Evaluating the Diagnostic Potential of Human Growth Hormone and RNase P Genes in Tuberculosis Detection: An Analytical Study Using Menstrual Blood and Sputum Samples

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Abstract: *Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB), remains a significant global health challenge, with diagnostic limitations, especially in extrapulmonary cases. Molecular diagnostics targeting specific genetic markers have emerged as promising tools for TB detection. This study investigates the diagnostic potential of the human growth hormone (hGH) and RNase P genes in detecting TB, using menstrual blood and sputum samples. A cross-sectional analytical study was conducted involving 50 patients presenting with TB symptoms. Menstrual blood and sputum samples were collected and analyzed using polymerase chain reaction (PCR) to detect the presence of MTB (targeting the IS6110 gene), human growth hormone (hGH), and RNase P genes. Sensitivity, specificity, and accuracy were calculated for each gene and sample type, and receiver operating characteristic (ROC) curves were generated. The hGH gene in menstrual blood samples demonstrated a sensitivity of 33.3% and specificity of 62.5%, while the RNase P gene in sputum samples showed a sensitivity of 40% and specificity of 80%. ROC curve analysis revealed that the RNase P gene, tested in sputum samples, had a higher area under the curve (AUC) compared to the hGH gene, which was tested in menstrual blood samples. This finding suggests that RNase P is more effective for TB detection in our study. The detection of hGH and RNase P genes in menstrual blood and sputum samples, respectively, indicates their potential as molecular markers for TB diagnosis. However, the observed sensitivities and specificities suggest that these markers alone may not provide sufficient diagnostic accuracy. Further research is warranted to explore their utility in combination with other diagnostic approaches to enhance TB detection, particularly in extrapulmonary cases.*

Keywords: ***Mycobacterium tuberculosis*****, Human Growth Hormone, Receiver Operating Characteristic, Sensitivity, Specificity.**

1. INTRODUCTION

Tuberculosis (TB), instigated by *Mycobacterium tuberculosis* (MTB), persists as a formidable infectious malady, afflicting millions globally. While pulmonary TB prevails as the predominant manifestation, extrapulmonary TB often eludes diagnosis due to the constraints of traditional methods like sputum microscopy and culture.[1] Alarmingly, TB claims more lives annually than any other single infectious agent. The imperativeness to monitor TB during treatment is underscored by the risk of drug misuse, which can precipitate drug resistance, notably multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. These resistant variants pose significant challenges to effective disease management. In 1993, the World Health Organization (WHO) declared TB a global crisis, aiming to heighten public and political awareness.[2]

In recent years, molecular diagnostics have emerged as promising tools for TB detection, leveraging specific genetic markers for rapid and precise identification. Genes such as the human growth hormone (hGH) and RNase P have demonstrated potential as molecular markers in various infectious diseases. However, their applicability in TB diagnosis, particularly concerning menstrual blood and sputum specimens, remains unexplored. This study endeavours to evaluate the relevance of these genes in TB detection and their prospective role in enhancing diagnostic efficacy.[3]

2. MATERIALS AND METHODS

2.1. Study design

This cross-sectional analytical study was collaboratively conducted by Swami Rama Himalayan University (SRHU), Jolly Grant, Dehradun, and DNA Labs- A Centre for Applied Sciences, located in East Hopetown, Dehradun, Uttarakhand. The research encompassed 50 patients exhibiting TB symptoms. Prior to participation, ethical approval was secured, and informed consent was obtained from all participants.

2.2. Sample collection

Menstrual blood and sputum specimens were collected from patients suspected of TB infection. Control samples from non-TB individuals were also obtained for comparison. The specimens were stored and processed in a biosafety laboratory Type II of DNA Labs.

