scientific reports

Check for updates

OPEN

The impact of gamma interferon on BK virus candidate microRNAs and related miRNAs in kidney transplant patients with BK infection

Maryam Changizi[1], Gholamreza Motalleb[1]✉, Ramin Yaghobi[2], Afsoon Afshari[3] & Jamshid Roozbeh[3]

Kidney transplant recipients (KTRs) with impaired immune systems may develop BKV nephropathy (BKVN). BKVN and allograft rejection may harm transplanted kidneys. BKV replicates via miR-B1-5p and 3p in order to escape from host's immunological response. BKV alters KTR and viral gene expression and miRNA profiles. In an inflammatory setting, IFN-γ may initiate the removal of pathogens by inducing an immune response. It has antiviral immunity, which may prevent the virus from replicating by preventing the synthesis of BK virus proteins. Antiviral miRNAs like miR-29a are also produced in response to IFN-γ activation. Thus, we investigated these modifications as putative biomarkers for evaluating viral infection and the regulatory web that arises from their expression during infection and the emergence of post-transplant problems. This study was carried out on KTRs. Our research, which aimed to quantify and examine the amounts of cellular miRNA-29a, IFN-γ gene, BKV-miR-B1-5p and 3p from urine and blood in KT patient groups, has the potential to guide future research in the field. Patients with BKVN (BK-), patients without an active BKV infection (BK-), patients with a history of transplant rejection (Reject), patients without an active history of transplant rejection (Non reject), and a control group were among these groups. The Syber green real-time PCR was employed for the measurements and analysis. The findings of our investigation demonstrated that BK virus-caused kidney tissue damage (tissue), patients with an active BK virus infection (BK+), and KTRs who had previously experienced transplant rejection all showed less IFN-γ gene expression in comparison with control. These patients showed upper levels of miR-29a gene expression than the control group. Furthermore, these patients' gene expressions of miR-B1-5p and 3p showed higher in comparison with those of the control group. To date, there is no report on the effect of IFN-γ on the expression of BK polyomavirus miRNAs and related miRNAs in kidney transplant recipients with nephropathy compared to kidney transplant recipients without nephropathy in the Iranian population. Therefore, the results of this study can be used as a strategy to combat viral infections and pathogenesis caused by BK polyomavirus in kidney transplantation.

KT is the most effective cure in ESRD or end stage renal disease. BKVN and allograft rejection are prominent causes of KT. BKVN is a high-prevalence polyomavirus whose specific host is humans. The virus has double-stranded DNA with early, late, and non-coding regulatory regions (NCCR)[1]. MicroRNA production plays an important regulatory role for infection of BKV[2]. BKV makes two fully grown miRNAs, which are called BKV-miR-B1-5p and 3p which help to stop virus infected cells destroying through NK or natural killer cells[3]. Post-transplant immunosuppression is the main cause of BKV reactivation, which can cause BKV-associated BKVN. 10% of KTRs develop BKVN, and 50% lose their transplant. Diseases related to this virus are usually found in hematopoietic stem cells and donated kidneys of recipient patients[4]. Inflammatory interstitial nephropathy, the hallmark of BKVN, manifests itself 10–13 months after KT[5]. BKVN happens when either latent BKV is

[1]Division of Cell and Molecular Biology, Department of Biology, Faculty of Science, University of Zabol, Zabol 98613-35856, Islamic Republic of Iran. [2]Shiraz Transplant Research Center, Shiraz University of Medical Sciences, Shiraz 71937-11351, Iran. [3]Shiraz Nephro-Urology Research Center, Shiraz University of Medical Sciences, Shiraz 71937-11351, Iran. ✉email: reza.motaleb@uoz.ac.ir; rezamotalleb@gmail.com

released in the receiver or BKV infection spreads within the transplant and the immune system is too weak to fight it[2]. Both humoral and cellular immune responses activated against capsid antigens control viral activity in immunocompromised individuals, creating a balance between virus and immune defenses. However, when the body's immune system weakens due to drug treatment before and after organ transplantation or immunosuppression related to HIV, this balance is lost. It is responsible for the reactivation of the virus, which results viremia and viruria[6]. The immune response in the inflammatory environment is activated by IFN-γ, leading to pathogen eradication. It prevents immune system overactivation and tissue damage[7]. Recent research indicates that IFN-γ may inhibit viral protein synthesis, genome replication, and provide non-cytotoxic viral genome clearance in host cells[8]. IFN-γ regulates hundreds of genes. Also, a group of transcription factors dependent on IFN and support signaling can control gene expression differently. However, these transcription factors may decrease viral gene expression and proliferation by affecting viral promoters[9]. Many miRNAs are induced by increasing IFN-γ in different situations[10]. For instance, the expression of miR-29a/b is enhanced after being stimulated by interferon gamma and STAT-1 protein activation. miR-29a/b upregulation leads to the targeting of CDK6 molecules, resulting in enhanced production of antiviral genes and reduced viral immunity[10,11]. After solid organ transplantation, complications such as organ rejection are common in transplant patients. Acute graft rejection is formed following a strong immune system response by T cells, macrophages, and antibodies[11]. As the main mediators of immune responses to transplants, T cells can cause acute rejection through various cytokines[12]. Moreover, immune factors and non-immune agents, like the toxic effects of immunosuppressive drugs and infections, also play a role in transplant rejection[13]. Many factors can cause graft rejection and graft loss after KTx. These danger agents can be categorized into donor-connected, recipient-connected, donor-recipient adaptability, and peri- and post-operative factors[14]. There is no data on the impact of IFN-γ on the expression of BK polyomavirus and related miRNAs in kidney transplant patients with nephropathy compared to those without nephropathy in the Iranian population. Our work addresses significant BKVN issues relating to kidney transplant recipients. By focusing on molecular and cellular BKV recurrence factors, this study could lead to new ways to diagnose and treat the disease. Meanwhile, BKVN is still the main reason for graft defeat; information about its causes improves the treatment consequences. Our study aims to guide more personalized and successful treatment selections, thereby reducing viral complications and improving kidney transplant outcomes.

Results

This work aimed to examine the IFN-γ gene, miR-29a, miR-B1-3p and 5p differential expression in KTRs. BKV infection in patients with and those without, as well as those with and without a history of organ rejection after transplant, were compared with respect to these expressions. In two groups, we subsequently analyzed the levels of expression for the IFN-γ gene, miR-29a, miR-B1-3p and 5p. Table 1 summarizes the clinical and demographic data of the KTRs.

First group:
A- KT patients are currently infected with the BKV (BK+) were the subjects of this study.
B- Nephropathy tissue associated with the BKV (Tissue).
C- Blood and urine samples of KT patients without active BKV infection (BK-).
D- Blood and urine samples of people who did not undergo transplantation (Control).
Second group:
A- Blood and urine samples from KT patients with transplant rejection history (Reject).
B- Blood and urine samples from KT patients without transplant rejection history (Non reject).

Group	Control	BK+	Reject	Non reject BK-	Tissue
Number	20	10	10	10	10
Sex	Male = 3 Female = 17	Male = 8 Female = 2	Male = 5 Female = 5	Male = 6 Female = 4	Male = 8 Female = 2
Age (average years)	31.2 ± 6.1	49.2 ± 10.9	40.1 ± 14.1	49.1 ± 15.0	39 ± 14.1
Group of blood	O + = 6 A + = 6 AB + = 2 B + = 4 B- = 2	O + = 3 A + = 2 B + = 2 B- =1 O- =2	O + = 2 A + = 1 A-=3 B + = 2 B- =1 O- =1	A + = 2 B- =5 O + = 3	O + = 3 A + = 2 AB + = 2 B + = 1 B- = 1 O- = 1
BUN (ml/dl)		26.5	34.9	36	34.6
Cr (ml/dl)		3.05	3.2	3.8	2.2
Viral load (copies/ml)		9000			
Hypertension			10%	20%	
Other disease			10%	40%	50%
Types of rejection			ABMR = 6 TCMR = 3 ABMR and TCMR = 1		

Table 1. Demographic and clinical information in the of KTRs BK+, BK-, reject, Non-reject, tissue, and control group. ABMR: antibody mediated rejection; TCMR: T cell mediated rejection.

