion: Treatment with appropriate antimicrobial drugs is crucial for patients with typhoidal infections and the antimicrobial susceptibility of such isolates must be reported as soon as possible.

Medical and Health Science Journal.

Introduction

Enteric fever is a major public health concern around the world and endemic in low- and middle-income countries like, India. Typhoid fever and Paratyphoid fever, which are both life-threatening illnesses, are caused by *Salmonella Typhi* and *Salmonella Paratyphi A*, respectively.[1,2] Typically, it spreads through contaminated food or water. *Salmonella Typhi* proliferate and spread throughout the bloodstream seeding multiple organs in the body.[1] The disease's signs and symptoms are likely to appear gradually, one to three weeks after contact. Incidence data of culture confirmed Typhoid cases is approximately 377 per 100000 population with an approximate case fatality rate of 1% in India.[3] Early disease management can be aided by quick diagnosis, and precise antibiotic sensitivity testing guiding the treatment protocol.[4] Empirical therapy is usually followed when laboratory confirmation is not done in many outpatients setup. Typhoid fever morbidity and mortality have decreased dramatically in industrialised countries as a result of improved housing conditions and the use of drugs.[1] The management of cases are hampered due to emerging the drug resistance of isolates because of rampant and misuse of antibiotics.

Chloramphenicol, ampicillin, and cotrimoxazole are no longer frequently used to treat typhoid fever in endemic areas, and quinolones have taken their place as the drug of choice. This is because Multi-Drug Resistant (MDR) strains have emerged.[5] Nalidixic acid-resistant bacteria linked to decreased sensitivity to fluoroquinolones in patients treated with quinolones have been observed more frequently over the past few years. Alternative possibilities for effective medication and management of enteric fever cases are becoming essential. Ceftriaxone and azithromycin are being increasingly used for complicated and uncomplicated typhoid, respectively.[2,6,7] Over the counter use of these medications can cause emergence of resistance to these subsequently limiting their efficacy to treat. Consequently, this study investigates to analyse the current pattern of antibiotic susceptibility among Salmonella isolates from cases of enteric fever seen at ESIC Medical College & Hospital, Faridabad.

Methods

This is a retrospective, cross-sectional study. Blood cultures from patients with suspected enteric fever from Jan 2017 to Dec 2019 were included. The blood cultures were processed using the BacT/Alert automated blood culture system. Organisms were isolated and identified using standard microbiological methods.[8] Antimicrobial Susceptibility Test (AST) was done using the Kirby Bauer disc diffusion method and interpreted using CLSI guidelines.[3]

Statistical Analysis: The Statistical Package for Social Sciences was used to enter and analyse information on the bacterial isolates, their susceptibility to different antibiotics, and other details (SPSS). Distribution based on percentages is used to illustrate the results. "Significant" was defined as a p value less than 0.05.

Results

During the study period, a total of 4064 blood culture specimens were received from the patients suspected for fever, out of which 66.85% (2717/4064) showed blood culture positive and remaining 33.14% (1347/4064) samples were showed no growth on blood culture media.

Out of a total of 2717 culture positive samples, 373 (13.73%) were found positive for the growth of *Salmonella enterica* [*Salmonella typhi 87.13% (325/373), Salmonella paratyphi A (12.86%)]*, confirming the enteric fever. Other non-enteric pathogens that were isolated included Methicillin Resistant *Staphylococcus aureus* (MRSA) *12.59%*, Acinetobacter species 5.89%, *Klebsiella pneumoniae* 8.91%, *Escherichia coli* 9.42%, *Enterococcus* species 9.16%, *Citrobacter* species 5.37%, Coagulase-negative *Staphylococcus 9.16%, and Pseudomonas aeruginosa* 10.97% (Table 1).

Table 2 showed AST data of the *Salmonella* isolates. Among the *Salmonella Typhi,* majority isolates were resistant to Nalidixic acid. Ampicillin and Ciprofloxacin resistance was observed in more than 60% isolates. Moderate resistance to Amikacin, Azithromycin & Chloramphenicol (24%). Low level resistance was seen towards Cotrimoxazole, Meropenem, Ertapenem & Sulfamethoxazole. Whereas, 100% sensitivity was shown by a number of drugs, i.e., Ofloxacin, Cefixime, Cefotaxime, Ceftriaxone, Tetracycline, Imipenem, and Ticarcillin Clavulanate.

In contrast, *Salmonella paratyphi A* isolates showed only nalidixic acid (NA(R)) resistance was (75%), followed by ciprofloxacin (50%). The *Salmonella paratyphi A* were susceptible to majority of the other drugs testing. Table 3 showed statistically significant resistance of the Salmonella isolates to Nalidixic acid.

Table 1 Distribution of total number of isolates on the basis of gram staining

Organism	Number of isolates	Positive Percentage
Gram Negative bacilli	1475	54.29
Salmonella	373	13.73
Pseudomonas	298	10.97
E coli	256	9.42
Klebsiella	242	8.91
Acinetobacter	160	5.89
Citrobacter	146	5.37
Gram Positive Cocci	1242	45.71
MSSA	365	13.43
MRSA	342	12.59
CONS	286	10.53
Enterococcus	249	9.16
Total	2717	100

Table 2 Antimicrobial susceptibilities of *Salmonella enterica*.

Antibiotics	*Salmonella serotype Typhi* (n=325)			*Salmonella serotype Paratyphi* (n=48)		
	S (%)	*I* (%)	*R* (%)	*S* (%)	*I* (%)	*R* (%)
Ampicillin	235 (72.30%)	8 (2.46%)	82(25.23%)	48 (100%)	0	0
Nalidixic acid	17 (5.23%)	0	308 (94.77%)	12 (25%)	0	36 (75%)
Ciprofloxacin	95 (29.23%)	16 (4.92%)	214 (65.85%)	12 (25%)	12 (25%)	24 (50%)
Ofloxacin	325 (100%)	0	0	48 (100%)	0	0
Cotrimoxazole	257 (79.08%)	43 (13.23%)	25 (7.69%)	36 (75%)	12 (25%)	0
Cefixime	325 (100%)	0	0	48 (100%)	0	0

Cefotaxime	325 (100%)	0	0	48 (100%)	0	0
Ceftriaxone	325 (100%)	0	0	48 (100%)	0	0
Azithromycin	248 (76.31%)	0	77 (23.69%)	24 (50%)	24 (50%)	0
Tetracycline	325 (100%)	0	0	48 (100%)	0	0
Chloramphenicol	240 (73.85%)	8 (2.46%)	77 (23.69%)	48 (100%)	0	0
Imipenem	325 (100%)	0	0	48 (100%)	0	0
Amikacin	248 (76.31%)	8 (2.46%)	69 (21.23%)	36 (75%)	12 (25%)	0
Meropenem	317 (97.54%)	0	8 (2.46%)	48 (100%)	0	0
Ertapenem	283 (87.08%)	0	42 (12.92%)	48 (100%)	0	0
Sulfamethoxazole	274 (84.31%)	0	51 (15.69%)	48 (100%)	0	0
Ticarcillin-Clavulanate	325 (100%)	0	0	48 (100%)	0	0
Polymyxin B	325 (100%)	0	0	48 (100%)	0	0

S: sensitive, *I*: intermediate sensitive, *R*: resistant.

Table 3 Distribution of nalidixic acid (NA) resistant *S. Typhi* and *S. Paratyphi.*

Species	Nalidixic Acid (NA) Resistant		
	Resistant (%)	Sensitive (%)	p-value
Salmonella serotype Typhi (n=325)	308 (94.76%)	17 (5.23%)	
Salmonella serotype Paratyphi (n=48)	36 (75%)	12 (25%)	<0.05
Total(n=373)	344(92.22%)	29(7.77%)	

Discussion

Enteric fever is the most common cause of pyrexia of unknown origin. Limited laboratory diagnosis is the reason for under estimation of the true incidence of the disease in India. Data on culture positive typhoid cases is required to estimate the prevalence of the disease, its aetiology and antimicrobial susceptibilities.[9] Such data is must to formulate & focus policy decisions for control, preventing and managing the diseases. Over the counter use of antibiotics is also impact the AST data of the isolates in question. The study generated data by examining data of 373 Salmonella isolates over a period of 3 years. Among the positive blood cultures, *Salmonella enterica* was isolated in highest number [*Salmonella Typhi* and *Salmonella paratyphi* were 87.15% and12.86%, respectively].

The traditional 1st line agents (Ampicillin, Chloramphenicol, Cotrimoxazole), were found to have a better susceptibility profile. The current data shows a good response to the 1st line agent. This observation is also reported by studies done in India by Veeraraghavan B, Pragasam AK *et al* [10] and neighbouring countries i.e., Nepal.[11,12]

The postulated hypothesis for the recovery to the drug effect can be because of decrease clinical use of these agents for years, thus decreasing the selection pressure on bacteria. Such data strengthens the concept and importance of Antimicrobial Stewardship.

Among the 2nd line agents, ceftriaxone was universally susceptible in all isolates whereas, Azithromycin showed moderate level resistance. These agents are currently used for empirical therapy by most clinicians in India for typhoid cases. The data in our study corroborates with data projected from North India showing emergence of resistance towards Azithromycin among Salmonella isolates. In contrast, South Indian states have observed higher MIC of isolates towards ceftriaxone. The reason has

been attributed to the prescribing practices in these areas.[13]

Nalidixic acid resistance has been used as indirect evidence of increased Minimum Inhibitory Concentration (MIC) for Ciprofloxacin in *Salmonella Typhi* [14]. The present study reports very high number of NARST (Nalidixic Acid Resistance *Salmonella Typhi*) (>94%).

Similar to study in Central India showing 96% NARST isolates. These are higher in comparison to other Indian studies, which reports isolates in the range of 60-78%. [15,16,17] The limitation of this study was disc diffusion-based AST data, where MIC were not available. There Ciprofloxacin MIC of the NARST isolates cannot be correlated. But, considering the data reported in literature, NARST isolates found to have high chances of Ciprofloxacin failure in clinical use.[14] The untreated case mortality rate for typhoid fever is >10%, when patients with typhoid fever are treated with appropriate antibiotics, the rate should be <1.1. However, increasing resistance can cause difficulty in clinical management. Therefore, AST data survey and Antimicrobial Stewardship policies are need of the hour to control Typhoid related morbidity and mortality.

Conclusion

Treatment with appropriate antimicrobial drugs is crucial for patients with typhoidal infections and the antimicrobial susceptibility of such isolates must be reported as soon as possible. The changing trends to resistance to 1st and 2nd line drug can help formulate empirical therapy plans as per the epidemiologic profile of any given geographic area. Antibiotic Stewardship steps like rotation of drugs can be evidently useful to manage these cases. Evidence of antimicrobial resistance supports the need for continuous surveillance. Antibiotic resistance is here to stay, and our communities can only be saved by wise planning and an appropriate antibiotic policy.

Conflicts of Interest

The author started there is no conflict of interest

References

1. Typhoid. *[Internet] World Health Organization.* 2018. Jan 31, [2021 Mar 14;]. https://www.who.int/news-room/fact sheets/detail/typhoid Available from
2. Manandhar R, Raghubanshi BR, Neupane S, Lama R. Prevalence of Enteric Fever Pathogens Isolated from Blood Culture at a Tertiary Care Centre. JNMA: Journal of the Nepal Medical Association. 2021 Mar;59(235):256.
3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
4. Mogasale V, Ramani E, Mogasale VV, Park J. What proportion of Salmonella Typhi cases are detected by blood culture? A systematic literature reviews. Annals of clinical microbiology and antimicrobials. 2016 Dec;15(1):1-8.
5. Misra R, Prasad KN. Antimicrobial susceptibility to azithromycin among salmonella enterica typhi and Paratyphi A isolates from India. J Med Microbiol 2016; 65:1536-9.
6. Bhumbla U, Chaturvedi P, Jain S. Prevalence of Salmonella typhi in among febrile patients in a tertiary care hospital of South West Rajasthan. Journal of Family

Medicine and Primary Care. 2022 Jun 1;11(6):2852-5.

7. Patil N, Mule P. Sensitivity pattern of *Salmonella typhi* and paratyphi a isolate to chloramphenicol and other anti-typhoid drugs: An *in vitro* study. Infect Drug Resist 2019; 12:3217-25.
8. Mackie & McCartney Practical Medical Microbiology;14th Edition 2016 By JG Collee.
9. John J, Van Aart CJ, Grassly NC. The burden of typhoid and paratyphoid in India: systematic review and meta-analysis. PLoS neglected tropical diseases. 2016 Apr 15;10(4): e0004616.
10. Veeraraghavan B, Pragasam AK, Ray P, Kapil A, Nagaraj S, Perumal SP, Saigal K, Thomas M, Gupta M, Rongsen-Chandola T, Jinka DR. Evaluation of antimicrobial susceptibility profile in Salmonella typhi and Salmonella paratyphi A: presenting the current scenario in India and strategy for future management. The Journal of Infectious Diseases. 2021 Nov 15;224(Supplement_5): S502-16.
11. Barkume C, Date K, Saha SK, et al. Phase I of the surveillance for enteric fever in Asia Project (SEAP): an overview and lessons learned. J Infect Dis **2018**; 218:188–94.
12. Britto CD, Dyson ZA, Duchene S, et al. Laboratory and molecular surveillance of paediatric typhoidal *Salmonella* in Nepal: antimicrobial resistance and implications for vaccine policy. PLoS Negl Trop Dis **2018**; 12: e0006408.
13. Khan S, Kurup P, Vinod V, Biswas R, Pillai GK, Kumar A. Reconsidering azithromycin disc diffusion interpretive criteria for salmonellae in view of azithromycin MIC creep among typhoidal and nontyphoidal salmonella. J Lab Physicians **2019**; 11:39–44.
14. Kumar Y, Sharma A, Mani KR (2009) High Level of Resistance to nalidixic acid in Salmonella enterica serovar Typhi in Central India. J Infect Dev Ctries 3:467–469. doi: 10.3855/jidc.419.
15. Rodrigues C, Mehta A, Joshi VR (1999) Nalidixic acid-resistant Salmonella Typhi in Mumbai. Natl Med J India12:88.
16. Kadhiravan T, Wig N, Kapil A, Kabra SK, Renuka K, Misra A (2005) Clinical outcomes in typhoid fever: adverse impact of infection with nalidixic acid-resistant Salmonella Typhi.BMC Infectious Diseases5:37.
17. Kapil A, Das B (2002) Nalidixic acid susceptibility to screen ciprofloxacin resistance in SalmonellaTyphi. Indian J Med Res115: 49-54.

ORIGINAL ARTICLE

Transcriptional Analysis of Metalloprotease, Metallocarboxypeptidase, Isocitrate Lyase, Citrate Synthase, Malate Synthase and Dipeptidylpeptidase V of *Trichophyton Rubrum* Isolates from Dermatophytosis Patients

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ABSTRACT

Introduction: Dermatophytes are pathogenic fungi that cause cutaneous infection of human and animal and grow exclusively on the stratum corneum, nail and hair.
Methods: The present study was conducted on 160 samples from clinically diagnosed onycomycosis patients, further subjected to culture from nail samples of patients attending dermatology OPD of a tertiary care hospital, Delhi from January 2016 to December 2018
Results: In a soy protein culture, a substantial proteolytic activity was seen, which was secreted by *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum canis*. This proteolytic activity was 55–75% inhibited by O-phenanthroline, which authenticate that metalloproteases were secreted by all three species. A consensus probe was constructed on previously characterized genes, which encodes metalloproteases (MEP) of the M36 fungalysin family in *Aspergillus fumigatus, Aspergillus oryzae* and *M. canis*. From genomic libraries of *T. rubrum, T. mentagrophytes* and *M. canis, T. rubrum* & *T. mentagrophytes* a five-member MEP family was isolated and also secretes MCP A & MCP B, MEP 3, MEP4, IL, CS, MS AND DPP V of the M14 family according to the MEROPS proteolytic enzyme database.
Conclusion: This study shows that Metalloprotease, Metallocarboxypeptidase, Isocitrate Lyase, Citrate Synthase, Malate Synthase and Dipeptidylpeptidase V are expressed in Trichophyton Rubrum Isolates from Dermatophytosis Patients

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Introduction

Dermatophytes are pathogenic fungi which cause cutaneous infections in human and animal[1]. *Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum canis* are the most frequent human pathogenic species, accounting for 72–95% of the species isolated in hospitals and private practices[2]. Dermatophytes are characterized by their capability to grow exclusively in the stratum corneum, nails or hair, and able to digest components of the cornified cell envelope. The characterization of this disease is discoloration and thickening of the nail, frequenty with onycholysis, and may also affect the adjacent skin. Clinically onychomycosis is characterized by nail discoloration (yellow, white or, brown where the fungus is dense), nail dissociation from the nail bed (onycholysis), brittleness, nail thickening and subungual accumulation of scale. In more severe cases ridging and onychocryptosis (in grown nail) apears. All researched dermatophytes produce proteolytic activity in vitro[3]. From a single dermatophytic species, there are several reports of the isolation and characterization of one or two proteases [4,5, 6]. These proteases are generally described as keratinases and plays an important role in the provision of nutrients[7], host tissue invasion[8] and control of host defence mechanisms[9, 10.]

Multiple endoproteases are secreted by dermatophytes that are classified into two broad protein families, i.e., subtilisins (serine proteases), and fungalysins (metalloproteases) [11].

Various studies suggested that metalloproteases produced by *M. canis* and *T. rubrum* may be virulence-related factors involved in dermatophytosis, and dominate the two dermatophyte keratinase families[12]. There is a very high degree of similarity between five MEP genes with the MEP genes of *A. fumigatus* and the neutral protease I gene of *A. oryzae,* as all of which contain the HEXXH sequence motif. Researcher confirmed that the MEP genes of *T. mentagrophytes, T. rubrum,* and *M. canis* are highly homogeneous with 72–97% similarity between the gene sequences of various species [13].

Moreover to this dermatophytes also secretes two leucine aminopeptidases (Lap), Lap1 and Lap2, and two dipeptidyl-peptidases (DPP), DPP IV and DPP V. Lap1 and Lap2 are belongs to metalloproteases while DPP IV and DPP V are classified as serine proteases with a Ser, Asp, His catalytic triad[14]. Dermatophytes also secrete a metallocarboxypeptidase A (MCP A) and two serine carboxypeptidases (Scp), ScpA and ScpB. In a medium containing protein as a sole nitrogen and carbon source, *T. rubrum* & *T. mentagrophytes* secretes MCP A & MCP B of the M14 family according to the MEROPS proteolytic enzyme database. MCP A & MCP B is homologous to human pancreatic carboxypeptidase A, and is synthesized as a precursor in a preproprotein form[15].

Amongst the all virulent genes, the role of enzyme involved in glyoxylate cycle are also co-related with the pathogenicity of *T. rubrum*, although their functions and mechanisms remain undetermined. The primary response to phagocytosis in *S. cerevisiae* was the introduction of gene products associated with the

Glyoxylate cycle. The key enzymes of the Glyoxylate cycle, isocitrate lyase (IL) and malate synthase (MS), were highly induced in macrophages. Other transcripts, most notably those of the Tri carboxylic acid (TCA) cycle, were not induced under these conditions[16].

Real-time PCR analysis impart high levels of sub7 expression following growth on human nail, whereas all other virulence genes expression analysis were elevated following growth on human stratum corneum. So, in this study, we lookout at the m-RNA expression patterns and dynamics of genes encoding two major families of endoproteases, exoproteases: metallo-protease (MEP) 3, MEP 4, metallocarboxypeptidase (MCP) A, MCP B, and key enzyme of Glyoxylate cycle like Isocitrate lyase (IL), Citrate synthase (CS), Malate synthase (MS) and dipeptidyl-peptidases (DPP V) in *T. rubrum isolates* by real-time PCR from patients suffering from dermatophytosis in nail[17].

Materials and Methods

The present study was conducted on 160 samples from clinically diagnosed onycomycosis patients, further subjected to culture from nail samples of patients attending dermatology OPD of a tertiary care hospital, Delhi from January 2016 to December 2018.

The mean age of patients was 29.8 ±11.41 (with the range from 16 to 60 years). The duration of dermatophytic infection ranged from 3 months to 15 years (9.74 ± 6.81 months). Out of 160 samples, 100 were found to be KOH positive, of which 70 samples were culture positive for NDM & dermatophytes. Of the 70 isolates, 20 isolates were identified as *T. rubrum* and 50 isolates as NDM on phenotypic mycological assessment. *T. rubrum* was the predominant pathogen isolated from nail samples.

A portion of each clinical specimen was suspended in a drop of 40% potassium hydroxide (KOH) for processing of the nail respectively. KOH wet mount slides were viewed under 40X magnification. A portion of the sample was cultured on Sabouraud's dextrose agar (Hi-media, Mumbai) with antibiotics with chloramphenicol (0.05 g/l), gentamicin (20 mg/l) and cyclohexamide (0.5 g/l). All inoculated tubes were then incubated at 25°C for 3-4 weeks optimal growth. After growth, the etiological agent was confirmed by the characteristic morphology of the colony and by studying the microscopic appearance of the fungus on Lacto Phenol Cotton Blue (LPCB) mount and Urease test[18]. The molecular confirmation of isolates was done by PCR and sequencing using species-specific primers of *T. rubrum*.