2.3. Molecular analysis

DNA was extracted using Amplichain Nucleic Acid extraction kit, followed by PCR amplification by BIORAD T-100 Cycler, targeting the hGH and RNase P genes. Specific primers for gene amplification were designed to flank a region of the hGH gene, which spans approximately 2,893 base pairs (bp), but by selecting appropriate primers,[4] smaller fragments of approximately 572 bp segment were specifically amplified and analyzed, and RNase P Gene at approximately 120 bp, targeting IS6110 gene at 123 bp to detect the presence of MTB was confirmed through gel electrophoresis.[3],[5]

2.4. Statistical analysis

The study utilized statistical tools such as sensitivity, specificity, and predictive values to assess the diagnostic accuracy of the hGH and RNase P genes. Receiver operating characteristic (ROC) curves were generated to evaluate their diagnostic performance compared to conventional methods.

3. RESULTS

A total of 50 patients were included in the study, along with age, gender and symptom distribution as shown in Table 1. The majority of male patients fall within the 40-49 age group. Female patients are more evenly distributed across the 20-39 age range. The mean age of male patients is higher than that of female patients.

Table 1. Age, Gender and Symptom distribution

Patient ID.	Age	Gender	Symptoms	Sample Type
DNA 1	45	Male	Cough, Fever	Sputum
DNA 2	33	Male	Night sweats, Weight loss	Sputum
DNA 3	29	Male	Fatigue, Cough	Sputum
DNA 4	52	Female	Fever, Weight loss, Pain in abdomen	Menstrual Blood
DNA 5	41	Male	Chronic cough	Sputum
DNA 6	36	Female	Fever, Fatigue	Menstrual Blood
DNA 7	47	Male	Cough, Fever	Sputum
DNA 8	33	Female	Night sweats, Weight loss, Irregular menses, Infertility, Pain in abdomen	Menstrual Blood
DNA 9	50	Male	Fatigue, Cough	Sputum
DNA 10	28	Female	Fever, Weight loss, Anxiety	Menstrual Blood
DNA 11	38	Male	Chronic cough, Night sweats	Sputum

DNA 12	31	Female	Fever, Fatigue	Menstrual Blood
DNA 13	44	Female	Abdominal pain, Fever, Urinary tract infection	Menstrual Blood
DNA 14	35	Female	Night sweats, Weight loss	Menstrual Blood
DNA 15	42	Male	Fatigue, Chronic cough	Sputum
DNA 16	30	Female	Fever, Weight loss	Menstrual Blood
DNA 17	48	Female	Acute Cough, Night sweats	Sputum
DNA 18	37	Female	Fever, Fatigue	Menstrual Blood
DNA 19	45	Male	Chronic cough, Fever	Sputum
DNA 20	32	Male	Night sweats	Sputum
DNA 21	53	Male	Fatigue, Chronic cough	Sputum
DNA 22	26	Female	Fever, Weight loss, Irregular menses, White discharge	Menstrual Blood
DNA 23	38	Male	Cough, Night sweats	Sputum
DNA 24	29	Female	Fever, Fatigue	Menstrual Blood
DNA 25	50	Male	Chronic cough, Fever	Sputum
DNA 26	34	Female	Night sweats, Weight loss, Irregular menses	Menstrual Blood
DNA 27	41	Male	Fatigue, Chronic cough	Sputum
DNA 28	30	Female	Fever, Weight loss	Menstrual Blood
DNA 29	45	Male	Chronic cough, Night sweats	Sputum
DNA 30	36	Female	Fever, Fatigue	Menstrual Blood
DNA 31	44	Male	Chronic cough, Fever	Sputum
DNA 32	28	Female	Night sweats, Weight loss	Menstrual Blood
DNA 33	43	Male	Fatigue, Chronic cough	Sputum
DNA 34	30	Female	Fever, Weight loss	Menstrual Blood
DNA 35	47	Male	Chronic cough, Night sweats	Sputum
DNA 36	31	Female	Fever, Fatigue, Irregular menses	Menstrual Blood
DNA 37	50	Female	Irregular Fever, Excessive White discharge and abdomen pain	Menstrual Blood
DNA 38	33	Female	Night sweats, Weight loss	Menstrual Blood
DNA 39	40	Male	Fatigue, Chronic cough	Sputum
DNA 40	29	Female	Fever, Weight loss	Menstrual Blood
DNA 41	44	Female	Night sweats, Pain in Abdomen	Menstrual Blood
DNA 42	35	Female	Fever, Fatigue	Menstrual Blood
DNA 43	48	Male	Chronic cough, Fever	Sputum
DNA 44	32	Female	Night sweats, Weight loss, Pain in abdomen	Menstrual Blood
DNA 45	52	Male	Fatigue, Chronic cough	Sputum
DNA 46	27	Male	Fever, Weight loss	Sputum