C- Blood and urine samples of people who did not undergo transplantation (Control).

The demographic data of the healthy control and BK + and BK – and reject, non-reject, and tissue participants are summarized.

Demographic information and clinical characteristics: KTRs and health in blood and urine subjects are summarized.

Figure 1 shows the overall study design diagram and results during this investigation.

Analysis of IFN-γ and MiRNA expression (miR-29a, miR-B1-3p, miR-B1-5p) in blood, urine, and tissue samples from BK + and BK- patients and control subject

In the first group, we examined the expression data of the IFN-γ gene, miR-29a, miR-B1-3p, and miR-B1-5p in blood, urine, and tissue samples from BK + patients, BK- patients, and control subjects. Gene expression levels of IFN-γ were compared to GAPDH, and miR-29a levels were compared to miR-U6 within these groups. The results showed that IFN-γ in KT patients (BK+) was decreased in comparison with BK virus-related nephropathy tissue (Tissue) in urine and blood. In KTR (BK+) in comparison with KTR (BK-), there was a decrease in blood and urine. In KTR (BK+), there was a decrease in blood and urine in comparison with people who did not undergo transplantation (Control). Nephropathy tissue related to the BKV (Tissue) had a significance decrease in urine and blood ($p = 0.0350$) in comparison with people who did not undergo transplantation (Control). Nephropathy related to the BK virus (Tissue) decreased in urine and blood in comparison with KTR (BK-). KTR (BK-) had a decrease in blood and urine in comparison with people who did not undergo transplantation (Control). Statistical analyses showed that KTR (BK+) had a significance increase in urine and blood ($p = 0.0089$) in comparison with BKVN (Tissue). KTR (BK+) had a significance increase in blood ($p < 0.0001$) and urine ($p = 0.0089$) in comparison with KTR (BK-). KTR (BK+) showed significantly increase in blood ($p < 0.0001$) and urine ($p < 0.0001$) in comparison with people who did not undergo transplantation (Control). Nephropathy tissue related to the BK virus (Tissue) had a significant increase in the blood ($p < 0.0001$) and urine ($p < 0.0001$) in comparison with people who did not undergo transplantation (Control). Nephropathy tissue associated with BK virus increased significantly in urine and blood ($p = 0.0002$) in comparison with KTR (BK-). KTR (BK-) showed significant increase in blood and urine ($p = 0.0216$) in comparison with people who did not undergo transplantation (control), as shown in Fig. 2.

miR-B1-3p and miR-B1-5p in comparison with miR-U6 in blood, urine, and tissue of BK + and BK- groups

Our highly relevant study, which focused on comparing the expression levels of miR-B1-5p and 3p from different biological samples in KTR BK + and BK-, was conducted with meticulous attention to detail. Specifically, we compared these blood, urine, and tissue levels of BK + and BK- groups. Our analysis used miR-U6 as the reference point due to its stable expression across different conditions, allowing for more accurate sample comparisons. The statistical analysis showed that KTR (BK+) significantly increased urine and blood in comparison with nephropathy tissue related to the BK virus (Tissue). KTR (BK+) in comparison with KTR (BK-) in urine increased, and there was a significant increase in blood. KTR (BK+) significantly increased blood and urine in comparison with people who did not undergo transplantation (Control). Nephropathy tissue related to the BK virus and KTR (BK-) had a significant increase in blood and urine. BKVN tissue significantly increased blood and urine in comparison with people who did not undergo transplantation (Control). Statistical analysis showed that KTR (BK+) significantly increased in blood and urine in comparison with BKVN tissue. BKVN tissue significantly increased in comparison with KTR (BK-) in the blood and urine. BKVN tissue significantly increased in blood in comparison with people who did not undergo transplantation (Control), as shown in Fig. 3.

miR-29a and IFN-γ correlation in blood, urine, and tissue of BK + and BK- groups

It was found that miR-29a in comparison with IFN-γ in blood of KTR (BK+) increased significantly ($p < 0.0001$). This finding suggests that the blood may be a more reliable source for detecting miR-29a and IFN-γ changes in KTR (BK^+). However, there was no significant correlation between miR-29a and IFN-γ. miR-29a in comparison

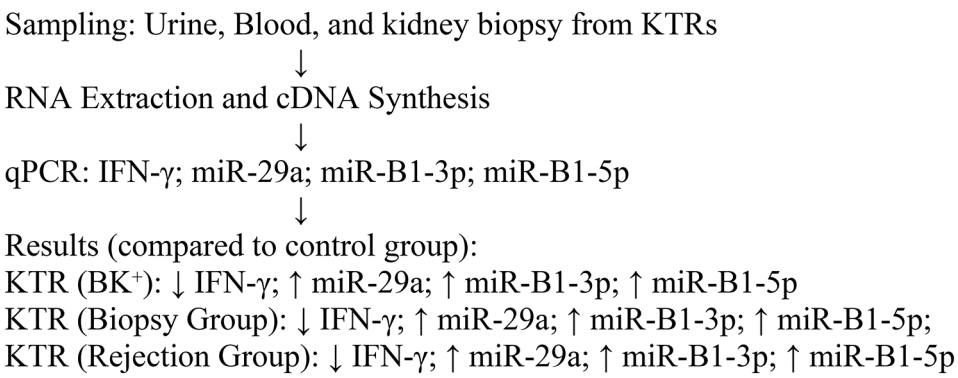

Fig. 1. Study design diagram.

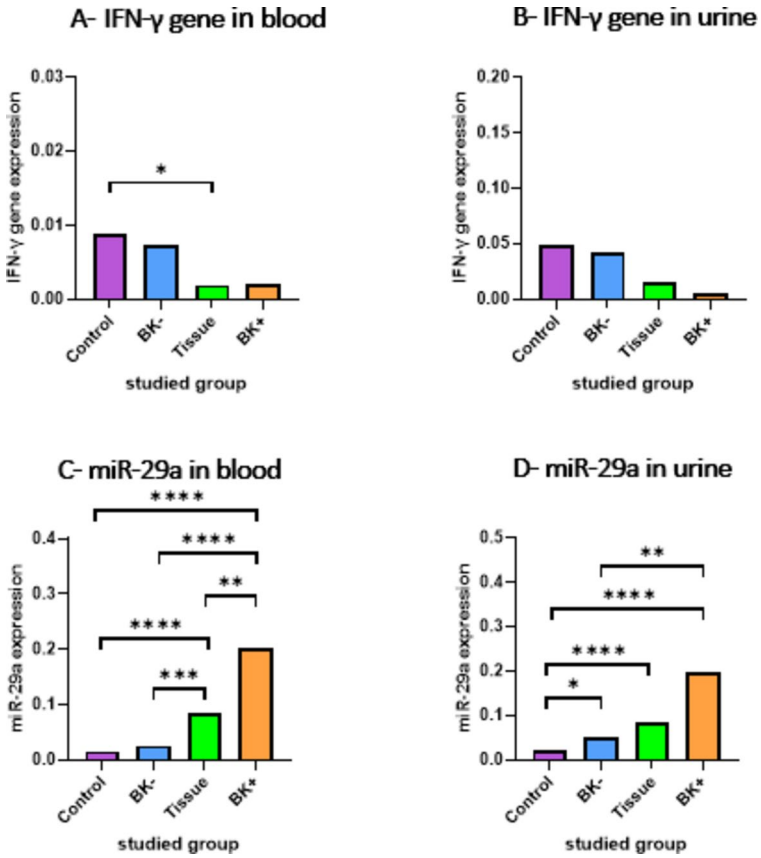

Fig. 2. Evaluation of IFN-γ and miR-29a in KTR (BK+), BKVN tissue, KTR (BK-), people who did not undergo transplantation (Control) (**A**) IFN-γ expression level in blood (**B**) IFN-γ expression level in urine (**C**) miR-29a in blood (**D**) miR-29a in urine.

with IFN-γ in the urine of KTR (BK+) increased significantly ($p = 0.0027$). This finding suggests that urine may also be a viable source for detecting these biomarkers. But we didn't find significant association between IFN-γ and miR-29a. miR-29a exhibited a notable rise relative to IFN-γ in the blood samples from KTR patients without BK infection, reaching statistical significance ($p = 0.0288$). Furthermore, a significant correlation was detected for miR-29a and IFN-γ in these samples ($p = 0.0490$).