DNA extraction and PCR

DNA was extracted from the cultures grown on SDA by using the commercially available DNA extraction kit (HiYield Genomic DNA Kit, RBC, Taiwan). PCR was performed with species specific primer of *T. rubrum*, forward GACCGACGTTCCATCAGGGGT and reverse TCAGACTGACAGCTCTTCAGAG (203bp) for amplification of the desired gene segment[19]. Each PCR tube contained a total volume of 25 µl which included 2.5 µl buffer (10X), 5 µl of Q-buffer, 0.5 µldNTPs (200 µM), $MgCl_2$ 0.5 µl (1.5 mM), 0.15 µlTaq polymerase, 1 µl of each primer, forward and reverse (10 µM) (Taq PCR Core Kit, Fisher Scientific – Qiagen, Germany), 5 µl of DNA

template and nuclease-free water to make up the volume. Amplification was performed in a Master cycler personal (Eppendorf, Hamburg, Germany). Initial denaturation was performed at 94°C for 10 min which was followed by 35 amplification cycles of 30 s at 95°C and 45 s at 65°C and 30 s at 72°C, and final extension of 10 min at 72°C. The amplified PCR products were analyzed by electrophoresis on 1.5% agarose gel, stained with Ethidium bromide and electrophoresed at 125 V and 15 mAh current in a 10 slot apparatus for 30 min. Molecular marker of 100 bp was used to determine the size of the amplicons.

Purification of PCR products was done by Sodium acetate method and DNA sequenc analysis was performed by comparison of the nucleotides with dermatophytes reference nucleotide sequence obtained from gene bank database (site http://www.ncbi.nih.gov/gene bank). On the basis of alignment of sequences of internal transcribed spacer region ITS 1 and 2 in the NCBI nucleotide database, the isolates were identified as *T. rubrum* with 99% similarity with reference strains. The representative sequences so obtained were submitted to gene bank database and accession numbers obtained were MH497367, MH497368 & MH497369.

RNA extraction and complimentary DNA synthesis

Total RNA was extracted from 20 culture isolates of *Trichophyton rubrum* using TRIzol™ Reagent (Invitrogen, USA). Briefly, 1 ml of TRIzol™ Reagent was added in the sample and mixed gently by micropipette to form a homogeneous cell lysate and incubated for 5 min at room temperature. 200µl of chloroform per ml of TRIzol™ reagent was added and centrifuged at 12,000 rpm for 15 min at 4°C to obtain a colorless upper aqueous phase. RNA containing aqueous phase was taken in a fresh 1.5 ml tube and washed with 500 µl of isopropanol. The eppendorf tube was centrifuged at 12,000 rpm for 10 min at 4°C and supernatant was discarded. 1 ml of 75% ethanol was added to the pellet and mixed by vortex. This was followed by centrifugation at 10,000 rpm for 5 min at 4°C. The supernatant was discarded and the pellet was resuspended in RNase free water, and incubated at 55-60°C for 15 min. The isolated RNA was stored at -80°C immediately. The integrity of the RNA samples was confirmed by agarose gel electrophoresis. Concentration and purity of the samples were assessed by Nano-drop (Eppendorf, USA). Extracted RNA was used as a template for c-DNA synthesis by Superscript reverse transcriptase II (Invitrogen, USA). Total RNA (2-5 µg) from each sample was reverse transcribed into c-DNA under thermal conditions, 25ºC for 10 min., 37ºC for 120 min., 85ºC for 5 min. and 4ºC for infinite time.

Expression study of virulence genes by Quantitative Real-Time PCR (qRT-PCR):

To quantify the expression of virulence genes, Metalloprotease 3 (MEP 3), Metalloprotease 4 (MEP 4), Metallocarboxypeptidase A (MCP A), Metallocarboxypeptidase B (MCP B), Isocitratelyase (IL), Citrate synthase (CS), Malate synthase (MS) and Dipeptidylpeptidase V (DPP V), qRT-PCR was performed on Light Cycler® 480 Instrument (Roche Diagnostics, Germany). Twenty confirmed isolates of *T. rubrum* and a reference strain of *T. mentagrophytes* (ATCC no. 28185) were used to estimate the expression of virulence gene. The final Real-Time PCR reaction of 20µl contained 2 µl of c-DNA, 10 µl

of SYBER Green Universal Master Mix, and 60M of random hexamer primers shown in table -1.a. The Real Time PCR conditions were standardized according to the melting temperature (Tm) of the primers as shown in **Table 1.b**.

Table 1.a-Primer sequences used for Real-time were as follows

GENE NAME	PRIMER
MEP 3	F 5'-AGCAGCACGCCAGCAACG-3'
	R 5'-GCAGACGGAAGGACTCGATGT-3'
MEP 4	F 5'-AGTCGGGACACCATTCTTCAG-3'
	R 5'-ATTTGGGCTTCTATGCTCTACG-3'
MCP A	F 5'-GCATTGAAGGCGGTGCAT-3'
	R 5'-GTCAACACTGTCTCCATTAACTTGGT-3'
MCP B	F 5'-GTTCTCGAGTGCAGTATGGCTACAACCAG-3'
	R 5'-GTTAGATCTTATTTTAACCTGAAAATAGGAT-3'
ISOCITRATE	F 5'-TGGAAAGATTCAAGATGGCGATA-3'
LYASE	R 5'-TCTGTGCATTTGATGGGTAATCA-3'
MALATE	F 5'-TCTTTCTCTCCTCTCTTTCTTTCCTTCCAACCAC-3'
SYNTHASE	R 5′-CAGAAGACGATTGACTTGGAATTTCTCAAGTGCTGTT-3'
CITRATE	F 5'-GAACATGGTAAATGGTCAGGTGAA-3'
SYNTHASE	R 5'-CGCCGAGGGTGAAGTCAA-3'
DPP V	F 5'-CTTAGATCTGTTCCTCCTCGTGAGCCCCG-3'
	R 5'-CTTGCGGCCGCTCATTCCTCTGCCCTCTCACC-3'
GAPDH	F-5'-CTTAGCACCCCTGGCCAAG-3'
	R-5'-TGGTCATGAGTCCTTCCACG-3'

Table 1.b: Thermal profile of Metalloprotease 3 & 4 (Mep3 & 4); Metallocarboxypeptidase A & B (Mcp A & B); Dipeptidyl-Peptidase V (DPP V); Isocitrate lyase; Citrate synthase; Malate synthase and GAPDH

Temperature	**Time**	**Cycles**	**Phase**
95°c	10 min	1	Initial Denaturation
95°c	20 sec	35	Denaturation
60°c	30 sec		Annealing
72°c	20 sec		Extension
95°c	10 sec	1	Melting temperature

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) served as an endogenous expression control for normalization of data and the threshold cycle (Ct) values were calculated by the software. The dissociation curve analysis was performed to verify that a single product was amplified. Amplification curve and melt peaks for each virulence gene were shown in Figure-2. The fold change of the expression of virulence genes in clinical isolates was compared to ATCC strain of *T. mentagrophyte* ATCC no. 28185 was calculated by $2^{-\Delta\Delta}$ Ct method (R) [19].

Result

The distribution of *T. rubrum* isolates confirmed by genotypic method, obtained from infected nail specimen. All samples phenotypically confirmed were reconfirmed genotypically by PCR & sequencing. The mean age and duration of disease of these 20 patients were 29.8 ±11.41 years and 9.74 ± 6.81 months, respectively. This result shows the infected nail in onycomycosis patients.

These 20 *T. rubrum* culture were confirmed phenotypic (LPCB) and genotypic (PCR) and reconfirmed by sequencing.

The real-time PCR analysis of virulence genes has shown that the expression of all the genes i.e. MEP 3, MEP 4, MCP A, MCP B, IL, CS, MS and DPPV were significantly up-regulated in all the clinical isolates as compared to reference strain of *T. mentagrophyte* ATCC no. 28185**..** The expression levels and the qualitative status of the virulence genes represented as Ct values in the 20 clinical isolates versus ATCC strain is shown in Table 2.

Table-2: Transcriptional analysis of differential expression level of virulence genes of *T. rubrum* calculated by fold change

T. R.	MEP 3	MEP 4	MCP A	MCP B	IL	CS	MS	DPP V
Avg. ΔCT of isolates	-2.61	-3.75	-3.13	-4.57	-1.36	-3.22	-2.48	-1.43
Avg. ΔCT of ATCC Strains	1.85	-1.76	2.29	-0.95	3.24	3.38	0.06	-0.88
Fold change(2^-$^{\Delta\Delta CT}$ of isolates)	**23.89**	**27.43**	**30.15**	**64.07**	**9.83**	**35.31**	**50.57**	**21.90**

Among three non- protease virulence genes, we found Malate Synthase as highly expressed (50.57 fold high), followed by Citrate Synthase (35.31) and Isocitrate Lyase (9.83), in clinical isolates of *T. rubrum* as compared to ATCC strain. Furthermore, among secreted protease gene encoding the major keratinases, Metallocarboxypeptidase B was strongly up regulated (64.07 fold high) followed by Metallocarboxypeptidase A (30.15) and DPP V (21.90), in dermatophytic patients as compared to ATCC strain. Furthermore, among Endoprotease gene encoding the major keratinases, Metallocarboxypeptidase B was strongly up regulated (64.07 fold high) followed by Metalloprotease 4 (27.43) and Metalloprotease 3 (23.89), compared to *T. mentagrophytes* ATCC no. 28185 strain.

Discussion

The dermatophytes species of Trichophyton and Microsporum are multiplication of an ancestral gene which encodes secreted fungalysin. These are the genes which invade keratinized tissues of an ancestral evolutionary gene of belongs to the family that encode secreted proteases.

Dermatophytes exclusively grow in the stratum corneum and utilized the keratin and structurally cross linked proteins of the cornified nail cell enclose as substrate. In the course of infection, dermatophytes produce various endo- and exo- proteases to degrade keratinized structure into short peptides and free amino acids, which is utilized as nutrients by the fungus.

The appearance of at least four gene duplication events in the putative ancestor of dermatophytes is required to explain the tree topology obtained (Fig. 3). A primary duplication produced the ancestral types of MEP 1 and MEP 5 on the one hand, and of MEP 2, MEP 3 and MEP 4 on the other hand. Subsequent duplications produced MEP 1 and MEP 5, MEP 4, and the ancestral type of MEP 2 and MEP 3. The duplication of the latter produced MEP 2 and MEP 3 along with the loss of three introns in MEP 2. Ancient gene duplications are known as one of the main forces in the generation of gene families and the creation of new functional capabilities[23].

In soy proteins culture medium, *T. rubrum, T. mentagrophytes* and *M. canis* secrets two major MEPs (MEP 3 and MEP 4) encoded by orthologous genes, although full length c-DNA of all MEP types was found to be present in the *T. rubrum* c-DNA library. In the MEPs, several putative glycosylation sites were identified (Table 2). The multiplicity of MEP 3 and MEP 4 protein bands can be explained by different levels of glycosylation. So in the present study, we have also done transcriptional study in MEP 3 & MEP 4 by real time PCR, to check their expression level, though in our study MEP 4 is more up-regulated (27.43 fold) in compare to MEP 3 (23.89 fold), which is concordant to the study done by Maranho et.al 2007 & Leng W et.al 2009. A comparison of the potential pathogenicity of five metalloprotease genes from *T. mentagrophytes* led to the proposal that MEP 4 and MEP 5 were possibly affect pathogenicity, which is determined in a guinea pig model and a keratin degradation test[24], whereas expression of only MEP 4 was significantly

upregulated after growth in vitro on keratin, collagen, elastin or human skin sections[25].

Among approximately 10 human pathogenic species dermatophytes isolated in Europe, *T.* rubrum, *T. mentagrophytes* and *M. canis* are most commonly observed, accounting for 72–95% of the species isolated in hospital and private practices[12]. All investigated dermatophytes produce proteolytic activity in vitro[13]. There is a report of the genome with perfect (or near-perfect) identity, the loss of one or various copies, or the acquisition of functional novelty through the accumulation of random mutations, also known as 'subfunctionalization'[24].

The isolation and characterization of five MEP and seven SUB genes from *T. rubrum* and *T. tonsurans* was demonstrated. The proteins (most of them being proteases) which are secreted in a medium containing proteins as the sole carbon and nitrogen source, likely represent the spectrum of enzymes that permit the degradation of keratinized tissues into assimilable compounds during the course of infection.

Burmester et.al 2011 identified DPP V as the *T. tonsurans* allergen Tri t 4, Tri r 4 of *T. rubrum* and Tri m 4 of *T. mentagrophytes.* Strikingly, when *T. benhamiae* was co-cultured with keratinocytes, expression of DPP V was up-regulated, but there is no change in the expression of the other exoproteases described above, but in our study exoprotease, such as MCP B is more up-regulated compared to MCP A & DPP V which is discordant from study of Burmester et.al 2011.

T. rubrum secretes two zinc-dependent metallocarboxypeptidases, viz. MCP A and MCP B, (M14A family), when grown on protein medium. Analysis of the dermatophytes revealed the presence of four M14 metallocarboxypeptidase genes in the genome of all isolates, except *T. benhamiae,* which possessed five such genes.

Metalloprotease genes of *T. rubrum* are most predominant genes for pathogenicity and the ability of dermatophytes to invade keratinized tissues and to be essentially confined to keratinize structures. It can be presumed that keratinolytic proteases (keratinases) might be significant virulence factors. Therefore, the characterization of keratinase appears to be a major step for a better understanding of dermatophytic infection, pathogenesis and subsequently the host-fungus relationship. Some keratinases have been isolated from *T. rubrum*[24, 25,26, 27].

Although keratinases are supposed to be involved in dermatophytic pathogenicity, only a few studies have evaluated there in vivo production[22]. Moreover to this, only one protease with keratinolytic activity, a recombinant *T. rubrum* protease was characterized at the gene level. In fact, most of the authors have only reported enzymatic activities of dermatophyte proteases on various macromolecular substrates and co-related there in vitro keratinolytic activity to the ability of dermatophytes to invade keratinized structures in vivo[26].

The MEP 4 and MEP 5 defective strains were the least pathogenic, while the MEP 3 mutant showed pathogenicity levels that were similar to the wild-type strain. The data suggested that the proteases coded for, by the MEP 4 and MEP 5 genes were more virulent than the other MEP genes, and were also the predominant proteases in the host invasion process of

T. mentagrophytes. Similar test results for the MEP 3 mutant and the wild-type strain suggested that MEP 3 metalloprotease expression in *T. mentagrophytes* may not be the same as in *M. canis* or *T. rubrum*[27, 28]. The MEP 1 and MEP 2 mutations had some effects on the pathogenicity of *T. mentagrophytes*; however, further research is needed to investigate their influence as inconsistent test results were observed. Furthermore research focusing on exocrine protein analysis of the transformants, variation in the protein composition, and transformation of other dermatophytes need further investigation of the functionality, mechanism, and specification of the MEP genes[29, 30].

Conclusion

This study shows that Metalloprotease, Metallocarboxypeptidase, Isocitrate Lyase, Citrate Synthase, Malate Synthase and Dipeptidylpeptidase V are expressed in Trichophyton Rubrum Isolates from Dermatophytosis Patients

Conflicts of Interest

The author started there is no conflict of interest

References

1. Heikkla, H.; Stubb, S. The prevalence of onychomycosis in Finland. Br. J. Dermatol. 1995, 133, 699–703.
2. Metalloprotease genes of Trichophyton mentagrophytes are important for pathogenicity Xinke Zhang, Yanchun Wang, Wanyi Chi, Yao Shi, Si Chen, Degui Lin∗ and Yipeng Jin∗ ClinicalDepartment,CollegeofVeterinaryMedicine
3. Scher, R.K.; Rich, P.; Pariser, D.; Elewski, B. The epidemiology, etiology, and pathophysiology of onychomycosis. Semin. Cutan. Med. Surg. 2013, 32, S2–S4.
4. Nouripour-Sisakht, S.; Mirhendi, H.; Shidfar, M.R.; Ahmadi, B.; Rezaei-Matehkolaei, A.; Geramishoar, M.; Zarei, F.; Jalalizand, N. Aspergillus species as emerging causative agents of onychomycosis. J. Mycol. Med. 2015, doi:10.1016/j.mycmed.2014.12.001.
5. Weitzman I, Summerbell RC. The dermatophytes. Clin Microbiol Rev 1995; 8: 240–259.
6. Yu RJ, Harmon SR, Blank F. Isolation and purification of an extracellular keratinase from Trichophyton mentagrophytes. J Bacteriol 1968; 96: 1435–1436.
7. Asahi M, Lindquist R, Fukuyama K et al. Purification and characterization of major extracellular proteinases from Trichophyton rubrum. Biochem J 1985; 232: 139–144.
8. MonodM,CapocciaS,LechenneBetal.Secretedproteasesfrom pathogenic fungi. Int J Med Microbiol 2002; 292: 405–419.
9. Monod M. Secreted proteases from dermatophytes. Mycopathologia 2008; 166: 285–294.
10. Brouta F, Descamps F, Monod M et al. Secreted metalloprotease gene family of Microsporum canis. Infect Immun 2002; 70: 5676–5683.
11. Maranhao FC, Paiao FG, Martinez-Rossi NM. Isolation of transcripts over-expressed in human pathogen Trichophyton rubrumduringgrowthinkeratin.MicrobPathog2007;43:166– 172.

12. JoussonO,LechenneB,BontemsOetal.Secretedsubtilisingene family in Trichophyton rubrum. Gene 2004; 339: 79–88.
13. Jousson O, Lechenne B, Bontems O et al. Multiplication of an ancestralgeneencodingsecretedfungalysinprecededspeciesdifferentiation in the dermatophytes Trichophyton and Microsporum. Microbiology 2004; 150: 301–310.
14. BroutaF,DescampsF,FettTetal.Purificationandcharacterizationofa43.5kDakeratinolyticmetalloproteasefromMicrosporum canis. Med Mycol 2001; 39: 269–275.
15. Giddey K, Favre B, Quadroni M et al. Closely related dermatophyte species produce different patterns of secreted proteins. FEMS Microbiol Lett 2007; 267: 95–101.
16. Giddey K, Monod M, Barblan J et al. Comprehensive analysis of proteins secreted by Trichophyton rubrum and Trichophyton violaceum under in vitro conditions. J Proteome Res2007; 6: 3081–3092.
17. Appelt L, Nenoff P, Uhrlaß S, Krüger C, Kühn P, Eichhorn K, Buder S, Beissert S, Abraham S, Aschoff R, Bauer A. Terbinafin-resistente Dermatophytosen und Onychomykose durch Trichophyton rubrum. Der Hautarzt. 2021 Oct;72(10):868-77.
18. Maranhao FC, Paiao FG, Martinez-Rossi NM. Isolation of transcripts over-expressed in human pathogen Trichophyton rubrum during growth in keratin. Microb Pathog. 2007; 43: 166–172.
19. Leng W, Liu T, Wang J, Li R, Jin Q. Expression dynamics of secreted protease genes in Trichophyton rubrum induced by key host's proteinaceous components. Med Mycol. 2009; 47: 759–765.
20. Danew P, Friedrich E, Mannsfeldt HG. Peptidase activity of skinpathogenic fungi. I. Determination of leucineaminopeptidase, arylamidase, carboxypeptidase and acylase activity in Trichophyton rubrum and Microsporum gypseum. Dermatol Monatsschr. 1971; 157: 232–238 [in German].
21. De Bersaques J, Dockx P. Proteolytic and leucylnaphthylamidase activity insomedermatophytes:preliminaryresults.ArchBelgDermatolSyphiligr. 1973; 29: 135–140.
22. Zaugg C, Monod M, Weber J et al. 2009. Gene expression profiling in the
23. humanpathogenicdermatophyteTrichophytonrubrumduringgrowthon proteins. Eukaryot Cell. 2009; 8: 241–250.
24. LatkaC,DeySS,MahajanSetal.Genomesequenceofaclinicalisolateof dermatophyte, Trichophyton rubrum from India. FEMS Microbiol Lett. 2015; 362: fnv039.
25. Marzluf GA. Genetic regulation of nitrogen metabolism in the fungi. Microbiol Mol Biol Rev. 1997; 61: 17–32.
26. Hensel M, Arst HN, Jr., Aufauvre-Brown A, Holden DW. The role of the Aspergillus fumigatus areA gene in invasive pulmonary aspergillosis. Mol Gen Genet. 1998; 258: 553–557.
27. Mercer DK, Stewart CS. Keratin hydrolysis by dermatophytes. Medical mycology. 2019 Jan 1;57(1):13-22.
28. Gupta AK, Stec N, Summerbell RC, Shear NH, Piguet V, Tosti A, Piraccini BM. Onychomycosis: a review. Journal of the European Academy of

Dermatology and Venereology. 2020 Sep;34(9):1972-90.

29. Cribier BJ, Bakshi R. Terbinafine in the treatment of onychomycosis: a review of its efficacy in high-risk populations and in patients with nondermatophyte infections. British Journal of Dermatology. 2004 Mar;150(3):414-20.
30. Piraccini BM, Alessandrini A. Onychomycosis: a review. Journal of Fungi. 2015 Jun;1(1):30-43.
31. Kawa N, Lee KC, Anderson RR, Garibyan L. Onychomycosis: A review of new and emerging topical and device-based treatments. The Journal of clinical and aesthetic dermatology. 2019 Oct;12(10):29.