DNA 47	39	Male	Chronic cough, Night sweats, Chest pain	Sputum
DNA 48	31	Female	Fever, Fatigue, Infertility	Menstrual Blood
DNA 49	46	Male	Chronic cough, Fever	Sputum
DNA 50	33	Male	Chronic cough, Fever	Sputum

In a cohort of 24 patients providing menstrual blood samples, 8 tested positive for MTB and exhibited the hGH gene at approximately 572 bp. Among 26 patients submitting sputum samples, 10 were MTB-positive, with the RNase P gene detected at approximately 120 bp. The detection of the hGH gene in MTB-positive menstrual blood samples suggests its potential as a molecular marker for TB diagnosis in female patients. Similarly, the presence of the RNase P gene in MTB-positive sputum samples further supports its role as a genetic marker in TB diagnostics.

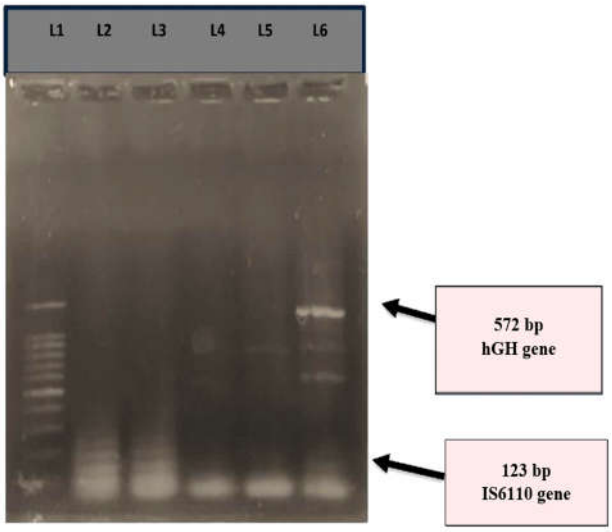

Figure 1. hGH gene can be visualized at approximately 572 bp in lane L6 and MTB target at 123 bp IS6110 gene in L6 guided by 50 bp Ladder in L1

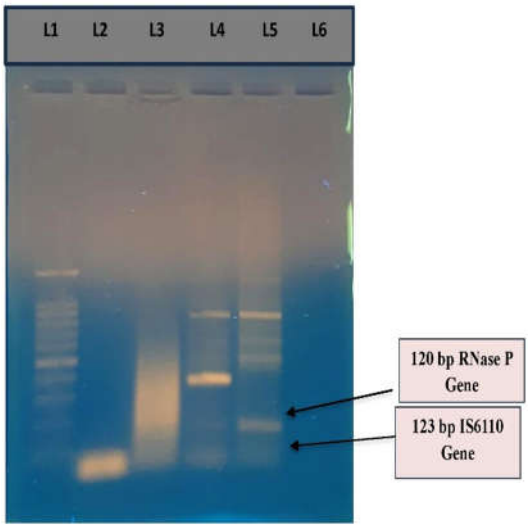

Figure 2. RNase P gene at 120 bp and MTB target at 123 bp IS6110 gene in L5, L4 guided by 50 bp Ladder in L1