Although, miR-29a was elevated in comparison with those of IFN-γ in the urine of KTR patients without BK infection, this increase did not correlate significantly with IFN-γ levels. Furthermore, in tissues affected by the BK virus, there was a substantial increase in miR-29a levels relative to IFN-γ ($p < 0.0001$); however, the correlation between these two markers was not significant. These findings are illustrated in Fig. 4.

IFN-γ and miR-B1-3p in BK + and BK- groups (blood, urine, and tissue)

The miR-B1-3p was higher in comparison with IFN-γ in the KTR (BK+) blood ($p = 0.0072$). miR-B1-3p in comparison with IFN-γ in KTR(BK+) urine increased significantly ($p = 0.0007$). However, the correlation between these two markers was not significant indicating a potential area for further investigation. In comparison with IFN-γ, miR-B1-3p in the KTR (BK-) blood increased significantly ($p = 0.0288$). However, the correlation between these two markers was not significant highlighting the need for more research. In comparison with IFN-γ, miR-B1-3p in the KTR (BK-) urine increased significantly ($p = 0.0021$). However, the correlation between these two markers was not significant, further emphasizing the need for additional studies. In comparison with IFN-γ, miR-B1-3p in BK virus-related tissue increased significantly ($p < 0.0001$). However, the correlation between these two markers was not significant, suggesting a different mechanism of action in tissue in comparison with blood and urine, as shown in Fig. 5.

miR-B1-5p and IFN-γ correlation in blood, urine, and tissue of BK + and BK- groups

Our comparison of IFN-γ and miR-B1-5p in BK + and BK- groups has revealed intriguing patterns and areas that require further investigation. We found that miR-B1-5p was significantly higher in comparison with IFN-γ in the KTR (BK+) blood ($p < 000.1$). Furthermore, there was a significant correlation ($p < 0.0001$) between miR-B1-5p and IFN-γ in the urine of KTR (BK+), adding to the complexity of our findings. However, there was no significant relation between miR-B1-5p and IFN-γ in the blood. The miR-B1-5p was decreased in comparison with IFN-γ in the blood and urine of KTR (BK-). This unexpected result raises interesting questions. However, these patients had no significant relation between miR-B1-5p and IFN-γ. The miR-B1-5p in comparison with

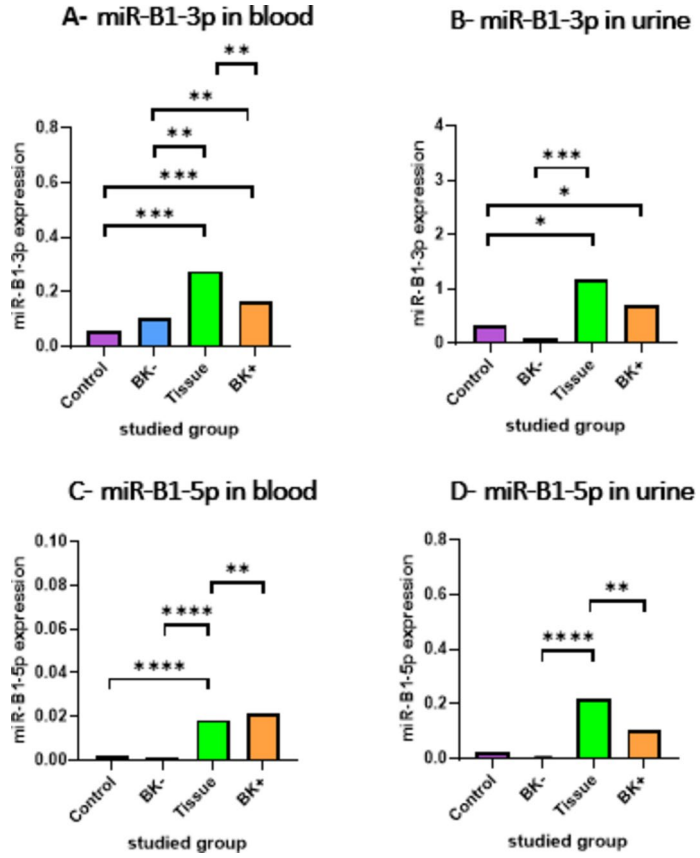

Fig. 3. miR-B1-5p and 3p evaluation of KTR (BK+), BKVN tissue, KTR (BK-), and people who did not undergo transplantation (Control) (**A**) miR-B1-3p expression level in blood (**B**) miR-B1-3p expression level in urine (**C**) miR-B1-5p expression level in blood (**D**) mi-B1-5p expression level in urine.

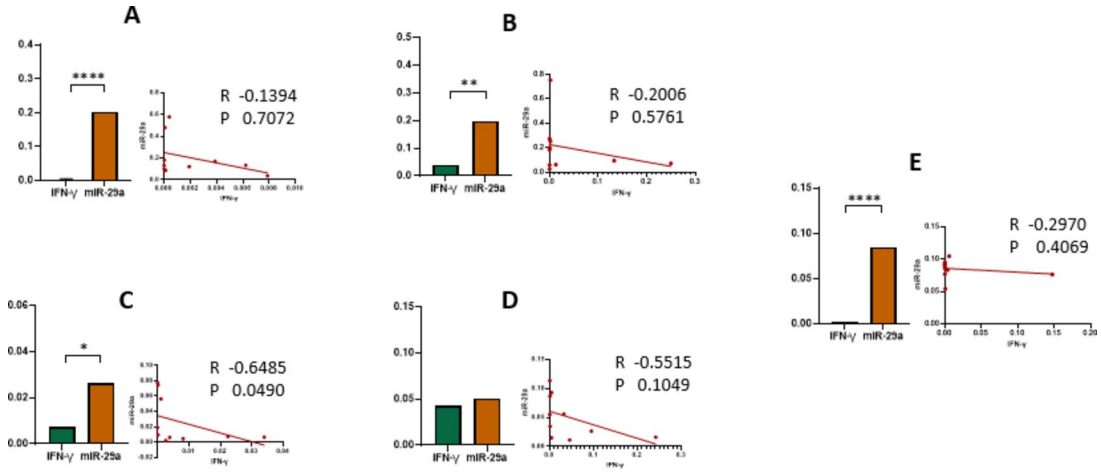

Fig. 4. Correlation of IFN-γ and miR-29a. (**A**) Blood of KTR (BK+). (**B**) In the urine of KTR (BK+). (**C**) Blood of KTR (BK). (**D**) Urine of KTR (BK-). (**E**) BKVN tissue.

IFN-γ in BKVN tissue increased significantly ($p < 0.0001$), but no significant relation was observed between them. These findings underscore the need for further research to fully understand the complex interplay between these genes in different contexts. It is given in Fig. 6.

No significant correlation was observed between BUN levels and IFN-γ gene expression in any of the investigated groups. In kidney transplant recipients (KTR) with BK+ ($p = 0.0431$), a significant positive correlation was observed between BUN levels and miR-29a expression. In other groups, there were no significant correlations observed between BUN and miR-29a expression. No significant correlation was observed between miR-B1-3p