REVIEW ARTICLE

Anesthesia Management in Laparotomy of Gastric Perforation Peritonitis: A Case Report and Literature Review

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ABSTRACT

Gastric perforation has the potential to induce acute peritonitis, leading to intense pain and a significant mortality risk. A female patient, aged 67, presented with symptoms of intense abdominal pain affecting all regions of the abdomen and extending to the shoulder. The pain does not alleviate with rest and is aggravated by physical activity and coughing. The Chest X-ray examination revealed the presence of cardiomegaly, characterized by an enlarged heart and aortic elongation. The Abdomen X-ray examination revealed the presence of a pneumoperitoneum. Laparotomy may be conducted in individuals with gastric perforation to identify the pneumoperitoneum's underlying aetiology. The utilization of a concurrent administration of spinal anaesthesia (SA) and general anaesthesia (GA) demonstrates the effective reduction of hemodynamic impact during pneumoperitoneum while avoiding any associated adverse effects.

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Introduction

Gastric perforation is a complex penetration of the wall of the stomach, large intestine, and small intestine, resulting in the intestine's contents flowing into the abdominal cavity.[1] Acute peritonitis from gastric perforation can result in excruciating discomfort. Seventy per cent of peptic ulcer disease-related deaths result from gastric perforation. The patient's mortality rate is frequently correlated with the perforation's diameter. Gastric resection or suturing of the perforation site are steps in managing gastric perforation after urgent surgery.[3] Patients with stomach perforation may undergo laparotomy to determine what led to the development of pneumoperitoneum. Additionally, a laparotomy might be therapeutic by sealing the punctured organ.[3]

The choice of anaesthesia modality for a laparotomy procedure is contingent upon several factors, including the patient's age, overall health status, general well-being, and the available resources and expertise of the surgical team, including surgeons, anaesthetists, and nurse

anaesthesiologists.[4] Individuals scheduled to undergo laparotomy have the option to receive either general anaesthesia or regional anaesthesia.[5] In a study conducted by Ghodki PS (2014)[6], it was demonstrated that the utilization of a concurrent administration of spinal anaesthesia (SA) and general anaesthesia (GA) effectively mitigates the hemodynamic consequences of pneumoperitoneum.[7] This combination approach was found to be devoid of any adverse effects. Utilizing both methodologies yields enhanced stability in cardiocirculatory function compared to the exclusive use of general anaesthesia.[6] This article presents a case report on peritonitis resulting from gastric perforation surgery performed under the administration of both general and spinal anaesthesia.

Case(s)

A 67-year-old female patient presented with symptoms of intense abdominal pain affecting all regions of the abdomen and extending to the shoulders, which had been ongoing for 5 hours before admission to the hospital. The pain sensation does not improve during rest periods but intensifies with physical activity and coughing. The patient also experiences a sensation of abdominal tightness and rigidity resembling a solid wooden surface. Pain is concomitant with symptoms such as muscular debility, abdominal distension, queasiness, lightheadedness, and perspiration characterized by decreased body temperature. The patient also disclosed a lack of bowel movements and flatus within the past 24 hours. In the past, patients frequently reported experiencing heartburn characterized by intermittent episodes of a burning sensation, which the administration of ulcer medications alleviated. The patient presents with a medical background of hypertension and diabetes mellitus, for which the prescribed treatment regimen consists of amlodipine 5 mg and metformin 500 mg regularly. The physical examination yielded the following findings: the individual's body weight was measured to be 60 kilograms, total body height was recorded as 150 centimetres, blood pressure was measured at 180/90 millimetres of mercury, pulse rate was observed to be 80 beats per minute, respiration rate was noted to be 20 breaths per minute, and their body temperature was measured to be 37.1 degrees Celsius. The findings from the assessment of the localized condition of the abdomen revealed diminished bowel sounds, a firm and rigid sensation akin to a board, tenderness throughout the abdominal area, diffuse tenderness, muscular guarding, pain upon percussion, and tympanic percussion. The laboratory analysis revealed a hematocrit level of 27%, hemoglobin concentration of 8.4 g/dl, leukocyte count of 19,900 ul, and blood glucose level of 193 mg/dl. The chest X-ray examination revealed the presence of cardiomegaly accompanied by aortic elongation, as depicted in Figure 1. The abdominal region was subjected to a plain X-ray examination, which revealed the presence of a pneumoperitoneum, as depicted in Figure 2.

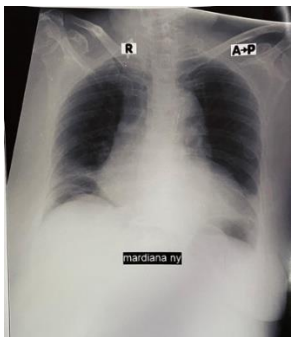

Figure 1. In the first admission, enlarged heart was seen, cardio thorax ratio was 58%.

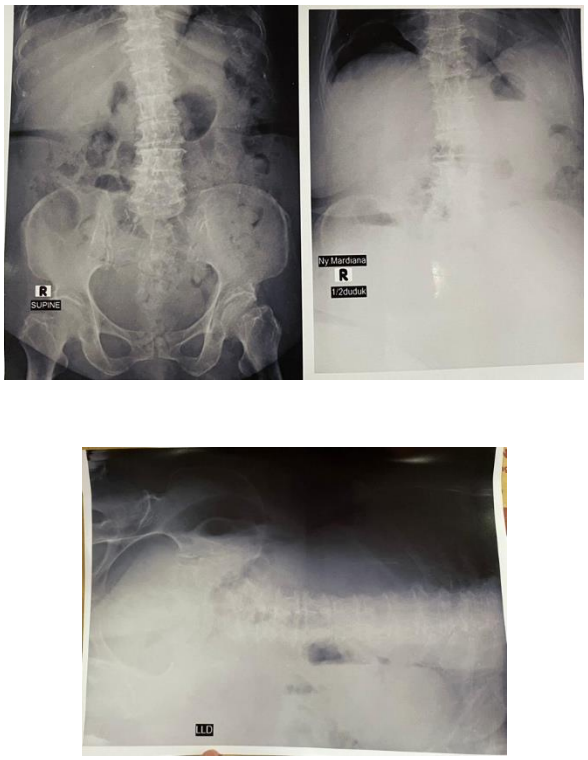

Figure 2. Abdominal x-ray showed pneumoperitoneum.

The patient is in a fasted state for 6 hours. The patient was premedicated with oral Ondansetron 4 mg and intravenous lactated ringer's solution 500 ml. Noninvasive blood pressure, oxygen saturation, electrocardiogram (ECG), and urinary catheter were monitored with oxygen intake. The initial vital signs were listed below: blood pressure (BP) 170/70 mmHg; heart rate (HR) 91 bpm; SpO2 99%. ECG showed a sinus rhythm of 91 Bpm. The patient was pre-oxygenated with 100% with 3lpm flow. Bupivacaine 0,5% 3 ml was injected as spinal analgesia. After approximately 10 minutes, rapid induction of general anesthesia for intubation was conducted with 1% propofol 100 mg, fentanyl 80 mcg, midazolam 1 mg, and Atracurium Besylate 20 mg. The patient was intubated using no.7 cuffed endotracheal tube. The cuff was inflated, and the tube was fluxed after checking bilateral air entry. Sevoflurane 1,5% was given as inhalation analgesia. The patient was steady during the surgery, with an average blood pressure of 146/80 mmHg, HR 87 times/minute, and SpO2 of 98 to 100%. The total amounts of intraoperative transfusion were plasma 200 ml and lactated ringer's solution 500 ml. The gastric resection was performed which lasted three hours. The total urine output was 100cc and blood loss was 200 ml.

After surgery, the patient was safely transferred to the Intensive Care Unit (ICU) to receive further treatment and later extubated after fully awake. With a follow-up in ICU, he was conscious and in good condition without complaints of headaches and nauseous. Vital signs were as follows: body temperature 36,6 ^{0}C, BP 151/78 mmHg, HR 91 bpm, SpO2 maintained at 98% by nasal oxygen 3 L/min.

Discussion

Gastric perforation refers to the pathological process wherein the integrity of the gastric wall is compromised, leading to the formation of an opening that establishes a communication between the gastric lumen and the peritoneal cavity. A peptic ulcer is identified as the predominant etiological factor contributing to gastric perforation. This disease typically manifests in the geriatric population, particularly among individuals with a documented history of nonsteroidal anti-inflammatory drug (NSAID) usage and those who engage in excessive alcohol consumption. Furthermore, gastric perforation can be attributed to malignancy and interventional procedures and may manifest spontaneously in neonates. The number of male patients exceeded that of female patients, with a higher prevalence observed among individuals aged 50 to 59 years.[2]

In this case, the patient is categorized as geriatric according to the World Health Organization's (2010) definition, which designates individuals aged 65 years or older as geriatric.[8] The utilization of pharmaceutical substances poses a heightened risk in the geriatric population, consequently augmenting the likelihood of encountering adverse effects associated with said medications.[9] Geriatric individuals undoubtedly undergo the process of aging, which is accompanied by a decline in the functioning of organ systems and a reduction in the capacity to respond to acute stressors. Hence, it is imperative to prioritize evaluating the patient's physiological state to establish a secure and efficient anaesthetic strategy before any medical procedure.[10]

The ASA scoring system, developed by the American Society of Anesthesiologists (ASA), serves as a means of categorizing and evaluating the physiological status of patients during the perioperative period. This classification system aids in the estimation of surgical risk. In this instance, the patient exhibited an ASA III score alongside the coexistence of hypertension and type II diabetes mellitus. This issue is of concern due to the notable correlation between elevating the ASA score and escalating mortality rates following anaesthesia.[11,12] The administration of intravenous anaesthetics has yielded favorable outcomes regarding blood glucose levels and complications among individuals diagnosed with type 2 diabetes, compared to inhalational anaesthetics. The administration of inhaled anaesthetics has been observed to induce impaired glucose tolerance and insulin secretion by inhibiting ATP-sensitive potassium channels in beta cells. Consequently, this mechanism can lead to hyperglycemia during the perioperative period.[11] Patients with a preexisting hypertension diagnosis may experience a worsening of their condition, known as severe hypertension, during the period following a surgical procedure.[13] Consequently, it is imperative to closely observe and provide appropriate cautionary measures to patients with severe hypertension, particularly if their management becomes challenging or uncontrollable.[12]

General anesthesia (GA) is the prevailing method employed for both laparoscopic and laparotomy procedures due to its ability to effectively manage surgical pain and enhance patient comfort during the pneumoperitoneum and Trendelenburg positions.[14] *General anesthesia* is a medical technique that ensures a protected airway and enables effective control of ventilation, thereby mitigating the occurrence of hypercarbia. Nevertheless, the patients experienced episodes of hypertension due to stress-induced sympathetic stimulation during intubation, in conjunction with the sympathetic activity triggered by pneumoperitoneum.[6]

Regional anaesthesia is widely acknowledged for its ability to mitigate the adverse effects associated with general anesthesia, including but not limited to nausea, vomiting, sore throat, tooth injury, sedation, postoperative atelectasis, and hypoventilation.[15,16] The potential adverse effects of general anaesthesia, including airway trauma, myalgias, and sore throats, can be mitigated through regional anaesthesia. Furthermore, regional anaesthesia has been shown to facilitate expedited cognitive recovery and oral intake during the immediate postoperative phase.[17] Regional anesthesia offers several potential advantages, including expedited recuperation and efficient management of postoperative pain. Regional anesthesia has been employed in laparotomy

procedures for patients exhibiting compromised cardiopulmonary function due to its minimal impact on cardiopulmonary function. The administration of regional anesthesia has potentially resulted in adverse effects, including significant hypotension and discomfort in the shoulder region due to irritation of the diaphragm.[18] Regional anesthesia is widely acknowledged for its ability to mitigate the adverse effects associated with general anesthesia, including but not limited to nausea, vomiting, sore throat, tooth injury, sedation, postoperative atelectasis, and hypoventilation. The potential adverse effects of general anesthesia, including airway trauma, myalgias, and sore throats, can be mitigated through regional anesthesia. Furthermore, regional anesthesia has been shown to facilitate expedited cognitive recovery and oral intake during the immediate postoperative phase. Regional anesthesia offers several potential advantages, including expedited recuperation and efficient management of postoperative pain. Regional anesthesia has been employed in laparotomy procedures for patients exhibiting compromised cardiopulmonary function due to its minimal impact on cardiopulmonary function. The administration of regio has resulted potentially resulted in adverse effects, including significant hypotension and discomfort in the shoulder region due to irritation of the diaphragm.[19] Previous studies have demonstrated that regional anesthesia can decrease metabolic response. This finding aligns with the research conducted by Ghodki PS (2014), which suggests that using both SA and GA can enhance hemodynamic stability among individuals undergoing laparoscopic hysterectomy.[20]

When systemic arterial (SA) administration is combined with general anesthesia (GA), the application of SA-induced sympathectomy can effectively restrict the rise in systemic vascular resistance (SVR), thus counteracting the elevation in mean arterial pressure (MAP). The study conducted by Ghodki PS (2014) corroborated these findings, indicating that the mean arterial pressure (MAP) was effectively sustained during pneumoperitoneum in the group that received a combination of spinal anesthesia (SA) and general anesthesia (GA), in contrast to the group that solely received GA.[7]

In this instance, postoperative bleeding was monitored within the recovery room. The individual exhibited complete consciousness, as indicated by an Aldrette score of 10. The patient was under observation in the Intensive Care Unit (ICU), and no instances of postoperative bleeding were observed. Subsequently, the patient was transferred to the hospital ward in a notably enhanced state.[20]

The patient's vital signs were monitored before, during, and after the operation, revealing that the patient's condition remained favorable and hemodynamically stable throughout the procedure, facilitated by the administration of general and spinal anesthesia.

Conflicts of Interest

The author started there is no conflict of interest

References

1. Hapsari TM. Penatalaksanaan Pasien Bedah Saluran Cerna (Perforasi Gaster) di Rumah Sakit Emanuel Banjarnegara. JNH (Journal of Nutrition and Health). 2021;9(2):8-12.

2. Qorina S. Profil Pasien Perforasi Gaster di RSUD Kolonel Abundjani Bangko pada Tahun 2020. Jurnal Ilmu Kesehatan Indonesia. 2021;2(1):17-20.
3. Andrian A, Ikhsan R, Siregar WY. Laporan Kasus: Perforasi Gaster. AVERROUS: Jurnal Kedokteran dan Kesehatan Malikussaleh. 2022;8(1):81-7.
4. Sirait RH. Buku ajar pemantauan hemodinamik pasien. UKI Press; 2020.
5. Mangku G, Senapathi TGA. Buku ajar ilmu anestesia dan reanimasi. Jakarta: Indeks. 2010.
6. Ghodki PS, Sardesai SP, Naphade RW. Combined spinal and general anesthesia is better than general anesthesia alone for laparoscopic hysterectomy. Saudi journal of anaesthesia. 2014;8(4):498.
7. Yu, G., Wen, Q., Qiu, L., Bo, L., & Yu, J. (2015). Laparoscopic cholecystectomy under spinal anaesthesia vs. general anaesthesia: a meta-analysis of randomized controlled trials. *BMC anesthesiology*, *15*, 176. https://doi.org/10.1186/s12871-015-0158-x
8. Organisation WH. Definition of an older or elderly person. 2010.
9. Singh S, Bajorek B. Defining 'elderly'in clinical practice guidelines for pharmacotherapy. Pharmacy practice. 2014;12(4).
10. Staheli B, Rondeau B. Anesthetic considerations in the geriatric population. 2021.
11. Hopkins TJ, Raghunathan K, Barbeito A, Cooter M, Stafford-Smith M, Schroeder R, et al. Associations between ASA Physical Status and postoperative mortality at 48 h: a contemporary dataset analysis compared to a historical cohort. Perioperative Medicine. 2016;5(1):1-6.
12. Daabiss M. (2011). American Society of Anaesthesiologists physical status classification. *Indian journal of anaesthesia*, *55*(2), 111–115. https://doi.org/10.4103/0019-5049.79879
13. Gill R, Goldstein S. Evaluation and Management of Perioperative Hypertension. [Updated 2023 Aug 7]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557830/
14. Bajwa, S. J., & Kulshrestha, A. (2016). Anaesthesia for laparoscopic surgery: General vs regional anaesthesia. *Journal of minimal access surgery*, *12*(1), 4–9. https://doi.org/10.4103/0972-9941.169952
15. Karcz, M., & Papadakos, P. J. (2013). Respiratory complications in the postanesthesia care unit: A review of pathophysiological mechanisms. *Canadian journal of respiratory therapy : CJRT = Revue canadienne de la therapie respiratoire : RCTR*, *49*(4), 21–29.
16. Xiong X-H, Chen C, Chen H, Gao R, Deng Q-Y, Cai X-W, et al. Effects of intravenous and inhalation anesthesia on blood glucose and complications in patients with type 2 diabetes mellitus: study protocol for a randomized controlled trial. Annals of Translational Medicine. 2020;8(13).
17. Lines D. Hypertension and anaesthesia. South African Family Practice. 2014;56(5):9-15.
18. Hwang JH, Kim BW. Comparison of General Anesthesia and Combined Spinal and Epidural Anesthesia for Gasless Laparoscopic Surgery

in Gynecology. JSLS: Journal of the Society of Laparoscopic & Robotic Surgeons. 2022;26(2).

19. Luchetti M, Palomba R, Sica G, Massa G, Tufano R. Effectiveness and Safety of Combined Epidural and General Anesthesia for Laparoscopic. Regional Anesthesia. 1996;21(5):465-9.
20. Long, E. F., & Mathews, K. S. (2018). The Boarding Patient: Effects of ICU and Hospital Occupancy Surges on Patient Flow. *Production and operations management*, *27*(12), 2122–2143. https://doi.org/10.1111/poms.12808

ORIGINAL ARTICLE

Evaluation of Molluscicidal Activities of Aqueous and Ethanolic Extracts of Onion Bulb (*Allium Sativum*) Against *Bulinus Wrighti*

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ABSTRACT

Introduction: Schistosomiasis is a disease of public health importance in the tropics and subtropics. However, the synthetic materials for prevention of the intermediate host of the disease are harmful, scarce, and toxic. Thus, an evaluation of molluscicidal activities of aqueous and ethanolic extracts of the bulb of *Allium sativum* against *Bulinus wrighti* were carried out.

Methods: Snails were exposed to various concentrations of plant preparations in laboratory conditions in a plastic aquarium containing 3L of de-chlorinated water for 96h continuously. Mortality was recorded at every 24hours interval for 96hours.

Results: The study shows that, molluscicidal activities are time and dose dependent against snails. The ethanolic extract was more toxic than aqueous extract. Ethanolic extract of *A. sativum* was found highly toxic to *B. wrighti* (24hrs. LC_{50}: 97.07mg/l; 96hrs: 21.70mg/l). Chemical profile of aqueous extracts of *A. sativum* showed the presence of some secondary metabolites. *A. sativum* extracts showed histopathological signs to hermaphrodite glands and the digestive tract of the treated snails.

Conclusion: This study showed that, this plant can be used as molluscicides. This study recommends the use of ethanolic and aqueous extract of *A. sativum* for the control of *B. wrighti.*

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Introduction

Schistosomiasis remained as a chronic and debilitating diseases due to the causative agent Schistosoma. It is a worldwide common infection occurring due to parasitic activity and is the most common after malaria in terms of effects on public health and socioeconomic burden in the tropical and subtropical areas of the world. On the other hand, it is still the most predominant waterborne disease, and a great risk to public health in rural settings of developing countries (Bala et al., 2012; In & Lee, 2017; Jibia & Sani, 2019). A free swimming form of the parasite at larval stage (cerariae) because of contact finds it ways into the guan skin and turned to mature form. Thereof, the parasite eggs are release through the urine or faces. Then hatched in the freshwater around and invade an aquatic snail (the intermediate host) (Dawaki et al., 2015; Gamde et al., 2022). For the continuity of the lifecycle, the eggs when in the snail, developed into cercariae to be disgorged into the water for the purposes of infecting human host (that usually contact freshwater such as pond, and stream) (Sing et al., 2016; Umoh et al., 2020; Johnbull et al., 2021).

Unfortunately, prior to the egestion of the eggs by human host (in the blood vessels in the urinary bladder); parts of them accumulate and affect (destroy) vital organs such as intestines, bladder, and quasi (Sady et al., 2015; Dawaki et al., 2016; Anyolitho et al., 2022). Infection due to schistosomiasis is known with severe morbidity such as bladder lesions, hematuria, kidney inflammation, urethral obstruction, and damage to internal organs (Asenahabi, 2019; Hamburg et al., 2021). It is worrisome to note that, about 700 million persons in the 74 endemic countries are at the risk of schistosomiasis probably contacted in their agricultural, recreational, and domestic activities that require contact with fresh water. Additionally, greater than the 207 million persons are infected with the disease across the various parts of the world (Dawaki et al., 2015; Dawaki et al., 2016; Aula et al., 2021). In this vein, children are affected with anemia, physical weakness, learning ability, and quasi (Akinneye et al., 2018; Anthony et al., 2019; Oyeyemi etal. 2020).