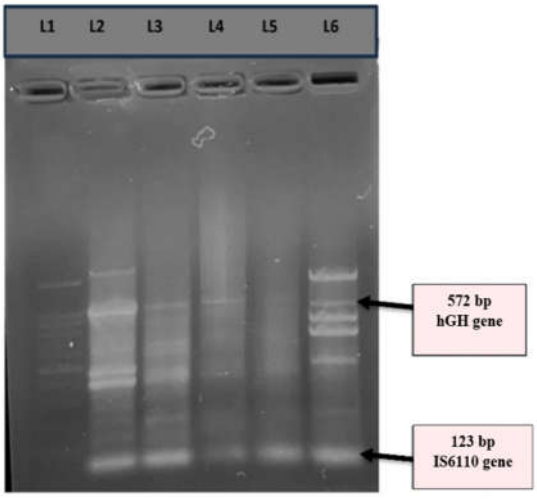

Figure 3. hGH gene can be visualized at approximately 572 bp in L6, L4, L3, L2 and MTB Target at 123bp IS6110 gene in L6 ,L5,L4,L3,L2 guided by 50 bp Ladder in L1

The hGH gene were amplified to produce a fragment of approximately 572 bp (Figure 3), depending on the specific primers used. When subjected to electrophoresis, this fragment migrated to a position between

the 550 bp and 600 bp markers of the 50 bp ladder, facilitating its identification. Similarly, the IS6110 gene, a common target for detecting MTB, is typically amplified to yield a 123 bp fragment. On the agarose gel, this fragment appeared just above the 100 bp marker, allowing for its distinction from other bands. The RNase P gene, often used as an internal control in PCR assays, produced a fragment of approximately 120 bp upon amplification. This fragment would also migrate to a position just above the 100 bp marker, close to the 123 bp band of the IS6110 gene. In gel setup, the 50 bp DNA ladder was loaded into lane 1 to serve as a reference. Subsequent lanes (e.g., Lanes 2 through 6) contained the PCR products of the hGH gene, displaying bands at approximately 572 bp. Other lanes contained the amplified products of the IS6110 gene, showing bands at 123 bp, and the RNase P gene, with bands at 120 bp.

The sensitivity (True Positive Rate, TPR) of a diagnostic test was calculated where TP (True Positives) represents correctly identified positive cases, and FN (False Negatives) represents missed positive cases. Sensitivity measures the ability of the test to correctly detect TB in affected individuals. The specificity (True Negative Rate) where TN (True Negatives) represents correctly identified negative cases, and FP (False Positives) represents incorrectly identified positive cases. This metric determines how well the test avoids false alarms by correctly identifying healthy individuals. The accuracy of the test, False Positive Rate (FPR), False Negative Rate (FNR) was also calculated. The Receiver Operating Characteristic (ROC) Curve representing test's performance by plotting Sensitivity (TPR) against FPR (1-Specificity). The area under the curve (AUC) quantifies the test's diagnostic ability, where an AUC value close to 1.0 indicates a highly accurate test, while an AUC near 0.5 suggests poor performance equivalent to random guessing.