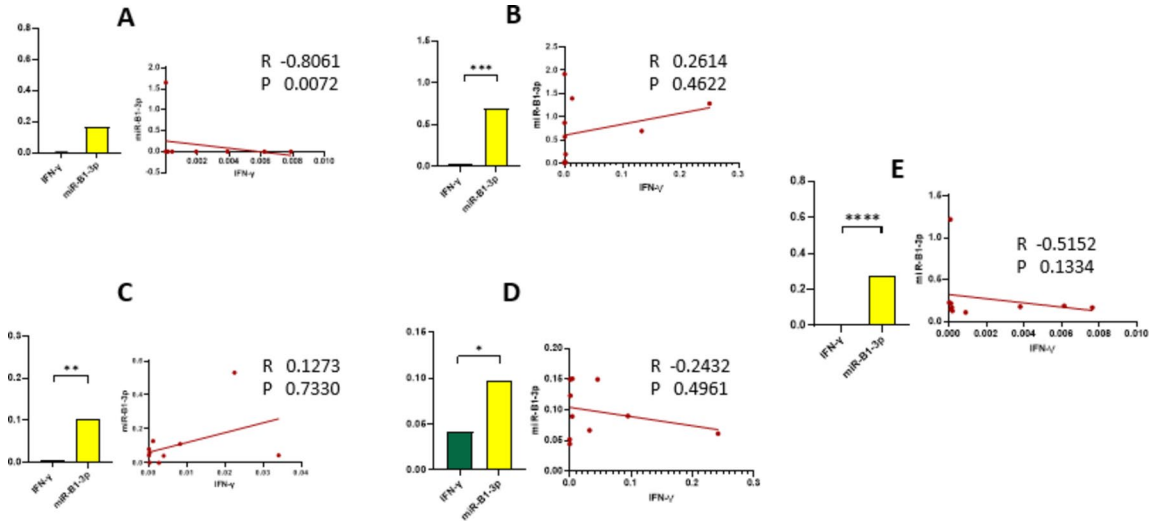

Fig. 5. Correlation IFN-γ and miR-B1-3p. (**A**) Blood of KTR (BK+). (**B**) Urine of KTR (BK+). (**C**) Blood of KTR (BK-). (**D**) Urine of KTR (BK-). (**E**) BKVN tissue.

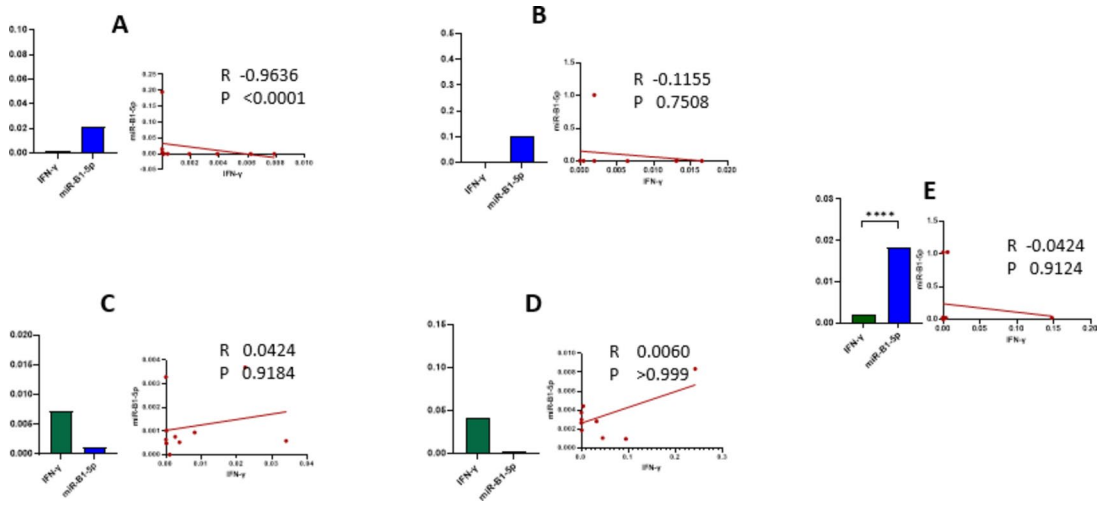

Fig. 6. IFN-γ and miR-B1-5p correlation. (**A**) Blood of KTR (BK+). (**B**) In the urine of KTR (BK+). (**C**) In the blood of KTR (BK-). (**D**) In the urine of KTR (BK-). (**E**) Nephropathy tissue associated with BK virus (Tissue).

expression and BUN levels in any of the investigated groups. BUN levels did not show a significant correlation with miR-B1-5p expression in any of the groups. There was no significant association between creatinine levels and IFN-γ gene expression in either the BK + or BK-groups. There was no significant association between creatinine levels and miR-29a expression in either the BK + or BK-groups. In KTRs with BK- ($p = 0.0347$), a clear negative correlation was observed between miR-B1-3p expression and creatinine levels. No significant associations were observed in other groups. No significant correlation was observed between creatinine levels and miR-B1-5p expression in any of the investigated groups (Supplementary Figures S1-S8).

IFN-γ gene expression characteristic curve analysis in blood, urine, and tissue of BK + and BK- groups

The sensitivity and specificity for the IFN-γ gene in KTR (BK+) and KTR (BK-) in blood at the cutoff point of 0.0080 are 30 and 100, respectively ($p = 0.2899$). Similarly, the sensitivity and specificity for the IFN-γ gene in KTR (BK+) and KTR (BK-) in urine at the cutoff point of 5.772 are 100 and 40, respectively ($p = 0.2568$). These findings, along with the sensitivity and specificity of the IFN-γ gene in nephropathy tissue related to BK virus (Tissue) and KTR(BK-) in the blood at the cutoff point of 0.0009 (60 and 70, respectively, $p = 0.4497$), and in nephropathy tissue associated with BK virus (Tissue) and KTR (BK-) in urine at the cutoff point of 0.0002 (90 and 60, respectively, $p = 0.0495$), highlight the significant interferon gamma act for BKV infection detection in KTR. These changes can help inform medical professionals about the sensitivity and specificity of the IFN-γ gene in different samples for detecting BK virus infection (Fig. 7).

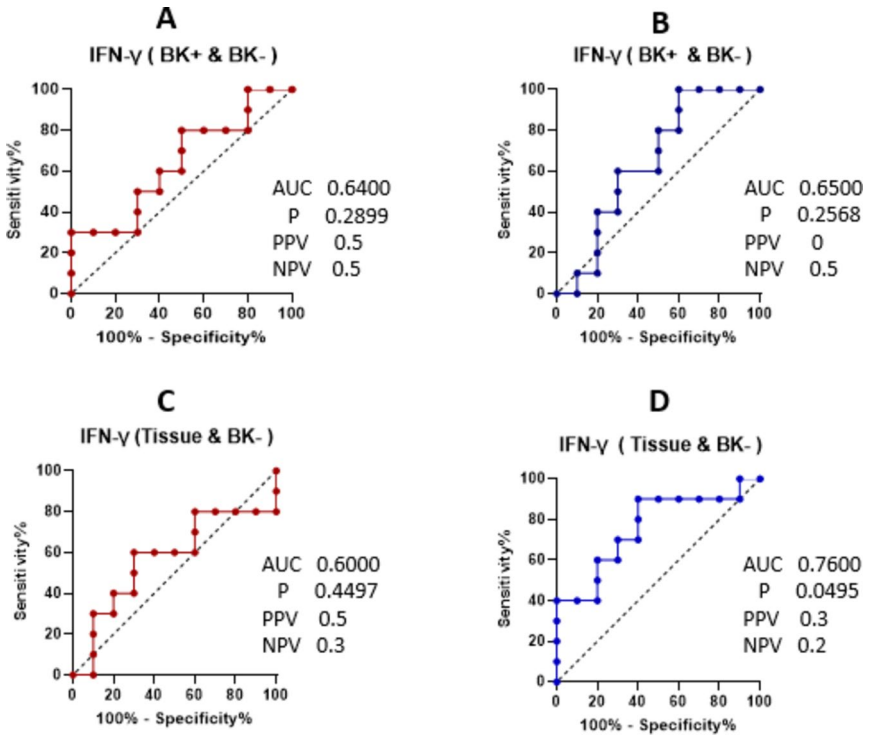

Fig. 7. ROC curve of IFN-γ gene expression. (**A**) KTR (BK+) and KTR (BK-) in blood. (**B**) KTR (BK+) and KTR (BK-) in urine. (**C**) BKVN tissue and KTR (BK-) in the blood. (**D**) BKVN tissue and KTR (BK-) in the urine.

miR-29a expression characteristic curve analysis in blood, urine, and tissue of BK + and BK- groups