Consequently, it is pertinent to seek for ways to prevent the spread of schistosomiasis in the country, especially in Sokoto to combat the challenges of poverty, malnutrition, and double burden of diseases (Sarkingobir et al., 2023ab; Suleiman et al., 2022). Infection prevention reduces the use of antibiotics and antimicrobial resistance, helps in ensuring clean and safe, environment, and enhances public health protection (Bala et al., 2012; Shaw, 2016; Dawaki et al., 2015; Johnbull et al., 2021). For the microorganism to spread and potentially incite the intermediate host, the snail (Shaw, 2016). The easiest wat to break the chain of transmission of schistosomiasis is to scuttle it through the developing of cheap and accessible plant-based material that kills the snail intermediate host (Shaw, 2016; Nelwan, 2019; Rinaldo et al. 2021); because, the synthetic products are expensive scarce, toxic (especially to non-target freshwater organisms or humans), it is pertinent to seek for an alternative (Singh & Tiwari, 2012; Suleiman et al., 2018ab). *Allium spp* is one of

the plants with the potential for the application in the prevention of schistosomiasis through killing of the snail (intermediate host) due to its phytochemical components. Because, the plant has been reported to be active on other biological beings, there is need to test it on intermediate snail of schistosomiasis for searching of cheap and effective prevention strategy in Sokoto and beyond (Noorshilawati et al., 2020). Thus, an evaluation of molluscicidal activities of aqueous and ethanolic extracts of the bulb of *Allium sativum* against *Bulinus wright* was the aim of this work.

Materials And Methods

Study Area

This study was carried out in Parasitology laboratory of Biological Sciences, Usmanu Danfodiyo University Sokoto, Sokoto State. Sokoto is the capital city of Sokoto State, lies between latitude 13° 3' 490N, longitude 5°14' 890E and at an altitude of 272m the sea level above. It is located in the extreme North Western part of Sokoto North and South local government areas and also some parts of Kware LGA from the North, Dange Shuni LGA from South and Wamakko LGA to the West. Sokoto metropolis is estimated to have a population of 427,760 people (NPC/FRN,2007) and by the virtue of its origin, the state comprises mostly Hausa/Fulani and other groups such as Gobirawa, Zabarmawa, Kabawa, Adarawa, Arawa, Nupes, Yorubas, Ibos and others. Occupation of city inhabitants include trading, commerce, with a reasonable proportion of the population working in private and public sectors (MOI, 2008). The Sokoto township is in dry Sahel surrounded by sandy terrain and isolated hills. Rainfall starts late that is in June and ends early, in September but may sometimes extend into october. The average annual rainfall is 550 mm with peak in the month August. The highest temperatures of 45°C during the hot season are experienced in the months of March and April. Harmattan, a dry cold and dusty condition is experienced between the months of November and February (Abdullahi et al., 2009). Modern Sokoto city is a major commerce center in leather crafts and Agricultural products (MOI, 2008).

Collection of Snails

Adult *Bulinus wrighti* were collected from their natural habitats from Kwalkwalawa local fresh water river in Sokoto metropolis. The snails were identified at the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. Size of the shell was ±2.00cm. The pH of the dechlorinated tap water was 7.2 and the temperature was 32.2^{o} C. Snails were acclimatized in the laboratory conditions for 72 hours in the Plastic aquaria containing dechlorinated tap water before being used for molluscicidal tests. Dead animals were removed quickly to avoid contamination of aquarium water.

Plant Materials Used in the Research

The bulb of *Allium sativum* were purchased from market during the period of March to July, 2013.Identified and confirmed by a senior plant taxonomist from Biological Sciences Department, Usmanu Danfodiyo University Sokoto. The

voucher numbers of the plant is: *Allium sativum*-UKM-B30093.

Preparation of Plant Extracts

Powder of *A. sativum* bulbs was prepared by peeling and slicing healthy cloves into 3mm thick, air dried and pulverizing into a mortar and pestle. The powder was kept dry, stored in air tight container in refrigerator and tested for molluscicide activity.

Aqueous Extraction

For aqueous extracts, the desired weight of plant powder was socked in 500mls dechlorinated tap water for overnight, stirred and filtered. The filtrate was used for toxicidal activity as shown in Table 1.

Ethanolic Extraction

Five hundred grams (500g) of air-dried *A. sativum* (bulbs), was extracted with 1.5 liters of ethanol. The extraction was kept in orbital shaker for 30 minutes. The extracts were filtered, using muslin cloth and concentrated to dry under reduced pressure in a rotary evaporator at 40^{o}C which yield ethanolic extract of *A. sativum*. The extracts were kept in fridge in Laboratory for further use.

Study of Toxicity of Preparation of Plant Derived Molluscicides

Toxicity experiments were performed by the method of Singh & Singh (1997). Ten experimental animals were kept in each aquarium containing 3 liters of dechlorinated tap water, and exposed continuously for 96h to different concentrations of plant materials and preparations (Table 1). Control animals were kept in similar conditions without treatment. During experimental period snails were kept in starved condition. As it was periodic sampling the mortality was recorded after every 24 hours interval up to 96 hours during exposure of the snails. Each experiment was replicated six times (Suleiman et al., 2018ab).

The toxic effect of the molluscicides was also studied against fish *Oreochromis niloticus* (Tilapia). In these experiments a group of 10 Tilapia were exposed in 6 liters of dechlorinated tap water as carried out by (Suleiman et al., 2018ab). The fishes were exposed for 24 hours LC_{90} (of snail) to 96 hours.

No response to a needle probe in case of snails, and no response against touch in case of fish (Tilapia) was taken as evidence of death. Dead animals were removed on each observation during exposure period to avoid any contamination of the aquarium water (Suleiman et al., 2018ab).

Table 1**:** Doses of plant extracts Tested on *B. wrighti* for Toxicity.

Name of Treatment	Concentration (mg/L)
Ethanolic extraction of *A. sativum*	15, 30, 40, 50
Aqueous extraction of *A. sativum*	300, 400, 500, 600

Histological Preparation

Treated and control snails were removed from their shells, and washed thoroughly with distilled water and fixed in 10% formalin. The snail tissues were processed for paraffin sectioning after embedded in paraplast at 50^0C. The 7um sections were stained with iron haematoxylin and eosin, and examined for tissue changes with light microscopy (Ahmad et al., 2014).

Statistical Analysis

Lethal concentration (LC_{50}) value, lower (LCL) and upper (UCL) confidence limits, slope value, t- ratio, g- value and heterogeneity were calculated according to the method of POLO (probit or logit) computer programme of Suleiman etal., (2018a). Tables and charts were prepared for presentation of results.

Results And Discussion

Molluscicidal Activity of Plant Products against Snail *B. wrighti*

This part of the result deals with study of molluscidal properties of preparation of *A. sativum* against *B. wrighti*. The freshwater snails were exposed to different concentrations of preparations (Table 1). Mortality was recorded after 24h, 48h, 72h, and 96h during the exposure period. *Tilapia* Fish were exposed to (24h LC_{90} against *B. wrighti*) of molluscicidal formulation for 96h to observe any toxic effect against non-target animals in aquatic environment. Toxicity evaluation of all the plant derived formulations and their combinations showed that the molluscicidal activity of these preparations against *B. wrighti* was time and dose dependent. This result revealed that *B. wrighti* was susceptible to the plant extracts at different concentrations.

Aqueous and ethanolic extract of *Allium sativum* (Galic)

Table 2 and 3, showed toxicity of aqueous and ethanolic extract of *A. sativum* (Garlic) against *B. wrighti* respectively. The aqueous extracts of *A. sativum* that killed 50% (LC_{50}) *B. wrighti* decreased from 807.00mg/l (24h), to 386.00mg/l (96h). The ethanolic extracts of *A. sativum* that killed 50% *B. wrighti* (LC_{50}) decreased from 97.07 mg/l (24h) to 21.70 mg/l (96h) indicating the molluscicidal activity of this extract against *B. wrighti* was time and dose dependent. The toxicity of aqueous extracts of *A. sativum* against *B. wrighti*, in Table 2, indicated the LC_{50} at 96h was 386.00mg/l. while the toxicity of ethanolic extracts of *A. sativum* against *B. wrighti*, (Table 3), indicating the LC_{50} at 96h was 21.70mg/l. This result showed that ethanolic extracts of *A. sativum* possessed high molluscicidal properties compared to the aqueous extracts of *A. sativum* against *B. wrighti.*

In the control experiment, no mortality was observed. The slope value observed in this study was steep. A value of t- ratio was greater than 1.96. The ‘g value is less than 0.5. Heterogeinity factor was less than 1.0. In the control experiment, no mortality was observed.

Phytochemical Screening of Plant Extracts

Phytochemical screening was carried out to determine the specific compounds present in the Plant

extract, which may be responsible for the observed biological effects. The result of phytochemical analysis of aqueous extract of *Allium sativum* (Garlic) showed that saponins, glycoside, alkaloids, steroids, volatile oils, and cardiac glycosides are present in the Plant extract while flavonoid, tannins, saponin glycoside, balsams and anthraquines are absent as shown in (Table 4).

Table 2: Toxicity of Aqueous Extract of *Allium Sativum* (Garlic) Against *B. 30right.*

Exposure Time	**LC_{50}(mg/l)**	**LCL**	**UCL**	**Slope Value**	**t-ratio**	**g-Value**	**Heterogeneity**
24hr.	807.00	147.40	654.00	5.76±1.39	4.12	0.22	0.26
48hr.	770.00	114.6	660.00	3.57±0.93	3.80	0.26	0.29
72hr.	530 .00	590.00	491.00	5.42±0.85	6.31	0.09	0.30
96hr.	86.00	406.00	365.00	8.95±0.99	8.96	0.04	0.45

Batches of ten snails were exposed to different concentrations of Aqueous extract of *A. sativum* powder. Mortality was recorded at every 24hrs. Each set of experiment was replicated six times. Concentrations given were the final concentration (w/v) in the aquarium water.

A value of t- ratio greater than 1.96 indicate that regression is significant.

levels (90, 95, 99) as it was less than 0.5.

Value of heterogeneity factor was less than 1.0 denotes that in the replication test of random samples, the concentration response line would fall within 95% confidence limits and thus the model fits the data adequately. The indices of significance of potency estimation ‘g value’ indicate that the value of mean was within the limits at all probability

Table 3**:** Toxicity of Ethanolic Extraction of *Allium sativum* (garlic) against *B. wrighti*

Exposure Time	LC_{50} mg/l	LCL	UCL	Slope Value	t-ratio	g-.Value	Heterogeneity
24hr.	97.07	63.78	419.18	1.90±0.56	2.58	0.33	0.28
48hr.	58.51	44.98	113.60	1.84±0.47	3.84	0.26	0.20
72hr.	25.80	22.13	29.23	3.18±0.46	6.88	0.08	0.35
96hr.	21.70	18.92	24.26	5.25±0.57	9.20	0.06	1.31

Batches of ten snails were exposed to different concentrations of ethanolic extract of *A. sativum* powder. Mortality was recorded at every 24hr. Each set of experiment was replicated six times. Concentrations given were the final concentration (w/v) in the aquarium water.

A value of t-ratio greater than 1.96 indicate that regression is significant.

Value of heterogeneity factor was less than 1.0 denotes that in the replication test of random samples, the concentration response line would fall within 95% confidence limits and thus the model fits the data adequately.

The indices of significance of potency estimation 'g value' indicate that the value of mean was within the limits at all probability levels (90, 95, 99) as it was less than 0.5.

Table 4: Phytochemicals present in Aqueous Extract of *Allium sativum*

PHYTOCHEMICAL	TEST	OBSERVATION	INDICATION
Flavonoid	Ferric chloride	+ve (green color)	Absent
Tannins	Ferric chloride	+ve (blue-green color)	Absent
Saponins	Frothing test	+ve (frothing)	Present
Glycosides	Fehling's solution	+ve (brick-red precipitate)	Present
Alkaloids	Wagners	+ve (turbidity or precipitate)	Present
Cardiac glycosides	Kellerkilliani	+ve (reddish-brown color)	Present
Steroids	Chloroform	+ve (reddish-brown color)	Present
Volatile oils	Dilute HCL	+ve (white precipitate)	Highly present
Saponin glycosides	Fehling's solution A&B	+ve(bluish-green precipitate)	Absent
Balsams	Alcoholic ferric chloride	+ve (dark green color)	Absent
Anthraquines	Borntrager's test	+ve(pink, red or violet color)	Absent

Histopathological Changes

Treatment of snails with *A. sativum* bulb extracts showed histopathological signs (Figures 1-3) to the hermaphrodite glands and the digestive tract of the snails. The histological changes were a function of extract concentrations.

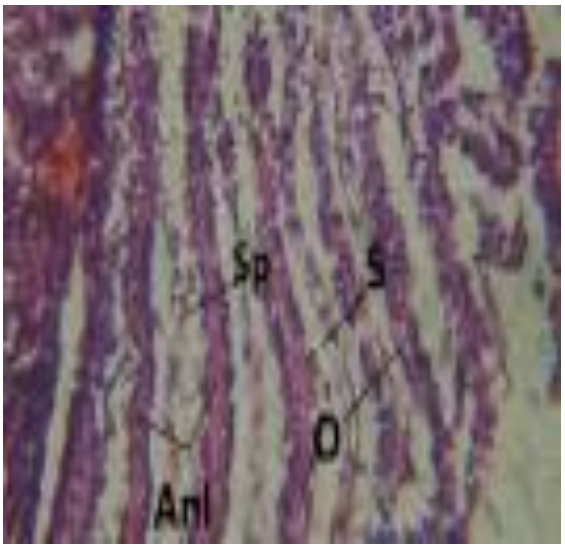

Figure 1a: T.S. in control *B. wrighti* (Hermaphrodite region). Anl= ancel's layer Sp= sperms S= spermatocytes O= oocyte X= 200

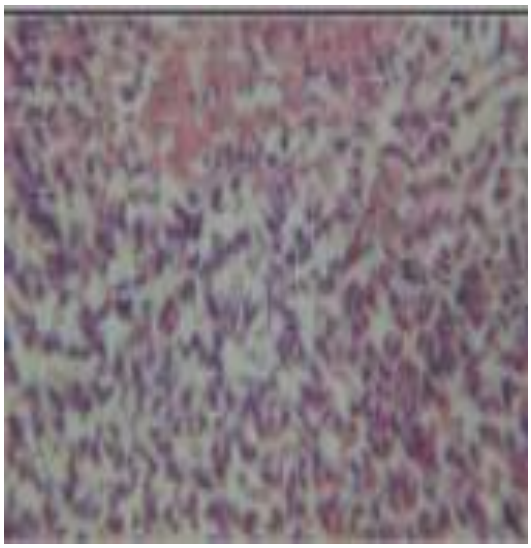

Figure 1b T.S. in control *B. wrighti* showing digestive epithelia. X = 200

Cells of hermaphrodite glands and digestive glands are all intact and normal. No changes were observed in control, all vacuoles and follicular membrane are intact and normal in shape and thickness.

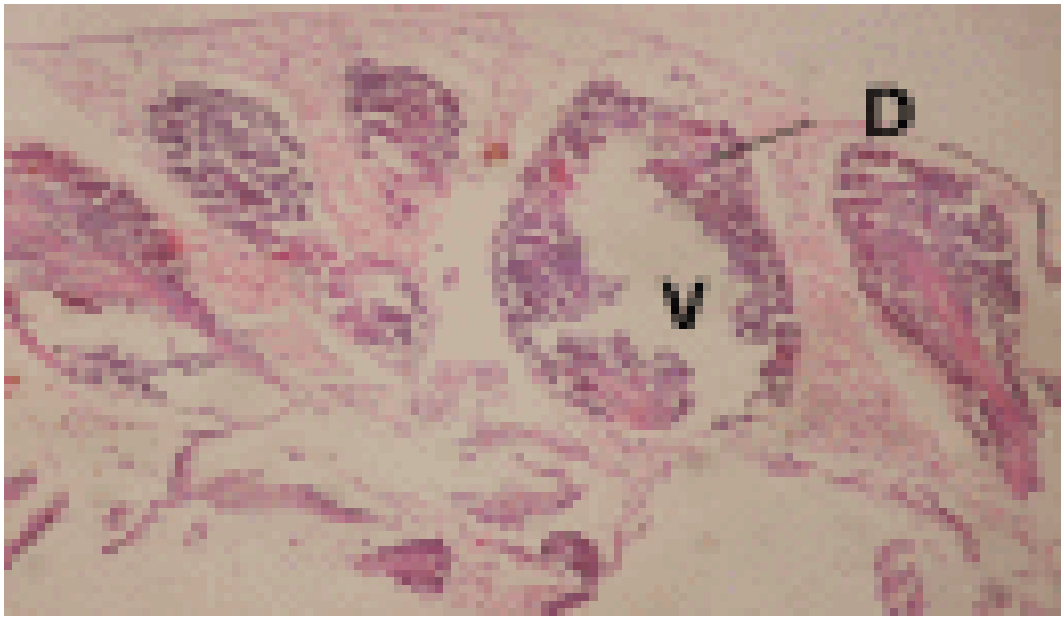

Figure 2: Ts.S. in treated *B.wrighti* with 50 mg/l ethanolic extract of *A. sativum* (Hermaphrodite region). D: degeneration V: vacuoles X= 200

Treated *B. wrighti* with a concentration 50mg/l ethanolic extraction of *A. sativum* showed large vacuoles and degeneration in the hermaphrodite glands, destruction in the follicular membrane and the matured ovum showed losing of the nucleolus.(Figure 2).

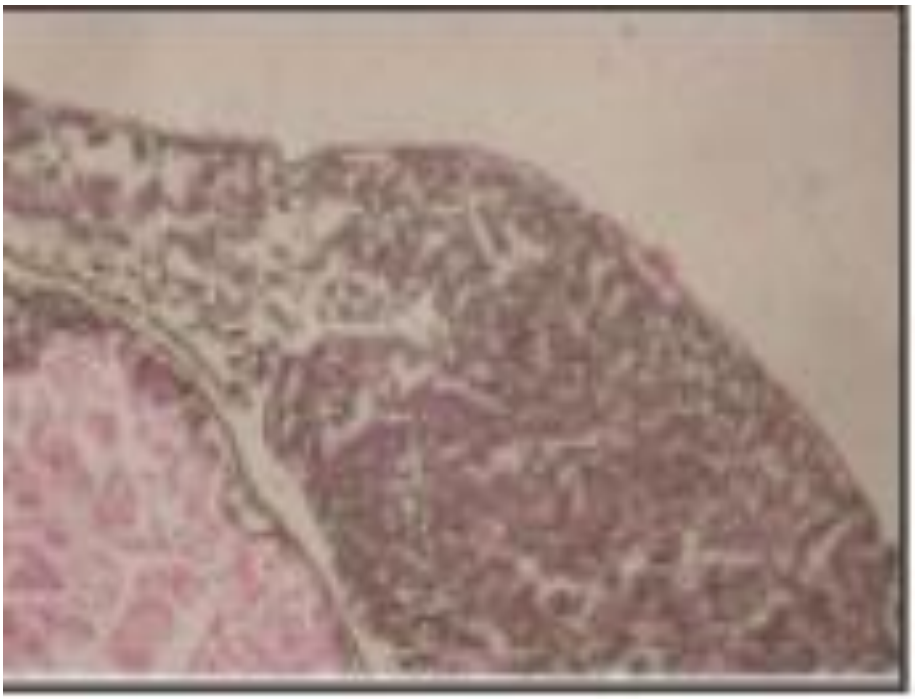

Figure 3: T.S. in treated *B. wrighti* with 50 mg/l of ethanolic extract of *A. sativum* (digestive acini).Large vacuoles and great destruction of digestive acini. X= 200

Large vacuoles and great destruction was observed in the digestive acini and the columnar epithelial cells (Figure 3) when compared with control.

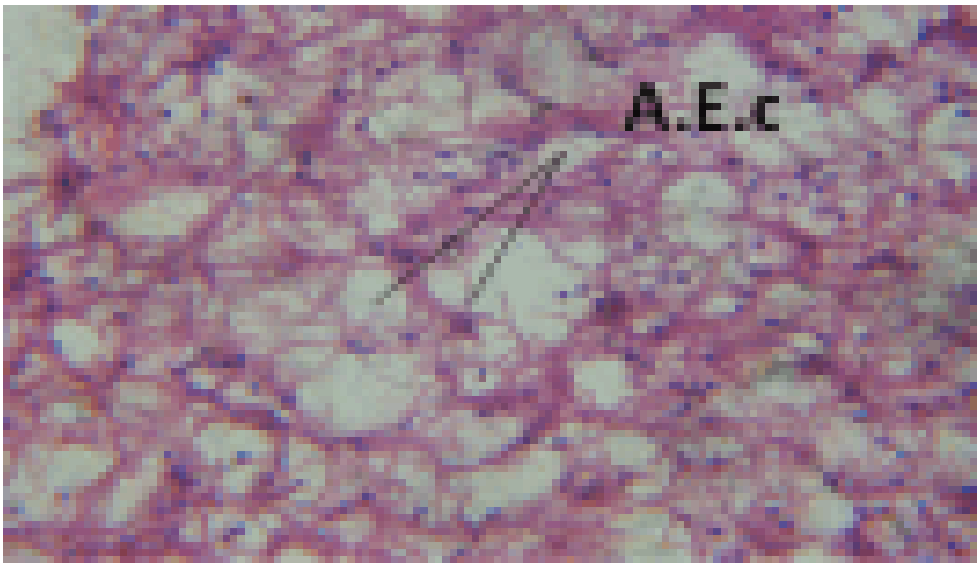

Figure 4 T.S. in treated *B*. wrighti with 50 mg/l of ethanolic extract of *Allium satium* showing digestive epithelia. A.E.c: evacuated epithelial cells X = 200

In the digestive epithelial, there observed large evacuated epithelial cells (Figure 4).