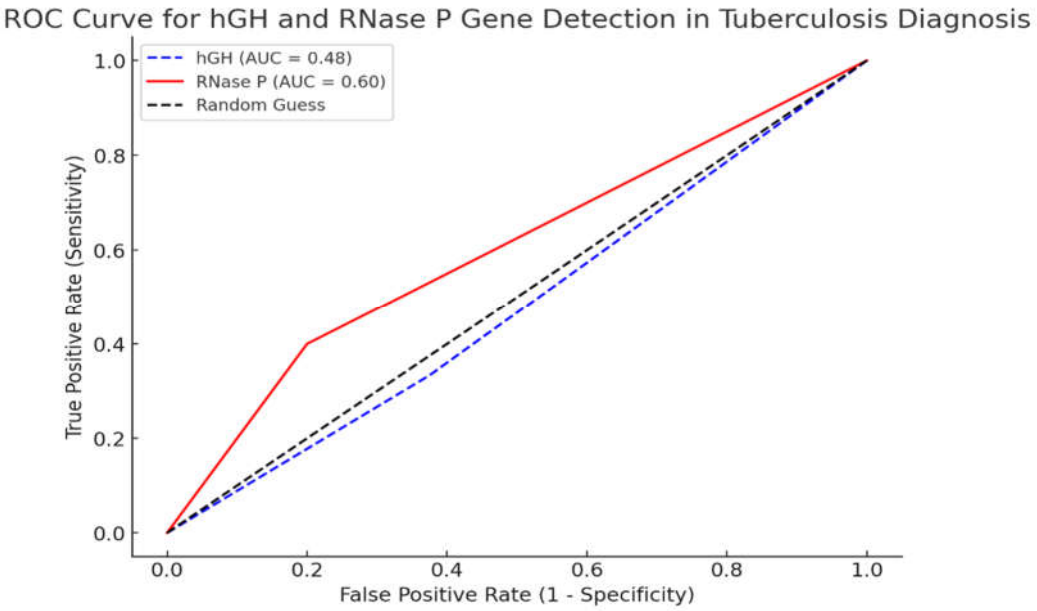

Figure 4. ROC Curve for hGH and RNase P gene detection in TB diagnosis

Given in the Figure 4, ROC curve comparing the diagnostic performance of the hGH and RNase P genes in TB detection. The AUC values indicate that RNase P (red line) has a higher diagnostic accuracy compared to hGH (blue dashed line). The AUC closer to 1.0 indicates better diagnostic test performance. AUC near 0.5 indicates poor test performance, equivalent to random guessing. In our analysis, RNase P (sputum) has a higher AUC than hGH (menstrual blood), confirming its superior diagnostic capability.

Table 2. Sensitivity, Specificity and Accuracy of Gene and Sample Type

Gene & Sample Type	True Positives (TP)	False Negatives (FN)	True Negatives (TN)	False Positives (FP)	Sensitivity (%)	Specificity (%)	Accuracy (%)
hGH (Menstrual Blood)	8	16	10	6	33.3%	62.5%	75%
RNase P (Sputum)	10	15	12	3	40%	80%	88%

As per table 2, hGH gene detection in menstrual blood has a low sensitivity (33.3%), meaning it misses a significant number of true MTB-positive cases. However, its specificity (62.5%) indicates that it can correctly identify MTB-negative cases at a moderate rate. Its overall accuracy is 75%, suggesting potential but limited diagnostic reliability. RNase P gene detection in sputum shows higher sensitivity (40%) and higher specificity (80%), making it a more reliable molecular marker for TB. With an accuracy of 88%, it demonstrates better diagnostic performance compared to hGH gene.

4. DISCUSSION

The findings indicate that molecular markers such as the hGH and RNase P genes may enhance TB diagnostic accuracy, particularly in cases of extrapulmonary TB. However, the calculated sensitivity and specificity values for these markers suggest limitations in their diagnostic performance. Specifically, detection of the hGH gene in menstrual blood samples demonstrated a sensitivity of 33.3% and a specificity of 62.5%. This low sensitivity implies a significant number of false negatives, indicating that the hGH marker may miss many true TB-positive cases. Similarly, the RNase P gene detected in sputum samples exhibited a sensitivity of 40% and a specificity of 80%. While the specificity is moderate, the sensitivity remains suboptimal, suggesting that RNase P may also fail to identify a considerable number of TB-positive cases.

The high false-negative rates associated with both markers underscore the necessity for additional diagnostic approaches or the identification of more reliable molecular markers to improve MTB detection accuracy. In related research,[7] the expression of the normal human growth hormone (hGH-N) gene in transfected cells revealed the secretion of two hGH proteins, with 91% migrating at 22 kilodaltons (kDa) and 9% at 20 kDa. This finding indicated that the 20 kDa hGH is produced through alternative splicing of the primary hGH-N gene transcript, where exon 3 is partially skipped. This study highlights the complexity of hGH gene expression and its regulation at the mRNA level.