The sensitivity and specificity for miR-29a in KTR (BK+) and KTR (BK-) in the blood at the cutoff point of 0.0826, which is the threshold for determining the presence of the virus, are 100 and 90, respectively ($p = 0.0004$). The sensitivity and specificity for miR-29a in KTR (BK+) and KTR (BK-) in the urine at the cutoff point of 0.0577, which is the threshold for urine-based detection, are 70 and 90, respectively ($p = 0.0102$). The sensitivity and specificity for miR-29a in BK virus-related nephropathy tissue and KTR (BK-) in blood at the cutoff point of 0.0753, the threshold for tissue-based detection, are 90 and 90, respectively ($p = 0.0007$). The sensitivity and specificity for miR-29a in BK virus-related nephropathy tissue and KTR (BK-) in the urine at the cutoff point of 0.0661, which is the threshold for urine-based detection in tissue are 70 and 90, respectively ($P = 0.0539$) (Fig. 8).

miR-B1-3p expression characteristic curve analysis in blood, urine, and tissue of BK + and BK- groups

The sensitivity and specificity for miR-B1-3p in KTR (BK+) and KTR (BK-) in the blood at the cutoff point of 0.0005 are 100 and 100, respectively ($p = 0.0002$). The sensitivity and specificity for miR-B1-3p in KTR (BK+) and KTR (BK-) in urine at the cutoff point of 0.1728 are 100 and 70, respectively ($p = 0.1306$). The sensitivity and specificity for miR-B1-3p in nephropathy tissue associated with BK virus (Tissue) and KTR (BK-) in the blood at the cutoff point of 0.1396 are 90 and 80, respectively ($p = 0.0046$). The sensitivity and specificity for miR-B1-3p in BK virus-related nephropathy tissue and KTR (BK-) in urine at the cutoff point of 0.1596 are 100 and 70, respectively ($P = 0.0013$) (Fig. 9).

miR-B1-5p expression characteristic curve analysis in blood, urine, and tissue of BK + and BK- groups

The sensitivity and specificity for miR-B1-5p in KTR (BK+) and KTR (BK-) in the blood at the precise cutoff point of 0.0003 are 90 and 70, respectively ($p = 0.1988$). The sensitivity and specificity for miR-B1-5p in KTR (BK+) and KTR (BK-) in urine at the precise cutoff point of 0.0048 are 90 and 40, respectively ($p = 0.8798$). The sensitivity and specificity for miR-B1-5p in nephropathy tissue associated with BK virus (Tissue) and KTR (BK-) in the blood at the precise cutoff point of 0.0048 are 100 and 100, respectively ($p = 0.0002$). The sensitivity and specificity for miR-B1-5p in nephropathy tissue associated with BK virus (Tissue) and KTR (BK-) in urine at the precise cutoff point of 0.0052 are 90 and 100, respectively ($P = 0.0003$) (Fig. 10).

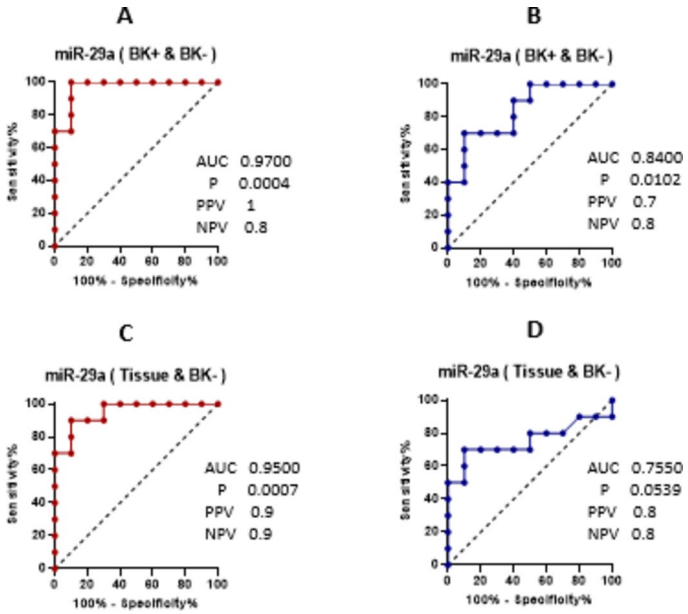

Fig. 8. ROC curve miR-29a expression. (**A**) KTR (BK+) and KTR (BK-) in blood. (**B**) KTR (BK+) and KTR (BK-) in urine. (**C**) BKVN tissue and KTR (BK-) in the blood. (**D**) BKVN tissue and KTR (BK-) in the urine.

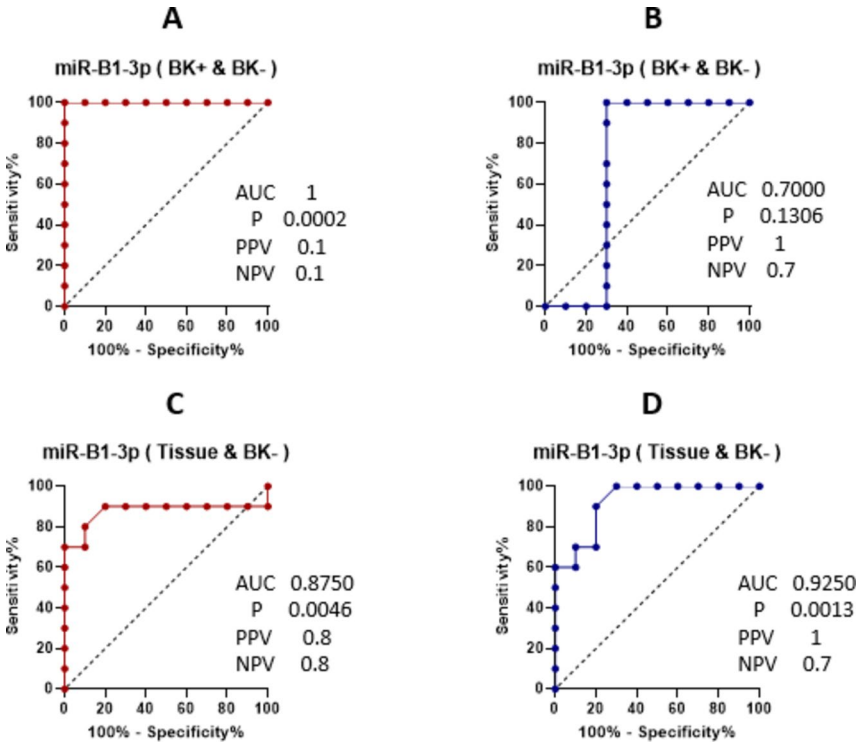

Fig. 9. ROC curve miR-B1-3p expression (**A**) KTR (BK+) and KTR (BK-) in blood. (**B**) KTR (BK+) and KTR (BK-) in urine. (**C**) BKVN tissue and KTR (BK-) in the blood. (**D**) BKVN tissue and KTR (BK-) in the urine.

Investigating the correlation among IFN-γ gene, miR-29a, miR-B1-3p, miR-B1-5p: blood and urine, tissue from BK + and BK- groups

It was found that there was no significant relationship between IFN-γ, miR-B29a, miR-B1-3p and mir-B1-5p in blood and tissue of BK + and BK- groups, but there was a significant relationship between miR-29a and miR-B1-5p in urine (Fig. 11).

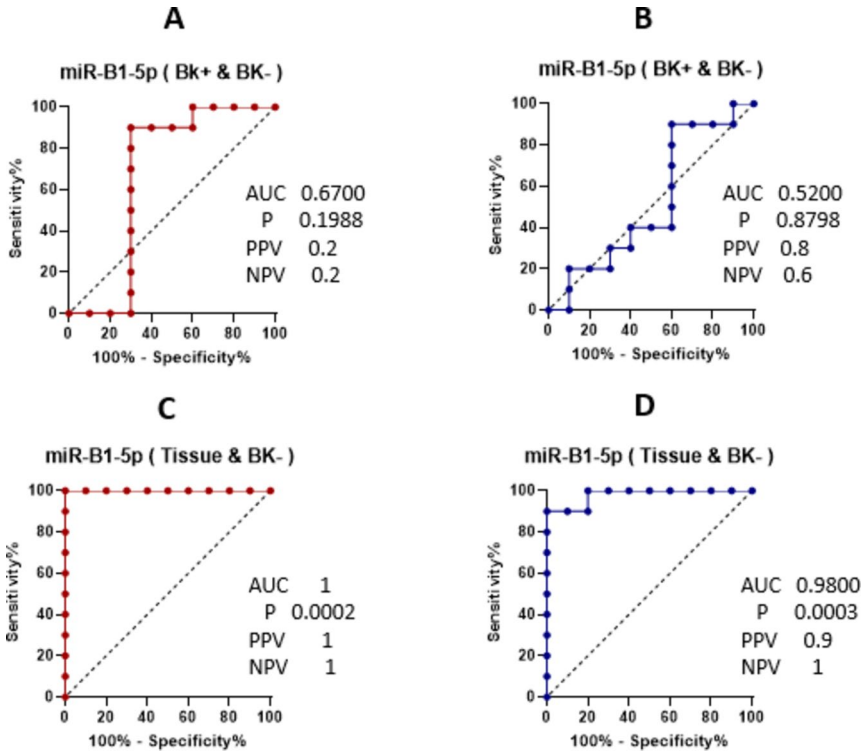

Fig. 10. ROC curve miR-B1-5p expression (**A**) KTR (BK+) and KTR (BK-) in blood. (**B**) KTR (BK+) and KTR (BK-) in urine. (**C**) BKVN tissue and KTR (BK-) in the blood. (**D**) BKVN tissue and KTR (BK-) in the urine.