The size of vacuoles was not affected by the decrease in concentrations of the extract. While in control snails no pathological changes were observed in the digestive gland and hermaphrodite gland.

The present study showed that aqueous extracts of *Allium sativum*, and ethanolic extracts of *Allium sativum* possessed molluscicidal properties. Their activities were time and concentration- dependent. Between the tested plants against *B. wrighti*, ethanolic extract of *A. sativum* showed the highest molluscicidal activity on the test snail species after 24hr -96hr of exposure period. The varying potencies of each plant may be due to the differences in concentration and or the type of the active ingredient (s) present in each plant (Brimer et al., 2007). Most of the plants species screened during this study for their molluscicidal activity more or less accumulate similar classes of compounds. Saponin is highly toxic and also exhibit hemolytic properties, which act as poison show cytotoxic or pesticidal activity (Hostettmann & Marston, 1995; Noorshilawati et al., 2020). In the present study, Saponin is present in *A. sativum* which may be responsible for the death of *B. wrighti.*

However, it has been established that not only saponins but also some sesquiterpenes, flavonoids, glycosides as well as phorbol esters possess molluscicidal properties (Hostettman et al., 1982; Rug & Ruppel, 2000; Al-zanbangi et al., 2001). Flavonoids and glycosides were also present in *A. sativum* which may be responsible for the death of *B. wrighti*, in the present study. The penetration of the toxicants also has a greater significant for the aquatic environment, because their whole body is bathed in a diluted solution of toxicants. To have maximum effect the plant must penetrate the organism and then transported to active site rapidly. It seemed the high titer of plant extract in snails may be due to rapid penetration of the plant molluscicides through soft foot of snails body and / or it may be possible the plant active component may change into more toxic form in the aquarium water in snail body which is

triggered by different enzymes and cause differential mortality (Singh & Tiwari, 2012; Suleiman et al., 2018ab).

Nevertheless, no mortality was recorded in Tilapia fish *(Oreochromis niloticus)* even at 24 hrs LC_{90} of the molluscicides investigated in this study which indicated that the use of these molluscicides may probably be safe to non-target animals such as Tilapia fish. It is reasonable to conclude that the concentrations of molluscicides does not produce symptoms of toxicity (mortality) in fish (*Oreochromis niloticus*) which possibly because the amount of drug was rapidly detoxified by fish or the fish has a different metabolic pathway than the snails.

The steep slope value observed in the toxicity studies demonstrated that a small increase in concentration of molluscicides cause a large mortality in the snails. A value of t- ratio greater than 1.96 indicated that regression is significant. Value of heterogeneity factor was less than 1.0 denoted that in the replication test of random samples, the concentration response line would fall within 95% confidence limits and thus the model fits the data adequately. The indices of significance of potency estimation 'g value' indicated that the value of mean was within the limits at all probability levels (90, 95, 99) as it was less than 0.5.

The results of phytochemical analysis of aqueous extract of *A. sativum* (bulb) showed the presence of saponins, glycoside, alkaloid, steroids, volatile oils and cardiac, glycoside while flavonoid, tannins, saponin glycoside, balsams and anthraquines are absent. This result was similar to the findings of Pavni et al. (2011) who reported that *A. sativum* contains flavonoids, monosaccharides, glycosides, saponins, tannin and free reducing sugar.

The molluscicidal activity of the plant extracts on the histology of the digestive gland of snails acting as intermediate host have been suggested in scattered cases in the literature (Brackenbury, 1999; Suleiman et al., 2018ab). In addition, several investigations have studied the histological and histochemical changes induced by mollucicides on the digestive gland of aquatic gastropods (Mulley & Mane, 1990; Sing & Tiwari, 2012; Noorshilawati et al., 2020).

Figures 1-4 shows the histopathological studies of the effects of the extracts on the *B. wrighti* snail. During the histological test of treated *B. wrighti* snail on 50mg of ethanolic extract *A. sativum*, large vacuoles, degeneration in the hermaphrodite glands and destruction in the follicular membrane were observed, which was similar to the observation of Ahmad et al., (2014), when *Biomphalaria alaxandrina* snail was treated with methanolic extract of *Callistemon viminalis* fruits, bark and leaves. Thus, the plant extract of *Allium sativum* can served as a cheap, affordable, available, and effective bio-friendly material for the prevention of bilharziasis in the area.

Conclusion

The results of this study showed that *A. sativum* possessed molluscicidal properties against *Bulinus wrighti* which is the vector for urinary schistosomiasis. Ethanolic extract of *Allium sativum* was the most effective. This plant molluscicides are readily available, inexpensive and environmentally

safer for controlling human urinary schistosomiasis. This will not only eliminate the economic burden of importing expensive synthetic molluscicides, but also stimulate growth of small-scale industries in Nigeria.

Recommendations

1. The study also recommends the use of ethanolic extract of *A. sativum* in controlling the *Bulinus wrighti* because the study reveals that ethanolic extracts of *A. sativum* are highly toxic against *B. wrighti.*
2. Government should encourage the use of indigenous small- scale industries of Plant molluscicides instead of importing expensive synthetic molluscicide.
3. Further research is recommended using different solvents for extraction and comparing their activity.

Conflicts of Interest

The author started there is no conflict of interest.

References

1. Abdullahi. K, Abubakar. U., Adamu. T., Daneji, A. I., Aliyu, R. U., Jiya, N., Ibraheem, M. T. O., & Nata'ala. S. U. (2009). Malaria in Sokoto, North Western Nigeria. *African Journal of Biotechnology,* 8 (24), Pp. 7101-7105
2. Ahmed, A. G.,Galal T. M., Sahar R. G., Walaa S. A., & Atef M. E.,(2014). Molluscicidal Activity of the Methanol Extract of *Callitemon viminalis* (Sol. Ex Gaertner) G.Don ex Loudon Fruits, Bark and leaves against *Biomphalaria alexandrina* Snails. *Iranian Journal of Pharmaceutical Research,* 13(2), 505-514. PMCID: PMC4157025.
3. Akinneye, J.O., Fasidi, M.M., Afolabi, O.J., & Adesina, F.P. (2018). Prevalence of urinary schistosomiasis among secondary school students in Ifedore Local Government, Ondo state, Nigeria. *International Journal of Tropical Diseases*, 1(1), 1-6.
4. Al-zanbangi, N.A.J. Barett, A. & A.A. Banaja, (2001). Laboratory Evaluation of Some Saudi Arabian *Euphorbiales* against *Biomphalaria pfeifferi. Acta tropica,* 78, 23-29.
5. Anthony, D., Emmanuel, B., Blessing, J.N., Enock, L., & David, Y.P. (2019). Schistosomiasis among primary school pupils in Pilagani District, Langtang North Local Government Area, Plateau State, Nigeria. *GSC Biological and Pharmaceutical Sciences*, 9(1), 5-14.
6. Anyolitho, M.K., Pels, K., Hyyse, T., Tumusiime, J., etal (2022). Knowledge, attitudes, and practices regarding schistosomiasis infection and prevention A mixed methods study among endemic communities of western Uganda; *Plos Neglected Tropical Diseases*, 16 (2), 1-10.
7. Asenahabi, B.M. (2019). Basics research design: A guide to selecting appropriate research design. *International Journal of Contemporary Applied Researches*, 6(5), 76-89.

8. Aula, O.P., McManus, D.P., Jones, M.K., & Gordon, C.A. (2021). Schistosomiasis with a focus on Africa. *Tropical Medicine and Infectious Diseases*, 6(109), 1-40.https://doi.org/10.3390/tropicalmed6030109.
9. Bala, A.Y., Ladan, M.U., & Mainasara, M. (2012). Prevalence and intensity of urinary schistosomiasis in Abarma village, Gusau, Nigeria: A preliminary investigation. *Science World Journal*, 7(2), 12-14.
10. Brackenbury, T.D. & Appleton,C.C. (1999). Structural damage to the foot-sole epithelium of *Bulinus africanus* following exposure to a plant molluscicide. *Malacologia*, 41, 393-401.
11. Dawaki, S., Al-mekhlafi, H., M. Ithio, I., Ibrahim, Abdulsalam, A.M., Ahmed, A. etal (2015). The menace of schistosomiasis in Nigeria knowledge, attitudes and practices regarding schistosomiasis among rural communities Kano state. *Plos ONE*, 10 (1) -1-14.
12. Dawaki, S., Al-mekhlati, H.M., Ithoi, L., Ibrahim, J., Abdulsalam, A.M., Ahmed, A. etal, (2016). Prevalence and risk factors of schistosmiasis among Hausa communities in Kano state, Nigeria Review. *Lastest Medicine in Tropical Sao Paulo*, 58 (54),1-9
13. Gamde, S.M., Garba, A., Tongvwam, P., Gamde, S.D., Agom, D.D., & Igbinosa, I.(2022). Prevalence, intensity, and risk factors of urinary schistosomiasis among primary school children in Silame, Sokoto, Nigeria. *Microbes and Infectious Diseases*, 4(2), 695-703.
14. Hamburg, S.D., Grobler, A.D., and Melarin, P.E. (2021). Knowledge, attitudes and practices on urinary schistosomiasis among Mandek Bay south. *Africa Journal of Parasitology Research* 2021-1-12.
15. Hostettmann, K., Marston, A. (1995). Saponins (chemistry and pharmacology of natural's products) Cambridge: *University Press.*
16. Hostettmann. K., Kizu, H., & Tomimori, T. (1982). Molluscicidal Properties of various Saponins. *Planta Medicine* 44, 34 – 35.
17. In, J. & Lee, S., (2017). Statistical data presentation. *Korean Journal of Anesthesiology*, 70(3), 268-276.
18. Jibia, A.B. & Sani, Z.R. (2019). Intensity of urinary schistosomiasis associated with gender-agedgroup of primary schools children in Sokoto south and Kware area, Sokoto state, Nigeria. *International Journal of Science for Global Sustainability*, 5(1), 54-64.
19. Johnbull, D., Panda, S.M., & Samaila, A.B. (2021). Epidemiological study of schistosomiasis in Basiraka, Gwaran local government area, Jigawa state Nigeria. *Science Forum (Journal of Pure and Applied Sciences) 21, 144-157.*
20. MOI (2008). Ministry of Information, Sokoto, Nigeria. *Diary*, p. 12.

21. Nelwan, M.L. (2019). Schistosomiasis life cycle, diagnosis and control. *Current Therapeutic Research*, (2019), 5-9.
22. Nelwan, M.L. (2019). Schistosomiasis Life cycle, diagnosis, and control. *Current Therapeutic Research*, 91, 5-9. Htps://doi.org/10.1016/j.curtheres.2019.06.001.
23. Noorshilawati, A.A., Nur Suraya, A. & Rosssiyah, S. (2020). Moluscicidal activity of *Ipomea batatas* leaf extracts against *Pomacea canaliculata* (Golden apple snail). *Food Research*, 5(5), 131-137.
24. Oyeyemi, O.T., Jeremias, W.J., & Grenfell, R.F.G. (2020). Schistosomiasis in Nigeria: Gleaning from the past to improve current efforts towards control. *One Health*, 11(100183), 1-10.
25. Pavni, K., Esha, B., Neha, J., Tushar, A.Shrey, K., Suchit,A.,Sarita, A.,Vibha,R., & Neeraj,W. (2011). *Journal of Pharmacy Research*, 4 (3), 902-908.
26. Rinaldo, D., Perez-Saez, J., Vounatsou, P., Utzinger, J., & Archand, J. (2021). The economic impact of schistosomiasis. *Infectious Diseases of Poverty*, 10(134), 1-12.https://doi.org/10.1186/s40249-021-00919-z.
27. Rug, M. & Ruppel, A. (2000). Toxic Activities of the Plant *Jatropha curcas* against Intermediate Snail Hosts and Larvae of *Schistosomes*. *Tropical Medical International Health*, 5, 423-430.
28. Sady, H., Al-mekhlafi, H.M. Atroosh, W.M., Al delaimy, A.K etal (2015). Knowledge, attitudes and practices towards schistosomiasis among rural population in Yemen. *Parasites*,8 (436), 1-14.
29. Sarkingobir, Y., Hiliya, A.A., Miya, Y.Y., Umar, A.I., Yabo, A.M., & Salah, N.M. (2023a). Some metals contained in well drinking water obtained from some secondary schools in Schools in Sokoto East District, Nigeria. *Journal of Community Service in Science and Engineering*, 2(1), 15-24. Doi: 10.36055/jocse.v2i1.19802.
30. Sarkingobir, Y., Umar, AI., Gidadawa FA., & Miya, Y.Y. (2023b). Assessment of food security, living condition, personal hygiene health determinants and relations among Almajiri students in Sokoto metropolis, Nigeria. *Thu Dau Mot Journal of Science*, 5(1),63-76. https://doi.org/10.37550/tdmu.EJS/2023.01.372. ISSN: 2615-9635. Publisher: Thu Dau Mot University Vietnam. https://ejs.tdmu.edu.vn.Home
31. Shaw, K. (2016). Prevention by breaking the chain of infection. *Nursing Times*, 112(40), 12-14.
32. Singh, K., Mudassiru, D., & Singh, J. (2016). Current status of schistosomiasis in Sokoto, Nigeria. *Parasite Epidemiological Control*, 1(3), 239-244.
33. Singh, V. & Tiwari, J.N. (2012). Effects of synthetic and extracts of plant on reproduction of snail *Lymnaea acuminata*. *Research journal of Pharmaceutical,*

Biological, and Chemical Sciences, 3(4), 705-710.

34. Suleiman, J., Singh K., Bala A.Y., Muhammad, M.T. & Yakubu, M.S. (2018b). Molluscicidal potential of column purified fractions of *Allium cepa* bulbs against intermediate host of urinary schistosomiasis (*Bulinus globosus)* in Sokoto, Nigeria. *Asian Journal of Research in Zoology*, 1(1), 1-6.
35. Suleiman, J., Singh K., Bala A.Y., Muhammad, M.T., Abdullahi, A., & Yakubu, M.S. (2018a). Comparison for the effectiveness of column purified fractions of *Allium cepa* bulbs and *Allium sativum* cloves against *Bulinus globosus* (intermediate host of urinary schistosomiasis) in Sokoto, Nigeria. *Asian Journal of Advanced Research and Reports*, 2(4), 1-6.
36. Suleiman, M., Ahmed, A., & Gobir, S.S. (2022). Haemo and gastrointestinal parasites in cattle slaughtered at Gwadabawa abattoir in Sokoto state, Northwestern Nigeria. *The Bioscientist*, 10(1), 55-64.
37. Umoh, N.O., Nwamini, C.F., Inyang N.J., etal. (2020). Prevalence of urinary schistosomiasis amongst primary school children in Ikwo and Ohaukwu communities of Ebonyi State, Nigeria. *African Journal of Laboratory Medicine*, 9(1), 1-5.https://doi.org/10.4102/ajlm.v9i1.812.

CASE REPORTS

Severe Calciphylaxis Secondary to end Stage Renal Failure: A Case Report

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ABSTRACT

Calciphylaxis is a vascular calcification disease causing skin necrosis which contributes to high morbidity and mortality. Its exact pathogenesis is currently unknown but is commonly associated with chronic renal failure, hypercalcemia, hyperphosphatemia, secondary hyperparathyroidism and a variety of hypercoagulable state. It is relatively rare but may occur in 1-4% of patients with End Stage Renal Failure (ESRF).[1] We are reporting a case of young lady with underlying ESRF presented with vascular and skin calciphylaxis.

Medical and Health Science Journal.

Introduction

Calciphylaxis is an uncommon condition affecting 1-4% of ESRF population.[1] Calciphylaxis was first introduced in 1962 by Selye when he was able to precipitate systemic calcification in nephrectomised rat.[2] However, its exact aetiology and pathogenesis have remained obscured until now.

Due to poor understanding regarding the disease, there are currently no definite diagnostic and therapeutic guidelines which may prevent devastating outcomes in calciphylaxis patients. The prognosis is generally poor with mortality rate as high as 60% in patients with ulcerative disease.[3] Patients who do not die of sepsis frequently had to undergo amputation of the affected limbs.

Case(s)

We are reporting a case of 30-year-old lady with underlying Type 1 Diabetes Mellitus complicated with retinopathy, neuropathy and ESRF secondary to Diabetic Kidney Disease for 3
years. She was on regular hemodialysis for 1 year and subsequently converted to Continuous Ambulatory Peritoneal Dialysis (CAPD) for 2 years.

She was initially presented with worsening bluish discoloration of all her fingers and toes for over 1 month. It was associated with severe pain and warmness. Some of the fingers slowly became black in colour. There was worsening ulceration over the right middle finger associated with pus discharge. She also had intermittent claudication of bilateral lower limbs. She had no fever, chest pain and abdominal pain at that time. She had no recent

admission prior to the presentation. She is a non-smoker and her family history were unremarkable.

Initial assessment noted dry gangrene of all fingers and toes. There was presence of right middle finger ulceration with surrounding erythema. Other systemic examinations were unremarkable.

She was seen by orthopaedic colleague and the impression was wet gangrene of the right middle finger complicated with osteomyelitis and dry gangrene of bilateral fingers and toes. She had undergone Ray's amputation of the right middle fingers and planned for conservative management for the rest of her bilateral fingers and toes dry gangrene. She was noted to have Methicillin Resistance Staphylococcal Aureus infection from the bone cultures and had completed IV vancomycin for 2 weeks and planned for oral T Bactrim and T Rifampicin for 6 weeks. She was discharged relatively well.

She presented again 2 weeks after discharge with complaint of generalized abdominal pain for 2 days. It was persistent in nature with no specific aggravating and relieving factor. The pain score was 9/10. It was associated with nausea, vomiting and loose stools for 2 days. There were no mucus and bloods in the stool and no history of black tarry stool. There was no fever. She was still able to continue with her CAPD and her peritoneal dialysis effluent was clear.

Abdominal examination revealed generalized abdominal tenderness with normal bowel sound. Per rectal examination was normal. Other systemic examinations were unremarkable.

Her blood investigations revealed leucocytosis with white cell count (WCC) of $30x10^9$/L and increased CRP indicating inflammation. Her intact parathyroid hormone (IPTH) level was 414 pg/ml, calcium 2.64 mmol/L, phosphate 2.07 mmol/L, alkaline phosphatase (ALP) 331 u/L. Unfortunately, autoantibodies associated with collagen disease was not sent. Other investigations were unremarkable.

She was initially treated as Peritoneal Dialysis (PD) peritonitis and intraperitoneal (IP) Cefazolin and IP Ceftazidime were initiated however she responded poorly to the treatment. Her abdominal pain was so severe that Acute Pain Service was involved, and she was started on IV Fentanyl infusion and later was changed to SC Morphine.

Her blood cultures and PD fluid cultures remained negative. Her PD fluid cell count was less than 100 cells/uL therefore not suggestive of PD peritonitis. Her abdominal pain did not improve despite adequate analgesia and few days on antibiotic. Her PD fluids remained clear. Therefore, an urgent abdominal CT scan was arranged.

Her abdominal CT scan noted extensive dense circumferential calcifications of the wall of the abdominal aortas and its branches (hepatic artery, celiac axis, superior mesenteric artery and inferior mesenteric artery), common iliac artery, external iliac artery and femoral artery. There was segmental filling defect within distal middle colic branches of superior mesenteric artery with dilated fluid filled jejunal loops. However, there is no definite evidence of acute bowel ischemia like enhancing bowel thickening or intramural air.

Her final diagnosis was severe calciphylaxis secondary to ESRF and she was subsequently managed conservatively with pain management. She had eventually died of Hospital Acquired Infection due to prolonged hospital stay.

Results

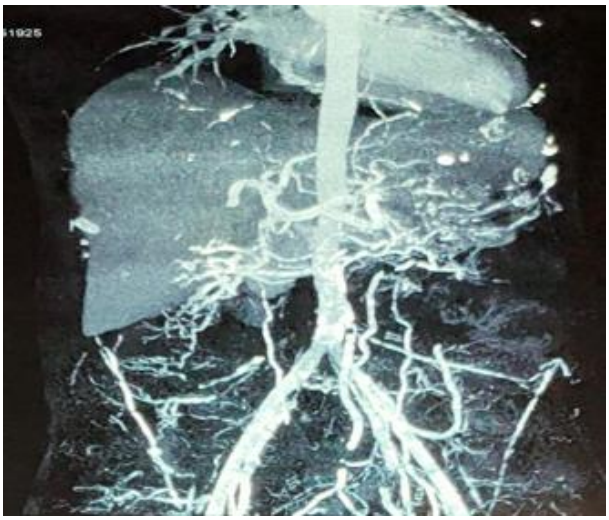

Figure 1. Coronal and sagittal view of abdominal CT scan showing extensive dense circumferential calcifications of the wall of the abdominal aortas and its branches (hepatic artery, celiac axis, superior mesenteric artery, inferior mesenteric artery), common iliac artery, external iliac artery and femoral artery.