Additionally, a study [9] revealed that human nuclear RNase P is essential for the transcription of tRNA and other small noncoding RNA genes by RNA polymerase III in HeLa cells. This function is beyond its well-known role in tRNA processing, suggesting that RNase P has broader regulatory roles in gene expression.

Recent research has also indicated that RNase P and its components have non-canonical functions in regulating chromatin assembly and the DNA damage response. These findings suggest that RNase P is involved in maintaining genomic stability and may play a role in the cellular response to DNA damage.[8],[9] Comparatively, a study evaluated the efficacy of point-of-care C-reactive protein (CRP) testing for TB diagnosis among HIV-positive individuals. The test demonstrated a sensitivity of 89% and a specificity of 72% for culture-confirmed TB cases.[10]

The ROC curve analysis provides a comprehensive evaluation of the diagnostic performance of the hGH and RNase P genes in TB detection. The AUC serves as a pivotal metric in this assessment, where an AUC value approaching 1.0 signifies an excellent diagnostic test, while an AUC near 0.5 indicates a performance no better than random chance.[11]

In our study, the RNase P gene, represented by the red line in the ROC curve, exhibits a higher AUC compared to the hGH gene, depicted by the blue dashed line. This disparity underscores the superior diagnostic accuracy of RNase P over hGH in TB detection. Specifically, the RNase P gene detected in sputum samples demonstrates a higher AUC than the hGH gene identified in menstrual blood samples, thereby confirming its enhanced diagnostic capability.

These findings suggest that the RNase P gene holds greater promise as a molecular marker for TB diagnosis, particularly when utilizing sputum specimens. The elevated AUC associated with RNase P

indicates its potential for more reliable identification of TB-positive cases, thereby contributing to improved diagnostic accuracy. However, it is imperative to acknowledge that while the RNase P gene shows superior diagnostic performance relative to the hGH gene, the overall sensitivity and specificity values observed in this study may still fall short of the optimal thresholds required for clinical application. Consequently, further research is warranted to validate these findings and to explore the integration of additional molecular markers or diagnostic approaches to enhance the accuracy and reliability of TB detection.

The ROC curve analysis highlights the RNase P gene's superior diagnostic performance over the hGH gene in TB detection, in sputum samples. These insights pave the way for future studies aimed at refining molecular diagnostic strategies for TB, ultimately contributing to more effective disease management and control. Additionally, a systematic review assessed the diagnostic accuracy of various microRNAs (miRNAs) as biomarkers for TB. Among the miRNAs studied, miR-31 exhibited the highest diagnostic performance, with a sensitivity of 96% and a specificity of 89%. These studies highlight the ongoing efforts to identify effective molecular markers for TB diagnosis.[12],[13],[14]

While our findings indicate potential for the RNase P gene in sputum as a diagnostic tool, the comparatively lower sensitivity and specificity underscore the need for further research to enhance its diagnostic accuracy.

5. CONCLUSION

In summary, while the hGH and RNase P genes have been investigated as potential molecular markers to enhance TB diagnostic accuracy, particularly in extrapulmonary cases, their diagnostic performance in this study was suboptimal. The hGH gene detected in menstrual blood samples exhibited a sensitivity of 33.3% and a specificity of 62.5%, indicating a considerable rate of false negatives. Similarly, the RNase P gene in sputum samples demonstrated a sensitivity of 40% and a specificity of 80%, which, despite being higher, still reflects limitations in accurately identifying TB-positive cases. These findings suggest that although RNase P shows relatively better diagnostic potential than hGH, neither marker alone provides sufficient sensitivity and specificity for reliable TB detection. Consequently, there is a pressing need for further research to identify more effective molecular markers or to develop combinatory diagnostic approaches that can improve the accuracy and reliability of TB diagnostics. The ROC in this study demonstrates the significance of hGH and RNase P genes in diagnosing TB from menstrual blood and sputum specimens. The molecular approach provides a promising avenue for improving TB detection, particularly in extrapulmonary cases. Further large-scale studies are needed to validate these findings and explore their clinical applicability in diverse patient populations.