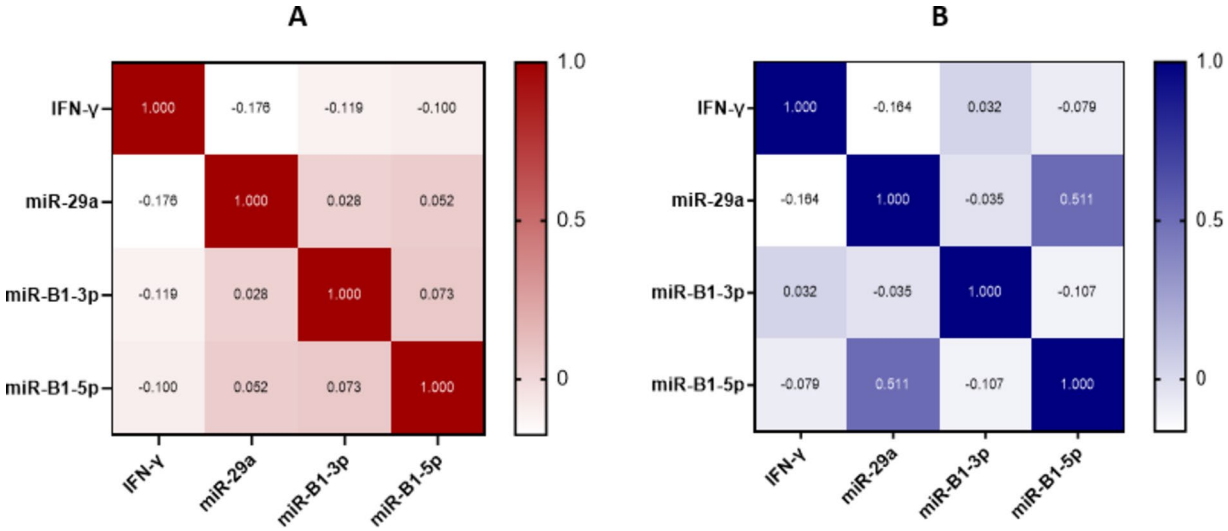

Fig. 11. Heatmap (**A**) For IFN-γ gene and miR-29a, miR-B1-5p and miR-B1-3p from tissue and blood in BK + and BK- groups. The lower correlation is shown in white and the higher correlation is shown in dark red. (**B**) For IFN-γ gene and miR-29a, miR-B1-3p and miR-B1-5p expressed from urine and tissue in BK + and BK- groups. The lower correlation is shown in white and the higher correlation is shown in dark blue. The dark areas show the spontaneous correlation between our study gene and miRNA and the coefficient range is from 0 to + 1.

Analysis of IFN-γ, miR-29a, miR-B1-3p, and miR-B1-5p expression in rejection, Non-Rejection, and control subjects

At this stage, we analyzed the IFN-γ, miR-29a, miR-B1-3p and miR-B1-5p from Reject, Non-reject, control subjects as second group. Our research is particularly relevant as we compare the IFN-γ gene expression level to the GAPDH, miR-29a, and miR-U6 in the blood and urine of the Reject and Non-reject groups, providing crucial

insights into the gene expression patterns in KTR. The data showed that IFN-γ in KTR (reject) in comparison with KTR (non-reject) decreased in blood and significantly decreased in urine ($P<0.0001$). A significant decreasing was found from urine ($p=0.000$) and blood ($p<0.0001$) of KTR (reject) in comparison with people who did not undergo transplants (Control). In KTR (non-reject), it also decreased in comparison with people who did not undergo transplant (control). The expression of miR-29a in KTR (Reject) in comparison with KTR (Non-Reject) increased in urine and significantly increased in blood ($p=0.0288$). A significant increasing from urine ($P=0.0053$) the blood ($p<0.0001$) in KTR (Reject) in comparison with people who did not undergo transplants (Control). KTR (non-reject), there was a significant increase in blood and urine in comparison with people who did not undergo transplant (control) ($p=0350$). It is given in Fig. 12. Our research is particularly relevant as we compare the IFN-γ gene expression level to the GAPDH gene and the miR-29a expression level to miR-U6 in the blood and urine of the Reject and Non-reject groups, providing crucial insights into the gene expression patterns in KTR. Statistical analysis revealed IFN-γ gene expression in KTR (reject) in comparison with KTR (non-reject) decreased in blood and urine ($p<0.0001$). The results showed a significant decreasing from blood ($P<0.0001$) also urine (0.000) of KTR (reject) in comparison with people who did not undergo transplants (Control). In KTR (non-reject), it also decreased in comparison with people who did not undergo transplant (control). The expression of miR-29a in KTR (Reject) in comparison with KTR (Non-Reject) increased in urine and significantly increased in blood ($P=0.0288$). Our results showed a significant increasing of urine ($P=0.0053$) also blood ($P<0.0001$) from KTR (Reject) in comparison with people who did not undergo transplants (Control). KTR (non-reject), there was a significant increase in blood and urine in comparison with people who did not undergo transplant (control) ($p=0350$) (Fig. 12).

miR-B1-3p, miR-B1-5p compared in miR-U6 from blood, urine between reject and Non-reject groups

The data indicated that miR-B1-3p in KTR (Reject) in comparison with KTR (Non reject) had an increase in blood with significant increasing of urine ($P=0.0337$). KTR (Reject) in comparison with people who did not undergo transplant surgery (Control), showed increasing in the urine and blood ($P<0.0001$). In KTR (Non-reject), in comparison with people who did not undergo transplant surgery (control), our data revealed increasing in the urine and blood ($P=0060$). The miR-B1-5p in KTR (Reject) increased in blood and urine in comparison with those without transplant rejection (Non reject). KTR (Reject) had a significant increase in blood ($p=0125$) and urine ($p=0038$) in comparison with people who did not undergo transplantation (Control).

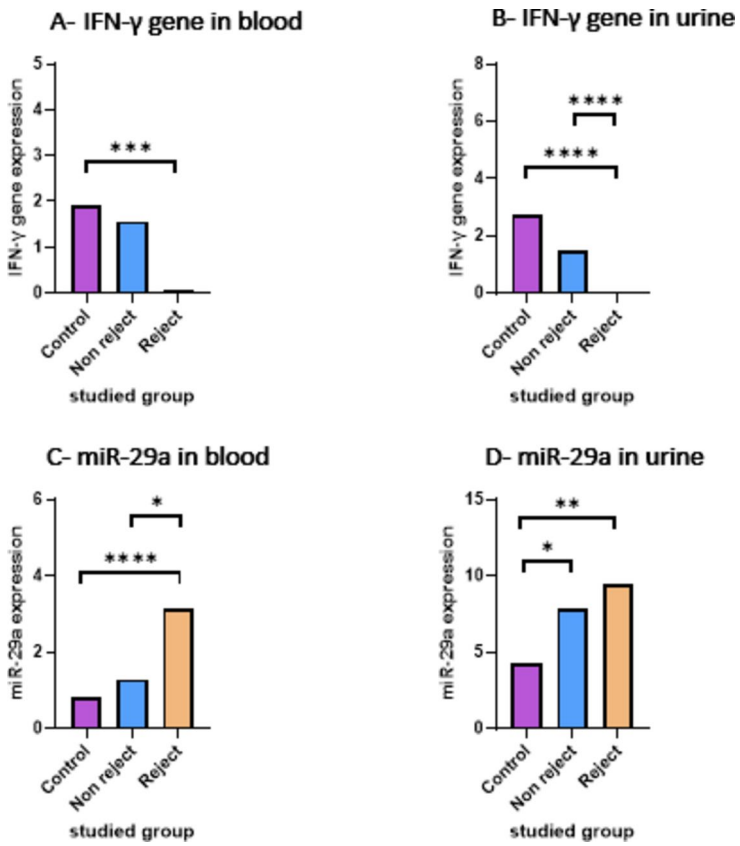

Fig. 12. Evaluation of IFN-γ gene and miR-29a expression level in KTR (reject), KTR (non-reject), people who did not undergo transplantation (Control) (**A**) IFN-γ expression level in blood (**B**) IFN-γ expression level in urine (**C**) miR-29a in blood (**D**) miR-29a in urine.