Discussion

Calciphylaxis is a rare necrotizing calcifying arteriolopathy which is generally uncommon but may affect up to 1-4% of ESRF patient populations. It was first introduced by Seyes in 1962 when he was able to demonstrate formation of calciphylaxis in nephrectomised rats.[2]

Its exact pathogenesis is unclear, but it is characterized by calcification of tunica media of small and medial arteries causing blockage of the lumen subsequently causing skin necrosis. It was suggested that uraemia-induced defects in nuclear factor-Kb (RANK), RANK ligand and osteoprotegerin may cause skeletal and extra skeletal mineralization defect subsequently leading to calciphylaxis.[4] Therefore, its risk factors may include prolonged dialysis with abnormal metabolism of calcium and phosphate as a result from renal hyperparathyroidism. Other risk factor may include connective tissue disease, chronic liver disease, diabetes mellitus, systemic hypercoagulability, and concomitant vascular disease.[5]

Patients commonly presented with intense pain and refractory skin ulcers. Early lesion may manifest as nonspecific violaceous mottling or as erythematous papules, plaques or nodules and may progress to stellate purpuric configuration with central cutaneous necrosis.[6] Bullous may also be seen as a rare manifestation of calciphylaxis.[7] They are at constant risk of infections and this disease generally has very poor prognosis. Patients with internal involvement may develop gastrointestinal haemorrhage or organ failure.

Our literature search has shown that there are only 4 reported cases of systemic calciphylaxis presented with gastrointestinal involvement like what is presented in our patient.[3] Therefore, high level of suspicion is needed so that proper investigation with CT scan, can be done to determine the diagnosis and prognosis.[8]

However, due to its rarity, there is currently no high-quality evidence for a guideline on how to properly investigate and to treat calciphylaxis patients. Imaging studies may include plain radiography that show a netlike pattern of arterial calcification. Incisional cutaneous biopsy is usually diagnostic and typically demonstrate calcification within the media and intima of small and medium sized arterioles with extensive intimal hyperplasia and fibrosis, but biopsy may result in a nonhealing wound.

This patient was diagnosed with severe calciphylaxis based on presence of gangrenous of

all fingers and toes as well as from the abdominal CT scan findings on top of her risk factor as an ESRF patient and her high level of calcium, phosphate and parathyroid hormone.

General management may include identification of risk factor, correction of abnormal metabolism of calcium and phosphate, prevention of infection and local management of ulcers usually by debridement. In patients with elevated calcium and phosphate, the levels must be brought to low-normal levels as quickly as safely possible.

Recently intravenous sodium thiosulfate administration has been gaining attention and has shown dramatic improvement in signs and symptoms in an ESRF patient and even a complete resolution of the disease in a non-uremic calciphylaxis patient.[9,10] It is a potent antioxidant that may increase the solubility of calcium deposits.

Another case report has also shown a better control of calciphylaxis in a dialysis patient with cinacalcet which act by inhibiting parathyroid hormone production via negative feedback to normalize the calcium and phosphate metabolism.[11] Studies have shown its efficacy in decreasing PTH, calcium and phosphate levels. Bisphosphonates like pamidronate may also be helpful by inhibiting arterial calcification. Aggressive wound care may also be necessary to avoid wound infection and sepsis. Other than that, it is also important to manage the pain appropriately.

Conclusion

Calciphylaxis is an important complication of ESRF which has high morbidity and mortality and they may also manifest as gastrointestinal complication. A good management in calcium and phosphate metabolism as well as maintaining dialysis adequacy is important to prevent this complication.

Conflict of Interest

The author started there is no conflict of interest.

References

1. Angelis, M., Wong, L. L., Myers, S. A., & Wong, L. M. (1997). Calciphylaxis in patients on hemodialysis: a prevalence study. *Surgery*, *122*(6), 1083–1090. https://doi.org/10.1016/s0039-6060(97)90212-9
2. Fader DJ, Kang S. Calciphylaxis Without Renal Failure. *Arch Dermatol.* 1996;132(7):837–838. doi:10.1001/archderm.1996.03890310129026
3. Weenig, R. H., Sewell, L. D., Davis, M. D., McCarthy, J. T., & Pittelkow, M. R. (2007). Calciphylaxis: natural history, risk factor analysis, and outcome. *Journal of the American Academy of Dermatology*, *56*(4), 569–579. https://doi.org/10.1016/j.jaad.2006.08.065
4. Baby, D., Upadhyay, M., Joseph, M. D., Asopa, S. J., Choudhury, B. K., Rajguru, J. P., & Gupta, S. (2019). Calciphylaxis and its diagnosis: A review. *Journal of family medicine and primary care*, *8*(9), 2763–2767. https://doi.org/10.4103/jfmpc.jfmpc_588_19
5. Galperin, T. A., Cronin, A. J., & Leslie, K. S. (2014). Cutaneous manifestations of ESRD. *Clinical journal of the American Society of Nephrology : CJASN*, *9*(1), 201–218. https://doi.org/10.2215/CJN.05900513
6. Sprague SM. Painful skin ulcers in a hemodialysis patient. *Clin J Am Soc Nephrol*. 2014;9(1):166-173. doi:10.2215/CJN.00320113

7. Nigwekar, S. U., Kroshinsky, D., Nazarian, R. M., Goverman, J., Malhotra, R., Jackson, V. A., Kamdar, M. M., Steele, D. J., & Thadhani, R. I. (2015). Calciphylaxis: risk factors, diagnosis, and treatment. *American journal of kidney diseases : the official journal of the National Kidney Foundation*, *66*(1), 133–146. https://doi.org/10.1053/j.ajkd.2015.01.034
8. Garin, N., Marti, C., Scheffler, M., Stirnemann, J., & Prendki, V. (2019). Computed tomography scan contribution to the diagnosis of community-acquired pneumonia. *Current opinion in pulmonary medicine*, *25*(3), 242–248. https://doi.org/10.1097/MCP.0000000000000567
9. Cicone, J. S., Petronis, J. B., Embert, C. D., & Spector, D. A. (2004). Successful treatment of calciphylaxis with intravenous sodium thiosulfate. *American journal of kidney diseases : the official journal of the National Kidney Foundation*, *43*(6), 1104–1108. https://doi.org/10.1053/j.ajkd.2004.03.018
10. Ning, M. S., Dahir, K. M., Castellanos, E. H., & McGirt, L. Y. (2013). Sodium thiosulfate in the treatment of non-uremic calciphylaxis. *The Journal of dermatology*, *40*(8), 649–652. https://doi.org/10.1111/1346-8138.12139
11. Colette B. Raymond., Lori D. W. (2008). Sodium thiosulfate, bisphosphonates, and cinacalcet for treatment of calciphylaxis, *American Journal of Health-System Pharmacy*, Volume 65, Issue 15, Pages 1419–1429, https://doi.org/10.2146/ajhp070546

ORIGINAL ARTICLE

Conservative Wound Treatment in DMT2 Patients Using Honey

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ABSTRACT

Introduction: The number of diabetic patients in Indonesia is on the rise. In contrast to typical wounds in non DMT2 patients, chronic wounds in DMT2 patients heal more slowly, which makes it difficult to achieve complete primary wound healing. Thus, surgery is frequently required to achieve optimal healing. Patients' physical condition, age, comorbidities, and financial circumstances such as high medical costs frequently limiting patients from receiving comprehensive care, resulting alternative treatments are required to treat chronic wounds in DMT2 patients whom prefer conventional medications, addressing all circumstances. Honey, in addition to having fructose compounds, which has the benefit of increasing glucose homeostasis and insulin response, resulting in lower insulin and plasma glucose levels, also has been shown to contain anti-inflammatory and antimicrobial substances that aid in wound healing. Thus, it may be an alternative therapy for chronic wound in DMT2 patients.

Methods : A Case-series studies of four DMT2 patients who were referred to Plastic and Reconstructive Outpatient ward with chronic wound were evaluated on a monthly basis following conservative treatment using Nusantara local honey-coated gauze.

Results : Secondary wound healing, which can be assessed from the epithelialization process started from the peri-wound area has been obtained through monthly observations.

Conclusion : Honey is used as an alternative therapy for patients with diabetic foot ulcers due to its anti-microbial and anti-inflammatory properties in the wound healing process. Furthermore, honey is considered less expensive and more affordable alternative for patients with co-morbidities that is impossible to operate, or with financial limitations.

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Introduction

Diabetes Mellitus (DM), is a clinical syndrome of metabolic disorders characterized by hyperglycemia caused by defects in insulin secretion, defect in insulin action, or both.[1] In Indonesia, diabetes' rising prevalence has resulted in various comorbidities. The most severe repercussions of hyperglycemia are microvascular complications (e.g nephropathy, neuropathy, and retinopathy); as well as macrovascular complications (e.g coronary artery disease, stroke, and peripheral arterial disease).[2]

Foot ulceration occurs in 15-20% of diabetics patients over their lifetime, and has been the leading cause of hospitalization. More than 15% of foot ulcers necessitate leg amputation. A range of 0.5-3% of diabetic foot ulcers occur annually, according to a number of other studies. The prevalence of foot ulcers has been reported to range from 2-10%. 45-60% of diabetic foot ulcers are solely neuropathic, while the remaining 45% are both neuropathic and ischemic.[2] In indonesia, diabetic ulcer patients make up about 15% of the population, with a 32% mortality rate and a 30% amputation rate.[3]

The fructose content of honey may play a role in one of the possible ways that may help people with diabetes. Reduced intestinal absorption[4], prolonged gastric emptying[5,6], and reduced food intake[7,8] are potential mechanism in this process. Chepulis and Starkey found that feeding honey to Sprague-Dawley rats for several weeks resulted in a significant decrease in HbA1C levels,[9] in contrast to rats that received glucose, in account that fructose improved glucose homeostasis and insulin response[10].

The use of topical honey also has been shown to increase the production of cytokines in tissue and influence the elimination of bacterial infection by increasing the mitogenic activity in B and T lymphocytes and neutrophils.[11,12] As a result, wound infections recover quickly, dead tissues are removed from wounds, and scar tissue is reduced. Additionally, they have a beneficial effect on epithelial growth, tissue granulation and angiogenesis.[12]

Case 1

A 53 years old female patient came to the Plastic and Reconstructive Outpatient Ward presented with an open wound on the sole of her left foot and primary complaint of swelling. History of DM (+) and currently not taking any prescribed medications. Patient stated that one week prior coming to the outpatient ward, during activites, she felt an excruciating pain on her foot along with warmness and swelling. These conditions was not alleviated with resting.

During physical examination on ventral pedis, an open wound with a tissue base, RSA (+) and slough was found without any necrotic tissue. The skin surface was not warm on palpation and no oedema was found. Patient was not feeling any pain during examination. ROM was limited.

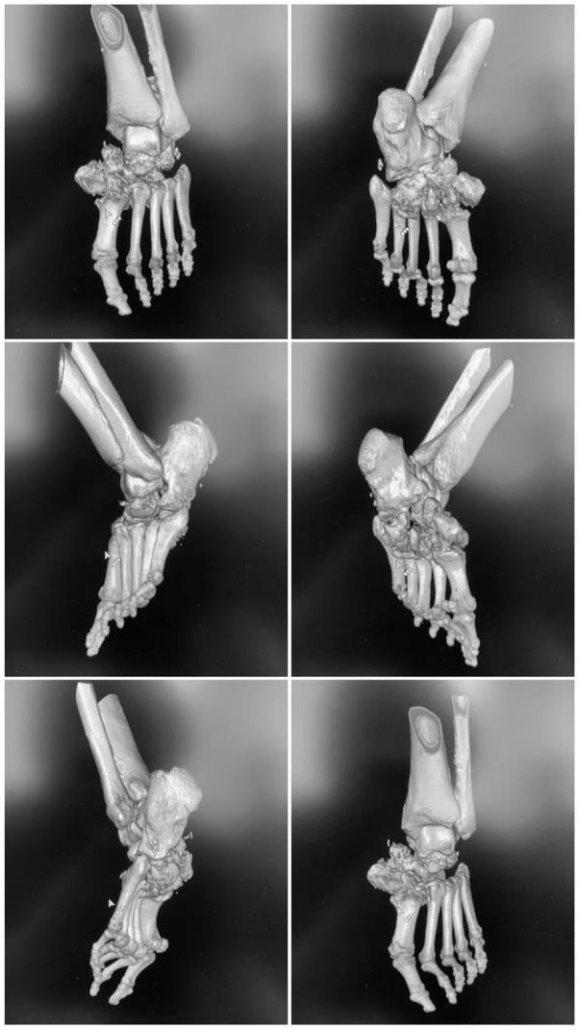

Figure 1 CT-Scan bone-view shown no abnormalities on bones and soft tissue.

CT-Scan of the leg did not reveal any tumor nor swelling on the gland. Bone and tissues were in normal condition.

Patient was advised to go on a surgery to perform debridement and secondary wound closure using skin graft or local flap, but she refused because she did not have enough money. Thus, the patient receives daily wound treatment using gauze coated in local Indonesian honey 'Nusantara' as an option. Follow-up is carried out once every 1 month.

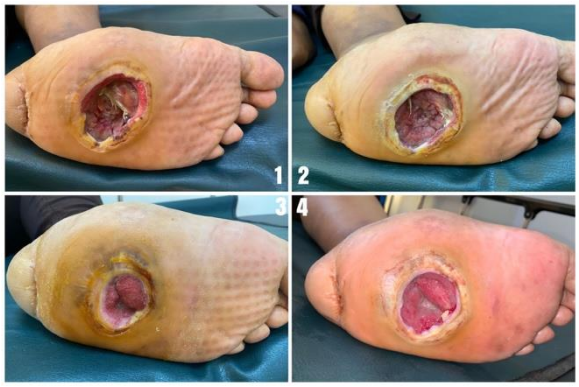

Figure 2 A monthly follow-up showing progression of epithelization started from periwound after conservative wound treatment using gauze coated in local Indonesian honey 'Nusantara'.

Case 2

80 years old male referred from the neurology outpatient ward with pressure sores due to prolonged bed-rest after a non-hemorrhagic stroke which causes the patient to experience weakness in all four extremities. History of MD (+) with basal insulin treatment and oral medication. During examination, the local status showed a grade III-IV pressure abrasion with exposed coccyx base. Patients denied any complaints of pain. Both defecation and urination are assisted by the family members by using diapers.

After consulting with specalist in internal medicine, cardiology, anesthesia, and neurology, it was determined that this patient posed a high-risk due to age-related risk factors, lab results, and a long history of anticoagulant consumption, have made it impossible to perform debridement in surgical room. Thus, it was decided to use a conservative treatment using gauze covered with honey everyday, followed with a monthly check-up.

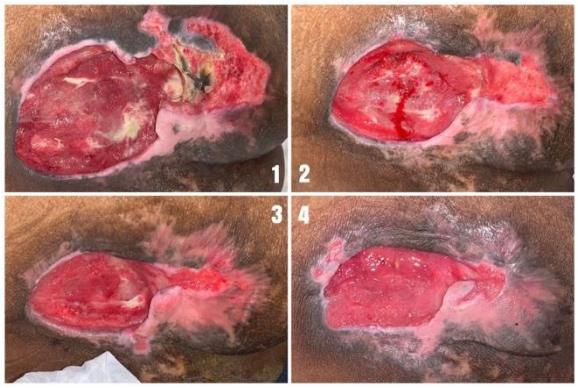

Figure 3 Routine follow-up results after 4 months of treatment. Notice how epithelialization process has occurred. A local necrotomy also has been carried out in the polyclinic to remove dead tissue for better wound-healing process.

Case 3

48 years old female came to the Plastic and Reconstructive Surgery Outpatient ward after receiving wound debridement in Emergency Unit two days prior due to history of trauma as a result of a traffic accident that caused multiple abrasions to the face and excessive skin loss on right leg. Patients had done an x-ray examination beforehand and no fractures in the calf has been found. Patient was then referred to the plastic and reconstructive surgery Outpatient ward for debridement and reconstruction with a local flap, but the patient refused to undergo surgery. History of DM (+) but patient refuses to take any medication and instead opt for traditional/herbal medicine.

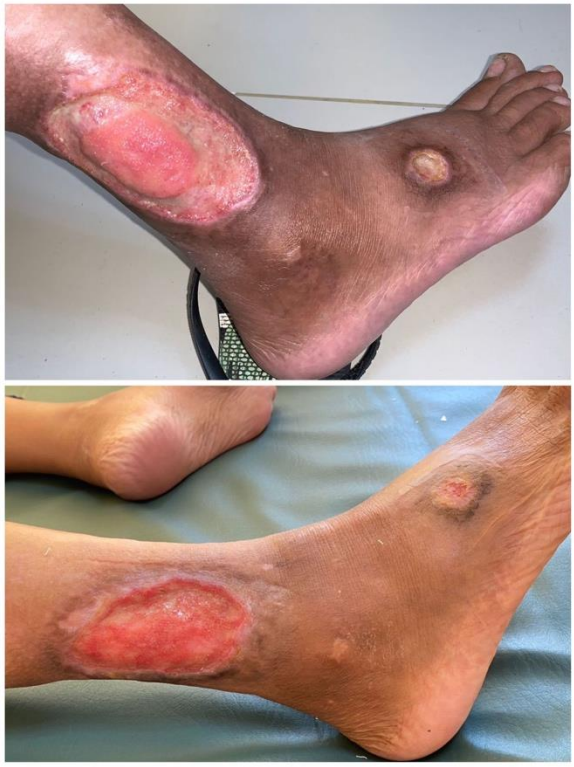

Figure 4 Chronic wound on lateral pedis (D) after 3 months follow-up.

Case 4

60 years old male referred from Orthopaedic Surgery Outpatient ward with chief complaint of chronic wound post-surgical reconstruction with tibial plate/screw due to traffic accident. History of DM run in the family was unknown, but during admission to the ER, it was found that patient' Random Blood Glucose result was above 200 and consulted to the internist. Patient currently treated with oral medication.

From physical examination, an open wound was found with plate and bone exposed, slough (+) and pus, necrotic tissue (-). Culture sample was taken, and it was found that patient was suffering from MRSA infection. Patient was then admitted to receive antibiotic IV using vancomysin for 5-7 days, prior planning a plate-removal surgery due to the secondary infection. However, the Orthopaedic surgeon recommends the plate to be

removed after one year of recovery. In the meanwhile, in order to achieve optimal epithelialization, patient was treated conservatively.

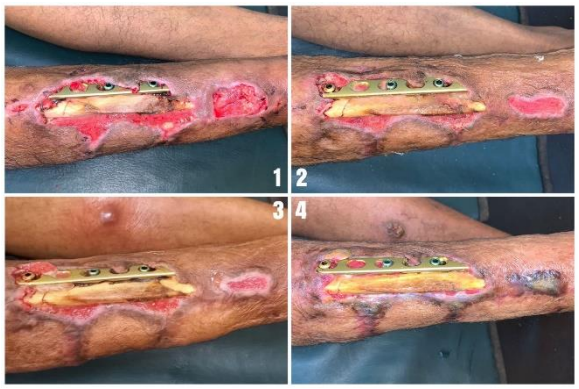

Figure 5 Monthly observation after 6 months treatment using Nusantara local honey. Epithelialization (+), slough (-), pus (-).

Discussion

Diabetes makes up the majority of non-traumatic lower limb amputations, which are widely preceded by ulcers which fails to heal. Hyperglycemia, which leads to abnormalities in the blood vessels and changes in the structure of peripheral blood vessels as a result of reduced blood flow to the skin, is the beginning in macroangiopathy complication. As a result, less blood is delivered to the distal areas; particularly the lower extremities, which further leads to sensory and motor neuropathy disorders. In addition, autonomic disorders alter the way of pressure distribution on the soles of feet. When this factor is combined with the lack of blood flow, it will make it easier for ulcers to form over time, thus making wound management more challenging.

The process of wound healing is diverse and influenced by numerous factors. Due to the excessive usage and tentative use of antibiotics in modern medicine, topical honey was used less as a wound treatment. Recent studies, however, have found antibiotic-resistant bacteria, such as *Methicillin Resistant Staphilococcus Aureus* (MRSA), in infected wounds, particularly within the past few years. This is due to the fact that antibiotic misuse leads to antibiotic resistance.

Apitherapy is the medical use of honey bee products, whether it's beeswax, pollen, royal jelly, propolis, or honey. Conversely, however, honey is extremely beneficial for wound healing due to its antibacterial and antiviral properties as it has an acidic pH of 3.2-4.5. It has been shown that a low pH may inhibit protease activity, reducing the amount of matrix destroyed that is needed for wound healing and tissue repair. In addition, an acidic environment may facilitate the ability for hemoglobin to release oxygen, which may assist in tissue regeneration. Although previous research has shown that microbes thrive in an acidic environment, the acidity of honey may hinder microbe reproduction. In comparison, honey is a hypertonic solution with an osmotic pressure of about 105 atmospheres. As a result, honey's high viscosity and osmolarity may aid in the formation of a barrier that prevents infections and prevents bacterial growth. Furthermore, the osmotic properties keep the wound surface moist, while simultaneously capable of absorbing pus and eliminate odor, in addition to restoring circulation and reducing both oedema and pain.

Because the majority of honey dressings are very effective at accelerating or reducing the size of the wound, honey should not be considered as a minor intervention. According to Muhammad Imran's research, the average wound healing time for diabetic ulcer patients was 18 days, compared to 29 days for other treatments (using a saline dressing, or conventional dressing).