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REFERENCES

[1] *S. Gagneux, "Ecology and evolution of Mycobacterium tuberculosis", Nature Reviews Microbiology., vol. 16, no. 4,* ***(2018)****, pp. 202–213.*

[2] *J. Carton, "Oxford Handbook of Clinical Pathology", 2nd ed., Oxford University Press, United Kingdom,* ***(2017)****.*

[3] *M. Saravanan, S. Niguse, M. Abdulkader, E. Tsegay, H. Hailekiros, A. Gebrekidan, et al., "Review on emergence of drug-resistant tuberculosis (MDR & XDR-TB) and its molecular diagnosis in Ethiopia", Microbial Pathogenesis., vol. 117,* ***(2018)****, pp. 237–242.*

[4] *C. Esteban, L. Audí, A. Carrascosa, M. Fernández-Cancio, A. Pérez-Arroyo, A. Ulied, et al., "Human growth hormone (GH1) gene polymorphism map in a normal-statured adult population", Clinical Endocrinology (Oxford)., vol. 66, no. 2,* ***(2007)****, pp. 258–268.*

[5] *N. Sharma, R. K. Singh, and P. Sharma, "Molecular mycobacteriology and expansion in disease diagnosis", Indian Journal of Clinical Biochemistry., vol. 31, no. 2,* ***(2016)****, pp. 138–147.*

[6] *B. Singh, V. Khandalwal, U. Agarwal, S. Mangla, U. Shankar, A. Goel, et al., "IS6110 gene in Mycobacterium tuberculosis complex in tuberculosis meningitis—clinical applications for disease monitoring", South Asian Journal of Biology., vol. 5, no. 1,* ***(2018)****, pp. 20–25.*

[7] *N. E. Cooke, J. Ray, M. A. Watson, P. A. Estes, B. A. Kuo, S. A. Liebhaber, "Human growth hormone gene and the highly homologous growth hormone variant gene display different splicing patterns", Journal of Clinical Investigation., vol. 82, no. 1,* ***(1988)****, pp. 270–275.*

[8] *N. Jarrous and R. Reiner, "Human RNase P: a tRNA-processing enzyme and transcription factor", Nucleic Acids Research., vol. 35, no. 11,* ***(2007)****, pp. 3519–3524.*

[9] *P. Wang, J. Lin, X. Zheng, and X. Xu, "RNase P: beyond precursor tRNA processing", Genomics, Proteomics & Bioinformatics., vol. 22, no. 1,* ***(2024)****, article ID qzae016.*

[10] *S. Leo, M. Narasimhan, S. Rathinam, and A. Banerjee, "Biomarkers in diagnosing and therapeutic monitoring of tuberculosis: a review", Annals of Medicine., vol. 56, no. 1,* ***(2024)****, article ID 2386030.*

[11] *F. S. Nahm, "Receiver operating characteristic curve: overview and practical use for clinicians", Korean Journal of Anesthesiology., vol. 75, no. 1,* ***(2022)****, pp. 25–36.*

[12] *A. Cohen, V. D. Mathiasen, T. Schön, and C. Wejse, "The global prevalence of latent tuberculosis: a systematic review and meta-analysis", European Respiratory Journal., vol. 54, no. 1,* ***(2019)****, article ID 1900655.*

[13] *E. L. Corbett, C. J. Watt, N. Walker, D. Maher, B. G. Williams, M. C. Raviglione, et al., "The growing burden of tuberculosis: global trends and interactions with the HIV epidemic", Archives of Internal Medicine., vol. 163, no. 9,* ***(2003)****, pp. 1009–1021.*

[14] *R. McNerney, M. Maeurer, I. Abubakar, B. Marais, T. D. McHugh, N. Ford, et al., "Tuberculosis diagnostics and biomarkers: needs, challenges, recent advances, and opportunities", Journal of Infectious Diseases., vol. 205, suppl. 2,* ***(2012)****, pp. S147–S158.*