In KTR (Non-reject), there was an increase in blood and urine in comparison with people who did not undergo transplantation (Control). This is shown in Fig. 13.

IFN-γ, MiR 29a association from blood, urine of reject and Non-reject groups

The comparison of miR-B29a and IFN-γ from the blood and urine of the two Reject and Non-reject groups was done according to their expression changes and the correlation between them. This information is essential in the field of transplantation and immunology. It was found that in the blood of KTR (Reject), the miR-29a gene expression increased significantly in comparison with the level of IFN-γ gene expression ($P < 0.0001$). But no significant association was found between miR-29a and IFN-γ. miR-29a increased in the urine of KTR (Reject) ($P < 0.0001$) comparison in the IFN-γ without any significant correlation. In KTR (Reject), the miR-29a gene expression increased in comparison with IFN-γ with a significant relation. In the urine of KTR (Non reject), miR-29a expression increased significantly ($p = 0.0007$) in comparison with IFN-γ gene expression without any significant correlation (Fig. 14).

miR-B1-3p and IFN-γ correlation from blood, urine in reject and Non-reject groups

The comparison of miR-B1-3p and IFN-γ from urine and blood (Reject) and (Non-reject) groups revealed intriguing findings that warrant further investigation. In the blood of KTR (Reject), miR-B1-3p increased significantly to compare IFN-γ ($P = 0.0029$) without significant association, indicating the need for more studies to see the performance of these genes in rejection. In the urine of KTR (Reject), miR-B1-3p expression increased significantly ($p < 0.0001$) in comparison with IFN-γ gene expression without correlation significantly. This correlation suggests a potential biomarker for rejection in urine; more research is needed to confirm this. In KTR (non-rejection), the miR-B1-3p was decreased in comparison with IFN-γ without any significant relation. Urine from KTR (non-reject), miR-B1-3p showed increasing compared to with IFN-γ ($P = 0.0341$) without significant association, indicating the need for further research to understand these findings' implications fully (Fig. 15).

Correlation of miR-B1-5p and IFN-γ from urine and blood in reject and Non-reject groups

In the blood of KTR (Reject), miR-B1-5p was increased to compare to IFN-γ ($P = 0.0029$) without association significantly. miR-B1-5p increased ($P < 0.0001$) in comparison with IFN-γ from KTR (Reject) urine without correlation significantly. In the blood of KTR (non-reject), the miR-B1-5p decreased in comparison with the level of IFN-γ gene expression without correlation significantly. From KTR (Non-reject) urine, miR-B1-5p expression was decreased in comparison with IFN-γ gene expression without significant correlation (Fig. 16).

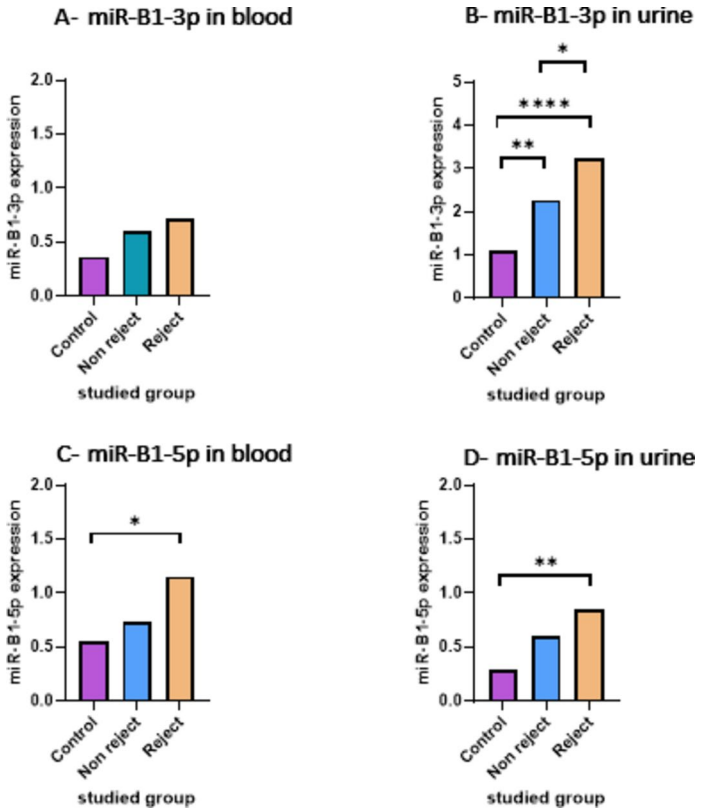

Fig. 13. mir-B1-5p and miR-B1-3p evaluation from KTR (reject), KTR (non-reject), people who did not undergo transplantation (Control) (**A**) miR-B1-3p expression level in blood (**B**) miR-B1-3p expression level in urine (**C**) miR-B1-5p expression level in blood (**D**) miR-B1-5p expression level in urine.

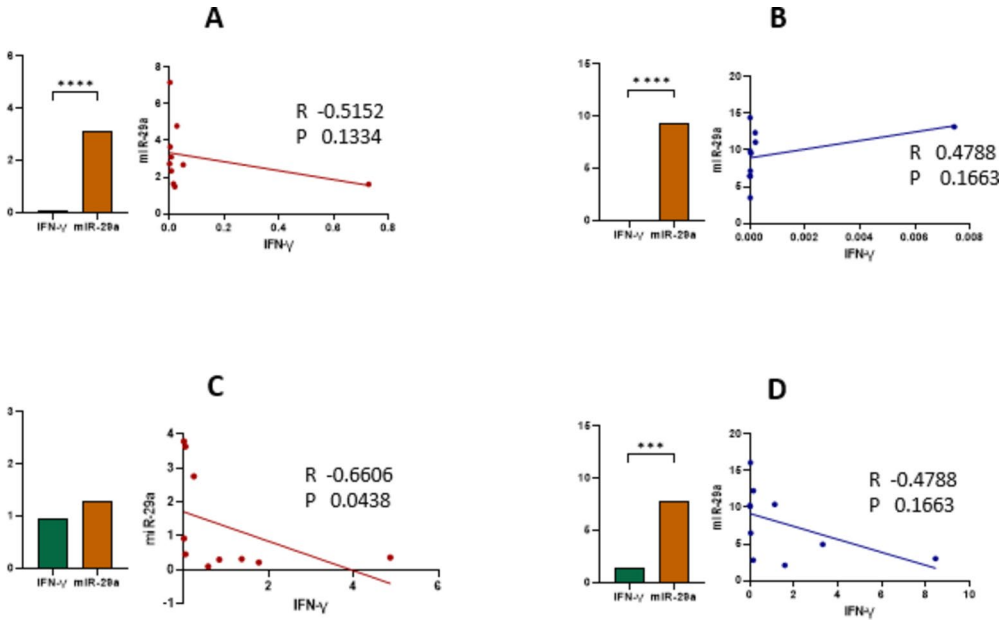

Fig. 14. miR-29a and IFN-γ correlation. (**A**) Blood of KTR (Reject). (**B**) Urine of KTR (Reject). (**C**) Blood of KTR (non-reject). (**D**) Urine of KTR (Non reject).