A recent study at Cipto Mangunkusumo Hospital in Jakarta, has compared the antibacterial activity of local Indonesian Honey Nusantara to Manuka Honey using the dilution method with steril Mueler Hinton Broth of each honey to obtain various concentrations of honey. The Minimum Inhibitory Concentration (MIC) is the lowest honey concentration that can prevent bacteria growth in the media. This can be determined by comparing the clarity levels of various concentrations of control media against the strains of bacteria P. Aeruginosa, S. Aureus and MRSA culture.

From this study, it has shown that Manuka Honey has a lower MIC than Nusantara local honey; implying that the methylglioxil substance in manuka honey is responsible for the honey's potent antibacterial properties. Meanwhile, the presence of methylglioxil in Indonesian honey has never been investigated. It is also known that local honey cannot be diluted below its MIC to exert its antibacterial properties, due to its high MIC. This is relevant, especially in wounds with a fair amount of exudate, since exudate may reduce the effectiveness of honey against bacteria. Hence, to prevent honey from becoming diluted, it is necessary to change the honey dressing on a wound that is highly exudative on a regular basis.

Conclusion

Honey is used as an alternative therapy for patients with diabetic foot ulcers due to its anti-microbial and anti-inflammatory properties in the wound healing process. Furthermore, honey is considered less expensive and more affordable alternative for patients with co-morbidities that is impossible to operate, or with financial limitations.

Conflicts of Interest

The authors report no conflicts of interest

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes care.* 2011. 34(1):562-(9).
2. Molan, P.C. The Evidence Supporting the Use of Honey as a Wound Dressing. *The International Journal of Lower Extremity Wounds.* 2006. 5(1), 40-54.
3. Flahr D. The Effect of Nonweight Bearing Exercise and Protocol Adherence on Diabetic Foot Ulcer Healing a Pilot Study. *Journal Wound Management.* 2010. 56(10):40-50.
4. Kellet G. L., Brot-Laroche E., Mace O. J. Sugar absorption in the intestine: the role of GLUT2. *Annual Reviews of Nutrition.* 2008;28(1):35–54. doi: 10.1146/annurev.nutr.28.061807.155518.
5. Moran T. H., McHugh P. R. Distinction among three sugars in their effects on gastric emptying and satiety. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*. 1981;241(1):R25–R30. doi: 10.1152/ajpregu.1981.241.1.R25.
6. Gregory P. C., McFadyen M., Rayner D. V. Relation between gastric emptying and short-term regulation of food intake in the pig. *Physiology & Bahaviour*. 1989;45(4):677–683. doi: 10.1016/0031-9384(89)90278-3.
7. Thibault L. Dietary carbohydrates: effects on self-selection, plasma glucose and insulin and brain indoleaminergic systems in rat. *Appetite.* 1994;23(3):275–286. doi: 10.1006/appe.1994.1059

8. Meirelles C. J., Oliveira L. A., Jordao A. A., Navarro A. M. Metabolic effects of the ingestion of different fructose sources in rats. *Experimental and Clinical Endocrinology & Diabetes.* 2011;119(4):218–220. doi: 10.1055/s-0031-1275276.
9. Molan P: Why honey is effective as a medicine. 2. The scientific explanation of its effects. Bee World 2001, 82:22-40.
10. Prieto P. G., Cancelas J., Villanueva-Peñacarrillo M. L., Valverde I. Plasma D-glucose, D-fructose and insulin responses after oral administration of D-glucose, D-fructose and sucrose to normal rats. *Journal of the American College of Nutrition.* 2004;23(5):414–419. doi: 10.1080/07315724.2004.10719386.
11. Al-Waili NS, Haq A. Effect of Honey on Antibody Production Against Thymus-Dependent and Thymus-Independent Antigens in Primary and Secondary Immune Responses. J Med Food 2004: 7(4):491-494. https://doi.org/10.1089/jmf.2004.7.491
12. Molan PC, Betts JA. Clinical Usage of Honey as a Wound Dressing: an Update. J Wound Care 2004; 13(9)353-356. https://doi.org/10.12968/jowc.2004.13.9.26708.

ORIGINAL ARTICLE

Kinetics IgM and IgG SARS-CoV-2 in Recovery Patients with Negative Evaluation RT-PCR

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ABSTRACT

Background: Coronavirus is the major pathogens at human respiratory system. The antibody is a response infected patients largely not clearly. We need to understanding of antibody responses to great diagnosis and treatment studies.
Objective: In this article the objective is to describe that kinetics of IgM and IgG SARS-CoV-2 in recovery patients.
Method: Cohort study used a total 19 subjects who had negative evaluation RT-PCR after confirmed. IgG and IgM of SARS-CoV-2 were detected by IFA (Immuno fluorosence assay) used serum. Serum were collected three times after first, second and fourth months, secound and fourth months negative evaluation RT-PCR. We profiled the serological responses (IgM and IgG) to SARS-CoV-2.
Result: Majority the IgM SARS-CoV-2 post evaluation RT-PCR were very low after one, second and fourth months negative evaluation. IgG SAR-CoV-2 patients post negative evaluation RT-PCR decreased after 4 months. The level IgM and IgG level increase at first week and decrease after 12 weeks.
Conclusion: IgM level lower than IgG level overtime. Quantitative IgG and IgM detection could be point of diagnosis and manifestation.

Medical and Health Science Journal

Introduction

Coronavirus disease (COVID-19) is one of major problem in human respiratory system. In late December 2019, a case of patients was admitted to hospital with initial symptoms like pneumonia.[1] The pathogenesis of COVID-19 infections is as follow.[2] In the last December 2019 five patients were hospitalized with acute respiratory syndrome and one of the patient died.[3] The China National Health Commission reported that first 17 deaths until January 2020. A total 1975 cases were confirmed COVID-19 infected in China with a total 56 deaths.[4] CDC reported first case human to human transmission of COVID-19 on January 30, 2020.[5]

COVID-19 is an infectious disease caused by the SARS-CoV-2 Virus. SARS-CoV-2 are a positive-sense, single stranded RNA (ssRNA) virus with genome length from 20-32 kb and about 125 nm in diameter that belongs to the Coronaviridae family.[6] The mortality rate of SARS-CoV-2 a novel beta coronavirus is about 3.4% but SARS-CoV-2 has potentially higher transmission than another Coronavirus.[7]

The diagnosis of COVID-19 is dependent on clinical symptoms, CT imaging and some

laboratory result. Although some clinical manifestations and laboratory result have indicated to lead as a COVID-19 disease, that not unique to SARS-CoV-2 infection. The rapid and accurate diagnostic of COVID-19 is needed to rapid and good management, accurate public surveillance, prevention and control disease. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) has been gold standard for diagnosis SARS-CoV-2.[8] Patients with COVID-19 infection have a mild disease and recover quickly after good clinical management. Some COVID-19 patients develop more severe disease, multiple organ failure and death in the short time.[7] Previous study reported that massive inflammatory responses induce the over-inflammation of T cells and be severe infection in SARS-CoV-2 pathogenesis.[9]

Serological examination of Covid 19 in survivors is not only helps identify affected cases but can provide important information regarding Covid-19 immunity to infected groups. In SARS-CoV-2 infection, specific IgG antibodies increase and reach a peak at 4 months after the onset of the disease and then decrease after 16 months.[10] Antibody level are generally regarded as protective, detrimental affected, as antibody dependent enhancement (ADE), hypothesis of SAR-CoV-2 infection.[11] It is still on investigation whether ADE play a role in pathogenesis of COVID-19. A study showed that patients in severity case with COVID-19 had higher total SARS-CoV-2 antibody titers compared mild patients SARS-CoV-2 infection.[12] However, weather higher neutralizing antibody (Nab) titers are associated with more lung manifestations to elucidated. Serological method another way to laboratory diagnostic, can diagnose disease by detecting antibody. Estimated decrease antibody to know antibodies circulated in the body. The aim this study to investigate the diagnostic value of serological method and the kinetic variance of IgG and IgM antibodies in patients with RT-PCR negative

Methods

Design and Subject Criteria

A total 19 patients were diagnosed by real-time PCR testing. After isolation the subject we informed to be subject in this study. Patients who agree with informed consent we included to this study. The patients were followed up level of IgG and IgM after negative result 1 month, 2 months and and 4 months after negative realtime-PCR testing. Demographic information and symptoms we got from patient interview. The diagnosis of COVID-19 and evaluation patients is based on report Health Office (Dinas Kesehatan Provinsi) Bengkulu Province, Indonesia. The study was ethical approved by Health Research Ethics Comitte of the Faculty of Medicine and Health Sciences Universitas Bengkulu, by numbers 189/UN30.14.9/LT/2020.

SARS-CoV-2 RNA Detection

A total of 19 patients were diagnosed with real-time PCR testing. Diagnosis of COVID-19 using the RT-qPCR method using a sore throat swab in the nasopharynx. The examination used primers and probes targeting NP and the SARS-CoV-2 open reading frame1b gene according to World Health Organization (WHO) guidelines.[13] Throat swab specimens were collected from all patients and samples were stored in viral transport media for laboratory testing.

Serology Testing

The specific IgG and IgM of SARS-CoV-2 were detected by IFA (Immuno fluorosence assay) used serum. The reagent used is COVID-19 IgG and IgM from (Frendcov, Nanoentech, Nanoentek America, Inc, USA). The catridge utilizas micro-fluicidics lateral flow technology where the analyte of interest in the sample forms immune complexes while moving through the fluidics pathway in the catridge. A well-mixed sample of 35 µL is transferred to the sample inlet of a single use frend COVID-19 total ab catridge. The catridge is then placed into the frend system, which is programmed to begin analysis once the sample has reacted with the reagents. IgM and IgG if present in the sample will bind to SARS-CoV-2 nucleocapsid fluorescent beads which then bind to the anti-IgM and anti-IgG in the test zone. All tests were performed according to the product manual.

Results

Demograpic and clinical characteristic patients

The study include total 19 subject with confirmed COVID-19 with RT-PCR diagnosis and were recovery. Among them 7 (36 %) subject is male and 12 (63,15 %) subject is female. The subject classified into two groups : mild (7 subjects, 36 %) and severe (12 subjects, 64%). The subject ages in the mild (28,85±4,3) and severe (34,25±4.9). There's np significant different age between mild and severe. But, the mean age of severe group more higher than mild group. The marriage status in mild 3 (15,78 %) subject and in severe group was 11 (57,9 %) subject. (Table 1)

Table 1. Demographic and clinical symptoms distribution

Paramaters	Mild N=7 (%)	Severe N=12 (%)
Gender		
Male	0 (0%)	4 (33.3)
Female	7 (100%)	8 (66.7)
Age	28.85±4.3	34.25±4.9
Marriage	3 (42.9)	11 (91.7)
Symptoms		
Febris	2 (28.6)	8 (66.7)
Cough	3 (42.9)	5 (41.7)
Headache	1 (14.3)	5 (41.7)
Anosmia	2 (28.6%)	7 (58.3)
Ageusia	2 (28.6)	7 (58.3)
Diarhea	1 (14.3)	2 (16.7)
Fatique	3 (42.9)	7 (58.3)
Dypsnoe	1 (14.3)	1 (8.3)
Comorbid	0 (0)	3 (25)

Most of subject had febris, cough, headache, anosmia, ageusia and fatique and dyspnoe of breath. Three subject in severe group had comorbidities. The comorbidities of 3 subject in severe group were diabetes mellitus. We investigated that severe group had higher percentages of clinical symptoms manifestation and comorbidities.

Most subject of mild group were isolated in isolation center (Bapelkes, Health office Bengkulu) (Figure 1). Most subject of severe group isolated at hospital. There are because of clinical symptoms of severe group subject need serious observation. There are no significant statistic different centralized isolation place

between mild group and severe group (Fisher exact, p>0.05)

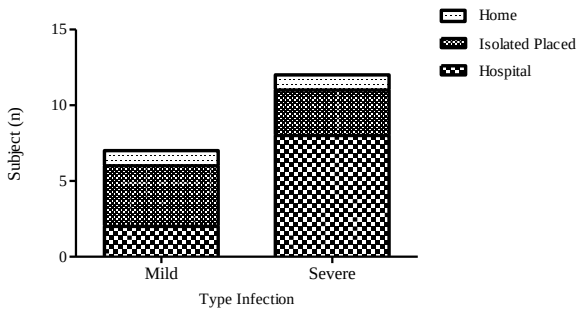

Figure 1. Distribution of centralized isolation

Recovery time between mild group and severe group not significant different (p>0.05, Mann-whitney test). Mean recovery time of severe group was longer than mild group. The maximum recovery time of severe group was 39 days. Maximum recovery time of mild group was 25 days. The minimum recovery time of severe group was 7 days and 10 days for severe group.

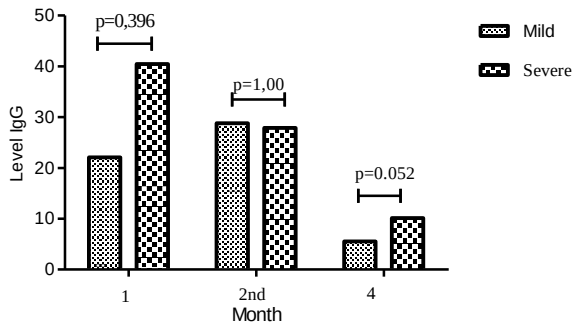

Figure 2. Recovery time based infection type

Analyzed cohort the IgM and IgG level antibodies in COVID-19 with RT-PCR negative result. The average of IgM and IgG level detection after 1st, 2nd and 4th months the RT-PCR was negative. After 1st month RT-PCR test negative the level of IgG in mild group lower than severe group. The result comparison IgG level between two group with Mann-Whitney test was not significant (0,396). After 2nd recovery the IgG level antibody in mild group same with severe group. There is not significant different IgG level between mild group and severe group (p=0.001). After 4th recovery the statistic analyzed with Mann-Whitney test was not significanat (p=0.052), but the mean IgG level antibody severity group higher than mild group. (Figure 3).

The figure 3a and 3b showed that the IgM level rate increase slightly at first and than decrease after the number of week form serological detected. In contrast, IgG level increase were higher than IgM overtimes.

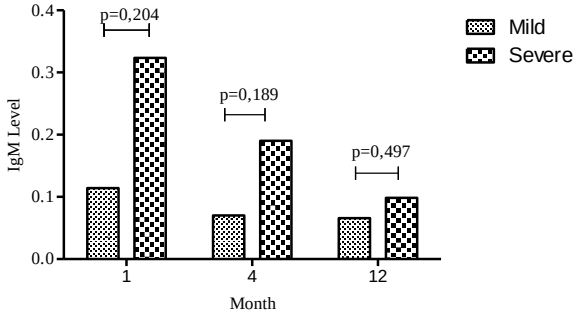

Figure 3. Comparison Kinetic (a) IgG (b) IgM with severity COVID-19

Discussion

Detection IgM and IgG SARS-CoV-2 during SARS epidemic allowed for serological diagnosis.[14] Identic antibody responses have been observed in SARS-CoV-2 infection patients, and the pattern is similar with acute viral infection.[15] Antibody diagnostic COVID-19 was rapid and sensitive for the diagnosis SARS-CoV-2 infection. Several detection test for antibody such as lateral flow immunoassay, CLIA, IFA and ELISA are currently available. In this study, the antibody respons were level IgM and IgG antibodies.We were analyzed at recovery COVID-19 subject and negative RT-PCR test.

Viral infection process with SARS-CoV-2, antibodies specific produced always consistent in

patients, except patients with immunodeficient. IgM antibody can detected after 3 days infection and first defense in humoral immunity, and IgG antibody are initiated and play a key role in long immune respond memory.[16] We investigated the IgM level subject. IgM level of subject were lower. Some subject not detect IgM in the serum. It was similar with 4 patients in previous study that the level IgM are very low.[17]

Data in this study showed that IgG was increased in 2 month after negative PCR resulted and than decrease after 4 month after negative PCR resulted. The kinetics of SARS-CoV-2 RBD IgGs and NtAb followed a predictable course[9], with antibody levels in both assays showing a consistent increase over time, and reaching a peak within the second and third week after onset symptoms for NtAb or slightly later for RBD-specific IgGs.[18]

Disregulated synthesis and release of pro-inflammatory cytokines is thought to be a pathogenetic hallmart of most severe forms of COVID-19.[19] In this study, the mean level IgM and IgG antibodies in mild group and severity group are different. Mean level IgM and IgG severe group higher than mean level IgM and IgG mild group. Quantitave result antibody detection are associated with the severity of COVID-19 and have potential value for use in predicting the disease prognosis.[17]

The limitations this study should be mentioned. Result this study need to be further the validated by studies in a lerge data of subject. The antibodies study need more sensitive method for detection comprehensive antibodies level.

Conclusion

The level IgM and IgG level increase at first week and decrease after 12 weeks. IgM level lower than IgG level overtime. Quantitative IgG and IgM detection could be point of diagnosis and manifestation.

Acknowledgments

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Conflict of Interest

No conflict of interest in this study

References

1. Bogoch, II, Watts A, Thomas-Bachli A, Huber C, Kraemer MUG, Khan K. Pneumonia of unknown aetiology in Wuhan, China: potential for international spread via commercial air travel. J Travel Med. 2020;27(2).
2. Du Toit A. Outbreak of a novel coronavirus. Nat Rev Microbiol. 2020;18(3):123.
3. Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chin Med J (Engl). 2020;133(9):1015-24.
4. Wang W, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. J Med Virol. 2020;92(4):441-7.
5. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;109:102433.

6. Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. J Adv Res. 2020;24:91-8.
7. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 2020;395(10223):507-13.
8. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395(10223):497-506.
9. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020;323(11):1061-9.
10. Liu W, Fontanet A, Zhang P-H, Zhan L, Xin Z-T, Baril L, et al. Two-Year Prospective Study of the Humoral Immune Response of Patients with Severe Acute Respiratory Syndrome. The Journal of Infectious Diseases. 2006;193(6):792-5.
11. Liu L, Wei Q, Lin Q, Fang J, Wang H, Kwok H, et al. Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI Insight. 2019;4(4).
12. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clin Infect Dis. 2020;71(16):2027-34.
13. Organization WH. Coronavirus disease (COVID-19) technical guidance : Laboratory testing for 2019-nCoV in humans. World Health Organization; 2020.
14. Woo PC, Lau SK, Wong BH, Tsoi HW, Fung AM, Chan KH, et al. Detection of specific antibodies to severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein for serodiagnosis of SARS coronavirus pneumonia. J Clin Microbiol. 2004;42(5):2306-9.
15. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3.
16. Racine R, Winslow GM. IgM in microbial infections: taken for granted? Immunol Lett. 2009;125(2):79-85.
17. Hou H, Wang T, Zhang B, Luo Y, Mao L, Wang F, et al. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. Clin Transl Immunology. 2020;9(5):e01136.
18. Gozalbo-Rovira R, Gimenez E, Latorre V, Frances-Gomez C, Albert E, Buesa J, et al. SARS-CoV-2 antibodies, serum inflammatory biomarkers and clinical severity of hospitalized COVID-19 patients. J Clin Virol. 2020;131:104611.
19. Allegra A, Di Gioacchino M, Tonacci A, Musolino C, Gangemi S. Immunopathology of SARS-CoV-2 Infection: Immune Cells and Mediators, Prognostic Factors, and Immune-Therapeutic Implications. International Journal of Molecular Sciences. 2020;21(13):4782.

ORIGINAL ARTICLE

Synchronization of Sputum Conversion and Resolution of Intensive Phase Lesion Areas on Thorax X-rays Determinants of Prognosis for Pulmonary Tuberculosis Therapy

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ABSTRACT

Background: Pulmonary tuberculosis (TB) is a chronic infectious disease caused by the bacterium Mycobacterium tuberculosis. Diagnosis of TB can be confirmed in two ways, namely bacteriological diagnosis (if AFB sputum is found (+) and clinical diagnosis is (if BTA sputum is found (-), but chest X-ray is (+) TB). This study was to determine the alignment of sputum conversion and extensive resolution of intensive phase lesions on chest radiographs which determine the prognosis of pulmonary TB therapy.
Methods: The study design was a retrospective cohort analytic with a retrospective longitudinal study design. Data from medical records of pulmonary TB patients who have undergone therapy for six months or more at the Pulmonary Polyclinic RSI Jemursari Surabaya. The number of samples was 48 patients aged 41-60 years. All of these pulmonary TB patients were smear positive (BTA+). X-ray examination was done before and after therapy.
Results: analysis using the Wilcoxon Signed Rank test to assess differences in the grade of lung lesions before and after therapy, obtained p = 0.003 (p <0.05) meaning there is a significant difference. Sputum conversion was also carried out after therapy, 89.6% of TB patients in this study experienced sputum conversion (BTA negative). To determine the alignment of sputum conversion with the resolution of lesion area, Kappa coefficient analysis K=0.033 (p>0.05) was performed with the results of 50% of patients, 47.9% showed improvement in lung lesions and sputum conversion, while 2.1% showed no improvement of lung lesions and no sputum conversion. The rest, 50% showed no congruence in the results of lung lesion repair and sputum conversion.
Conclusion: The results of Kappa coefficient analysis showed that K=-0.110 (p>0.05) showed that there was no congruence between the results of chest x-ray examination of lung lesions before and after therapy (improved or not) with sputum conversion

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Introduction

Pulmonary tuberculosis (pulmonary TB) is a chronic infectious disease that is still a major public health problem in the world, including Indonesia. Pulmonary tuberculosis is an infectious infectious disease caused by the bacterium Mycobacterium tuberculosis. The results of the Household Health Survey (SKRT) in 1995, pulmonary TB became the third cause of death after cardiovascular disease and respiratory disease in all age groups and the number one cause of death from respiratory infectious diseases.[1]

WHO estimates the incidence in 2017 was 842,000 or 319 per 100,000 population while TB-HIV was 36,000 cases per year or 14 per 100,000 population. According to the WHO report, Indonesia is in the list of 30 countries with the highest burden of tuberculosis in the world and ranks third highest in the world regarding the incidence of tuberculosis.

The incidence of tuberculosis in Indonesia in 2018 was 316 per 100,000 population or it is estimated that around 845,000 people suffered from tuberculosis in 2018.[2] WHO said there were around 1.7 million people who died from tuberculosis in the world, while in Indonesia it was estimated that 92,700 people died from tuberculosis, or about 11 people die of TB per hour. TB is not only experienced by adults, but people who have low immunity and have comorbidities, such as the elderly, children, diabetic patients PLWHA (People with HIV AIDS) are very at risk of being infected with TB (Indonesian TB)

The Indonesian Ministry of Health released the latest data on estimated cases of tuberculosis (TB) in Indonesia, the number of which fell by around 200 thousand, from around 1,020,000 cases in 2017 to 842,000 cases in 2018.[1]

Establishing the main TB diagnosis according to WHO is AFB sputum with results (+),(-) or no material (phlegm-), if sputum is not found, then it is diagnosed as TB based on chest X-ray supported by clinical symptoms (TB triad).[3]

The diagnosis of TB in 2 ways, namely by establishing a bacteriological diagnosis (if BTA (+) sputum is found), and clinical diagnosis (if AFB sputum is found (-), but chest x-ray (+) TB). TB therapy (according to WHO 2 category) category I (new case) and category 2 (relapse, tx failure, default), category 1 TB therapy takes 6 months.[4]

Is the harmony between sputum conversion and extensive resolution of intensive phase lesions can be a determinant of the prognosis of pulmonary TB therapy, which is a measure of the success of complete healing on time in patients with pulmonary TB. This is done by analyzing the conversion of AFB sputum in the intensive phase and analyzing the resolution results of serial chest X-rays obtained in the 2-month intensive phase.

Serial photo resolution is by looking at 2 photos to compare, namely the first chest photo obtained at the beginning of therapy and the second photo obtained at the end of the 2-month intensive phase and followed by the development of pulmonary TB infection healing for 6 months in order to find out how the prognosis of the patient's recovery after therapy for 2 and 6 months ended. The conversion of BTA sputum (change in AFB sputum (+) to sputum BTA (-) that occurs in the intensive phase.[5]

Methods

The study design was a retrospective analytic cohort with a retrospective longitudinal study design. Data from medical records of

pulmonary TB patients who have undergone therapy for six months or more at the Pulmonary Polyclinic RSI Jemursari Surabaya. The number of samples was 48 patients aged 41-60 years. All of these pulmonary TB patients were smear positive (BTA+). X-ray examination was done before and after therapy.

Results

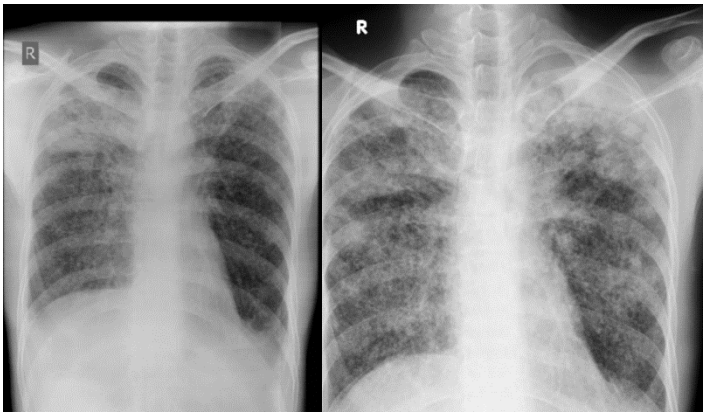

Figure 1. Patients with steady condition between before and two months after therapy

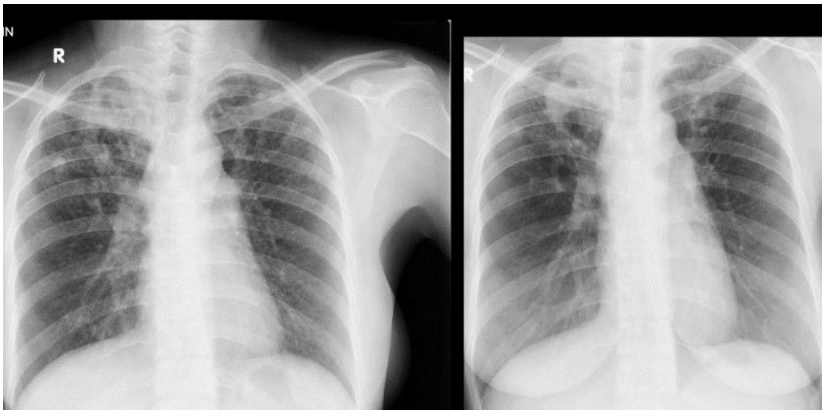

Figure 2. Patients with improved condition between before and two months after therapy

The picture above is 2 of the 48 samples used in this study. The first image represents a patient with a steady state between before and two months after therapy. In the second image, the patient's condition remained between before and two months after therapy. Several aspects examined in this study include the characteristics of patients with pulmonary TB, the description of the results of the chest x-ray examination, the results of the sputum examination (sputum conversion), and the alignment of the sputum conversion with the resolution of the extent of the lesion.

1. Characteristics of Pulmonary TB Patients

Table 1 Characteristics of patients with pulmonary TB

Characteristic	Category	Frequency (%)	∑x ± SD (Min-Max)
Age	21-40 years	17 (35,4%)	48,19 ± 15,24 (21 – 80) tahun
	41-60 years	20 (41,7%)	
	>60 years	11 (22,9%)	
Gender	Man	23 (47,9%)	
	Woman	25 (52,1%)	

Most of the pulmonary TB patients in this study were female and were in the 41-60 year age group.

From the results of sputum examination before therapy, it was found that all patients with pulmonary TB (100%) in this study were BTA positive (BTA+).

2. Overview of Thorax X-ray Examination Results

Table 2 Results of Thorax Photo Examination before and after therapy

Lung Lesion	Chest X-ray Examination Time	
	Before Therapy	After Therapy
Negative	0 (0,0%)	6 (12,5%)
Minimal	14 (29,2%)	23 (47,9%)
Moderate	32 (66,7%)	14 (29,2%)
Far advance	2 (4,2%)	5 (10,4%)
Total	48 (100,0%)	48 (100,0%)

The results of the chest x-ray examination showed that before therapy, most of the lung lesions were of moderate grade (66.7%) while after therapy, most of the lesions were minimal (47.9%) and only 12.5% were negative.

If you look at the changes in the chest radiograph that occur before and after therapy, the distribution is as shown in the following table.

Table 3 Changes in the picture of changes in lung lesions on chest X-ray examination before and after therapy

Lung Lesion Before Therapy	Lung Lesion After Therapy				Total
	Negative	Minimal	*Moderate*	*Far advance*	
Minimal	6 (12,5%)	3 (6,3%)	5 (10,4%)	0 (0,0%)	14 (29,2%)
Moderate	0 (0,0%)	20 (41,7%)	8 (16,7%)	4 (8,3%)	32 (66,7%)
Far advance	0 (0,0%)	0 (0,0%)	1 (2,1%)	1 (2,1%)	2 (4,2%)
Total	6 (12,5%)	23 (47,9%)	14 (29,2%)	5 (10,4%)	48 (100,0%)

In Table 3, the results of the analysis using the Wilcoxon Signed Ranks test showed that there were differences in the grade of lung lesions before and after therapy, $p=0.003$ ($p<0.05$). The data in Table 3 shows that most patients (56.3%) experienced improvement in the appearance of pulmonary lesions, 25.1% remained, and 18.7% experienced worsening.

3. Sputum Examination Results (Sputum Conversion)

Table 4 Sputum conversion after therapy

Conversion	Frequency	Percentage (%)
Yes	43	89,6
No	5	10,4
Total	48	100,0

Table 4 shows that most of the TB patients (89.6%) in this study experienced sputum conversion (BTA negative).

4. Alignment of Sputum Conversion with Resolution of Lesion Area

The analysis to determine the alignment of sputum conversion with the resolution of the lesion area obtained the following results.

Table 5 Results of alignment analysis of lung lesion repair with sputum conversion

Lung Lesion Repair	Sputum Conversion		Total
	Yes	No	
Yes	23 (47,9%)	4 (8,3%)	27 (56,3%)
No	20 (41,7%)	1 (2,1%)	21 (43,8%)
Total	43 (89,6%)	5 (10,4%)	48 (100,0%)

Kappa = -0,110 p = 0,258

The data in Table 5 shows that the concordance of results was obtained in 50% of patients, where 47.9% of patients showed improvement in lung lesions and sputum conversion, while 2.1% of patients showed no improvement in lung lesions and no sputum conversion. The rest, as many as 50% of patients showed no alignment of the results of lung lesion repair and sputum conversion.

The results of Kappa coefficient analysis showed that K = -0.110 (p>0.05) indicated that there was no congruence between the results of chest x-ray examination of lung lesions before and after therapy (improved or not) with sputum conversion. If the results of the chest x-ray examination are positive and negative, the results of the analysis of the alignment of the resolution of the lesion area with the conversion of sputum are as follows.

Table 6 Results of alignment analysis of chest x-ray examination with sputum conversion

Lung Lesion	Sputum Conversion		Total
	Yes	No	
Negative	6 (12,5%)	0 (0,0%)	6 (12,5%)
Positive	37 (77,1%)	5 (10,4%)	42 (87,5%)
Total	43 (89,6%)	5 (10,4%)	48 (100,0%)

Kappa = 0,033 p = 0,372

The data in Table 6 shows that concordance of results was obtained in 22.9% of patients, where 12.5% of patients showed negative lung lesions and sputum conversion, while 10.4% of patients showed positive lung lesions and no sputum conversion occurred. The rest, as many as 77.1% of patients showed no congruence between the presence of lung lesions and sputum conversion.

The results of Kappa coefficient analysis showed that K=0.033 (p>0.05) indicated that there was no harmony between the results of chest x-ray examination (positive or negative lesions) after therapy with sputum conversion.

Discussion

Inconsistency between the results of chest x-ray examination of lung lesions before and after therapy (improved or not) with sputum conversion. From the Sputum BTA factor of conversion patients (89.6%), from the chest x-ray factor 47.9% of patients with resolution experienced a reduction in lesions in general indicating the healing process, as well as 2.1% of patients with fixed lesions compared to the initial 2 months of therapy, but it can be concluded (47.9% + 2.1% = 48.8%) Radiological improvement. Broadly speaking, 50% of TB patients with AFB (+) experience conversion in their sputum, this is in line with TB patients with wider initial radiological features experiencing resolution, marked by infiltrates on radiological images, the area is narrowed and shows signs of the healing process.[6,7]

Meanwhile, the remaining 50% of patients who experienced conversion of their sputum were

statistically inconsistent with the resolution on the chest radiograph. It is suspected that 50% of patients with pulmonary TB with smear (+) who are not in harmony with the development of the reduction in the number of infiltrates on the radiographic picture of the chest radiograph have an X factor. From the point of view of TB patients who come with sputum smear examination (+) after intensive phase therapy for 2 months it appears on examination of sputum smear (-) as many as 89.6%, it is suspected that the number of pulmonary TB patients with sputum smear examination (-) after intensive therapy is caused by several things:

1) BTA (-) at the end of the Intensive Phase therapy, there were no TB germs found in the lungs anymore, because there was no AFB in the sputum sample, so the sputum samples did not find TB germs and smear staining results (-)
2) BTA (-) at the end of the Intensive Phase therapy, there were no TB germs found in the lungs in a dormant state, so that the sputum samples were not found to have TB germs and smear staining results (-)
3) BTA (-) at the end of the Intensive Phase therapy, TB germs were not found in the lungs, but the number of TB germs in the respiratory tract/lungs was relatively small, so that the sputum samples did not find TB germs and smear staining results (-)
4) BTA (-) at the end of the Intensive Phase therapy was evident in the lungs where TB germs were not found dormant, but the location of TB germs was far from the central airway/large channel, so that the sputum samples did not find TB germs and smear staining results (-)

Temporary conclusions from the results of Sputum Painting: reasons number 1) and 2) cannot be denied because logically there are no TB germs that are excreted through sputum, so the results of smear smear staining (-).[6]

However, for reasons number 3) and 4) there are still things that need to be revealed, namely why AFB germs cannot come out with phlegm. Sputum sample collection is influenced by several things:

1) The cough method for removing phlegm is good and correct in patients so that the coughed up phlegm can carry TB germs that are located far from the central airway / large channel or the number of TB germs in the respiratory tract / lungs is relatively small
2) The method of stimulating the production of good and correct phlegm in patients, so that more and more phlegm is produced, it is expected that the relatively small number of TB germs in the respiratory tract/lungs will accumulate and be carried along with the discharge of phlegm whose production is increasing.
3) The method of increasing the concentration of TB germs in the respiratory tract is less effective (ie collecting phlegm in the morning after waking up, without brushing teeth, without drinking, before bathing/ablution) is not carried out properly, so that TB germs accumulate in the respiratory tract in small numbers or there is not any
4) The method of increasing the frequency of sputum collection in one container for 24 hours as a one-time sample is not carried out properly, so the number of TB germs in the respiratory tract/lungs is relatively small. more and more

These results of the collection of phlegm Methods 1 to 4 should be applied to ensure that non-dormant TB germs can be expelled with sputum. This is the reason why out of 100% of TB patients with AFB (+) found 89.6% experienced conversion. The 89.6% of patients who underwent conversion, a chest X-ray was found 47.9% experienced resolution / experienced a reduction in lesions at the end of the 2-month intensive phase of therapy generally showing a healing process, as well as 2.1% of patients with persistent lesions at the end of the intensive phase 2 therapy. One month after show that TB germs in a stagnant position (Dorman) indicate a good body defense process. Temporary conclusions (47.9% + 2.1% = 48.8%) indicate the process leads to improvement. This results was similar from the theory from Jilani, 2023.[8]

Several references that also investigated the alignment of AFB sputum with chest X-rays,[9] among others, in the first literature the samples studied were 200 samples of pulmonary TB patients.[10] There were 100 samples of patients with positive smear results and 100 samples of patients with negative smear results. In the second literature, the samples studied were 147 samples of pulmonary TB patients.[11,12] The number of samples with positive smear test results were 38 samples and samples with negative smear results were 109 samples. Where in the third literature, the number of samples studied were 159 samples of pulmonary TB patients with negative smear results.[13,14]

The research action carried out in the first literature was a chest X-ray examination and AFB examination, the results of the chest X-ray obtained were interpreted by two radiology doctors independently.[3,15] In the second literature, only a chest X-ray was performed. The results of the chest X-ray examination were interpreted by two radiology doctors who were not aware of the results of the AFB examination in each patient.[16] In the third literature, sputum culture was repeated and chest X-ray was repeated in all samples. The results obtained from the chest x-ray examination were interpreted by two senior radiology doctors.

Conclusion

The reason why the Sputum Conversion occurs faster than the resolution of radiological photos, is presumably because the detection tool for the presence of lesions in the lungs is not influenced by mechanisms in the body, but is influenced by the sensitivity of the radiology tool and the experience of the radiographer, so radiology is a very sensitive and very detailed detection tool in detecting the presence of infiltrates or other features that indicate active lung lesions. Thus, the accuracy of radiology photos can detect the presence of an active disease process, so that it can be distinguished between severe, moderate, mild, and undetected lesions. Meanwhile, the detection of germs in the patient's sputum is strongly influenced by many factors.

Conflict of Interest

No conflict of interest in this study

References

1. Abdelaziz MM, Bakr WMK, Hussien SM, Amine AEK. Diagnosis of pulmonary tuberculosis using Ziehl-Neelsen stain or cold staining techniques? J Egypt Public Health Assoc. 2016;91(1):39–43.
2. Agarwal S, Caplivski D, Bottone EJ. Disseminated tuberculosis presenting with finger swelling in a patient with tuberculous

osteomyelitis: a case report. Ann Clin Microbiol Antimicrob. 2005;4:18.

3. Alzayer Z, Nasser Y Al. Primary Lung Tuberculosis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.

4. Asmar S, Drancourt M. Rapid culture-based diagnosis of pulmonary tuberculosis in developed and developing countries. Front Microbiol. 2015;6:1184.

1. Kementerian Kesehatan Republik Indonesia. Petunjuk Teknis Pemeriksaan TB Menggunakan Tes Cepat Molekuler. Jakarta: Gerakan Masyarakat Hidup Sehat; 2017.

2. Kementerian Kesehatan Republik Indonesia. Tuberculosis Control in Indonesia. Jakarta: Gerakan Masyarakat Hidup Sehat; 2022

3. Rai, D. K., Kirti, R., Kumar, S., Karmakar, S., & Thakur, S. (2019). Radiological difference between new sputum-positive and sputum-negative pulmonary tuberculosis. *Journal of family medicine and primary care*, *8*(9), 2810–2813. https://doi.org/10.4103/jfmpc.jfmpc_652_19

4. Bhalla, A. S., Goyal, A., Guleria, R., & Gupta, A. K. (2015). Chest tuberculosis: Radiological review and imaging recommendations. *The Indian journal of radiology & imaging*, *25*(3), 213–225. https://doi.org/10.4103/0971-3026.161431

5. Chao, WC., Huang, YW., Yu, MC. *et al.* Outcome correlation of smear-positivity but culture-negativity during standard anti-tuberculosis treatment in Taiwan. *BMC Infect Dis* **15**, 67 (2015). https://doi.org/10.1186/s12879-015-0795-1

6. Swai, H. F., Mugusi, F. M., & Mbwambo, J. K. (2011). Sputum smear negative pulmonary tuberculosis: sensitivity and specificity of diagnostic algorithm. *BMC research notes*, *4*, 475. https://doi.org/10.1186/1756-0500-4-475

7. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. Clin Infect Dis. 2017;64(2):111–5.

8. Jilani TN, Avula A, Zafar Gondal A, et al. Active Tuberculosis. [Updated 2023 Jan 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513246/

9. Williams, E., Cheng, A.C., Lane, G.P. and Guy, S.D. (2018), Delays in presentation and diagnosis of pulmonary tuberculosis: a retrospective study of a tertiary health service in Western Melbourne, 2011–2014. Intern Med J, 48: 184-193. https://doi.org/10.1111/imj.13551

10. Nachiappan AC, Rahbar K, Shi X, Guy ES, Barbosa M, J E, et al. Pulmonary Tuberculosis: Role of Radiology in Diagnosis and Management. RadioGraphics. 2017;37(1):52–

11. Singh SK, Tiwari KK. Clinicoradiological Profile of Lower Lung Field Tuberculosis Cases among Young Adult and Elderly People in a Teaching Hospital of Madhya Pradesh, India. J Trop Med. 2015;2015:230720.

12. Suryawati B, Saptawati L, Putri AF, Aphridasari J. Sensitivitas Metode Pemeriksaan Mikroskopis Fluorokrom dan Ziehl-Neelsen untuk Deteksi Mycobacterium tuberculosis pada Sputum. Smart Med J. 2018;1(2).

13. Hassane-Harouna, S., Braet, S. M., Decroo, T., Camara, L. M., Delamou, A., Bock, S., Ortuño-Gutiérrez, N., Cherif, G. F., Williams, C. M., Wisniewska, A., Barer, M. R., Rigouts, L., & de Jong, B. C. (2023). Face mask sampling (FMS) for tuberculosis shows lower diagnostic sensitivity than sputum sampling in Guinea. *Annals of clinical microbiology and antimicrobials*, *22*(1), 81. https://doi.org/10.1186/s12941-023-00633-8

14. Bahru Mantefardo, Gizaw Sisay, Ephrem Awlachew, "Assessment of Smear-Positive Pulmonary Tuberculosis and Associated Factors among Patients Visiting Health Facilities of Gedeo Zone, Southern Ethiopia: A Cross-Sectional Study", *Tuberculosis Research and Treatment*, vol. 2023, Article ID 2502314, 7 pages, 2023. https://doi.org/10.1155/2023/2502314

15. Muyoyeta M, Maduskar P, Moyo M, Kasese N, Milimo D, et al. (2014) The Sensitivity and Specificity of Using a Computer Aided Diagnosis Program for Automatically Scoring Chest X-Rays of Presumptive TB Patients Compared with Xpert MTB/RIF in Lusaka Zambia. PLOS ONE 9(4): e93757. https://doi.org/10.1371/journal.pone.0093757

16. Ebrahimzadeh, A., Mohammadifard, M., & Naseh, G. (2014). Comparison of chest x-ray findings of smear positive and smear negative patients with pulmonary tuberculosis. *Iranian journal of radiology : a quarterly journal published by the Iranian Radiological Society*, *11*(4), e13575. https://doi.org/10.5812/iranjradiol.13575