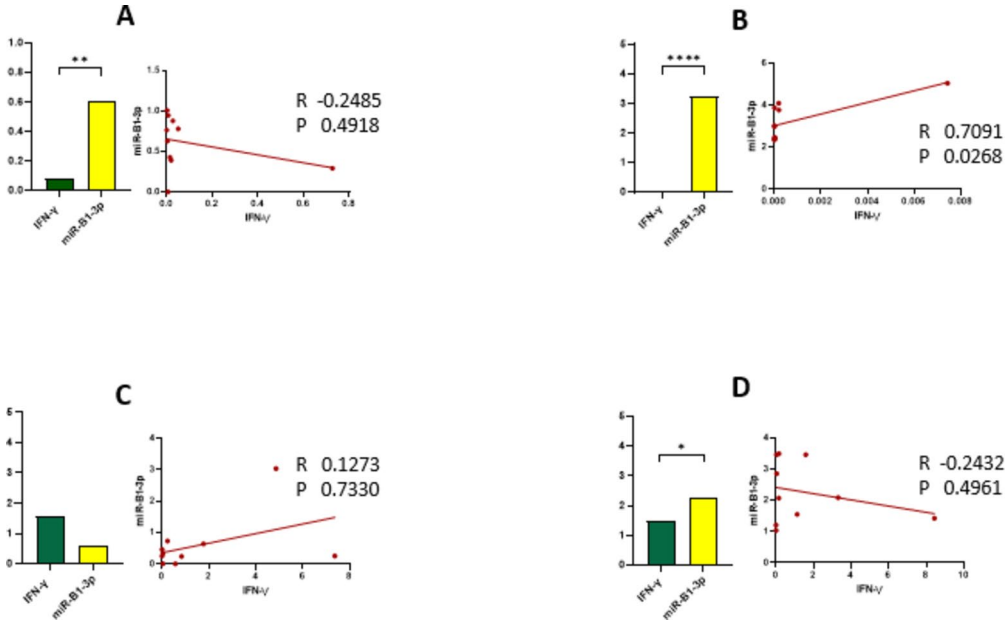

Fig. 15. Evaluation of correlation of IFN-γ and miR-B1-3p. (**A**) In the blood of KTR (Reject). (**B**) In the urine of KTR (Reject). (**C**) In the blood of KTR (non-reject). (**D**) In the urine of KTR (Non reject).

The study observed no significant correlation between BUN and IFN-γ expression in blood and urine samples from both the Reject and Non-reject groups. In all groups, there was no significant relationship between BUN levels and miR-29a expression. There was no significant correlation between BUN levels and miR-B1-3p expression in both the Reject and Non-reject groups. Similarly, no significant correlation was observed between BUN and miR-B1-5p expression in any of the groups. Our findings indicated no significant correlation between IFN-γ gene expression in blood or urine of either group and creatinine (Cr) levels. Furthermore, there was no significant correlation between Cr and miR-29a expression in either the Reject or Non-reject groups. Interestingly, while no significant correlation was observed in the other groups, in kidney transplant recipients of the non-reject group, an increase in creatinine levels was associated with a significant decrease in miR-B1-3p expression in blood ($p = 0.0348$) It was found with no significant relationship exists between Cr and miR-B1-5p in any of the groups (Supplementary Figures S9-S16).

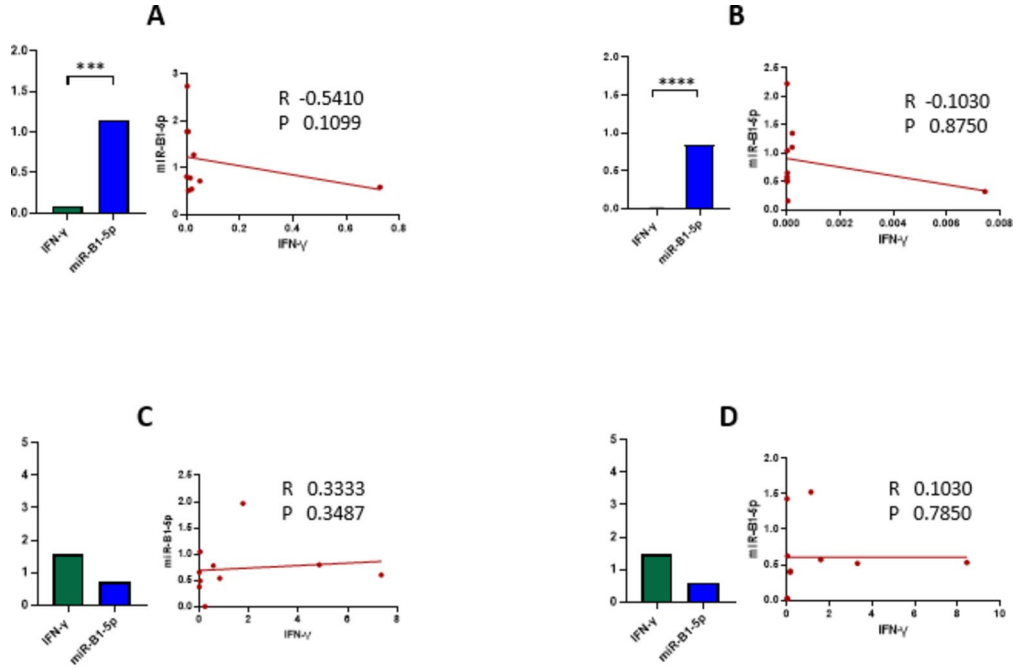

Fig. 16. Evaluation of correlation of IFN-γ and miR-B1-5p. (**A**) In the blood of KTR (Reject). (**B**) In the urine of KTR (Reject). (**C**) In the blood of KTR (non-reject). (**D**) In the urine of KTR (non-reject).

IFN-γ gene and miR-29a expression characteristic curve analysis in blood, urine of reject and Non-reject groups

The sensitivity and specificity for IFN-γ in KTR's blood were obtained at the cut point of 0.0302, they are 80 and 80, respectively ($p = 0.0494$). Similarly, the sensitivity and specificity for IFN-γ in KTR's urine were also derived with great accuracy: at the cut point of 0.0079, they are 100 and 90, respectively ($p = 0.0002$). For miR-29a at the cut point of 1/209, the sensitivity and specificity for KTR (Reject) and KTR (Non-reject) in the blood are 100 and 70, respectively ($p = 0.0284$). Similarly, in the urine of KTR, the sensitivity and specificity at the cut point of 5.69 are 90 and 40, respectively ($p = 0.3847$). The robustness of these results is further demonstrated by the analysis shown in Fig. 17.

miR-B1-3p and miR-B1-5p expression characteristic curve analysis in blood and urine of reject and Non-reject groups

miR-B1-3p sensitivity and specificity from blood of KTR (Reject) and KTR (Non reject) at the cut point of 0.7476 is 50 and 90, respectively ($p = 0.1509$). Sensitivity and specificity for miR-B1-3p in the urine of KTR (Reject) and KTR (Non reject) at the cut point of 2.221 are 100 and 60, respectively ($p = 0.0343$). The statistical data reveals that miR-B1-5p sensitivity and specificity of the blood from KTR (Reject) and KTR (Non reject) are not significantly different at the cutoff point of 0.6892 (70 and 60, respectively, $p = 0.1620$). Similarly, miR-B1-5p sensitivity and specificity from urine recipients, at the cut point of 0.5765, also show no significant difference (60 and 70, respectively, $p = 0.3847$). The robustness of these results is further demonstrated by the analysis shown in Fig. 18.

IFN-γ, miR-29a, miR-B1-3p, miR-B1-5p correlation in reject and Non-reject blood and urine groups

The results obtained through a rigorous methodology indicated a significant relationship between miR-29a and miR-B1-5p in blood. Our results demonstrate that there is no significant relationship between IFN-γ gene expression and three microRNAs in urine. The results are shown in Fig. 19.

Discussion

At the present study, the impact of gamma interferon on BKV candidate microRNAs and related miRNAs in kidney transplant patients with BKV infection was investigated. A kidney transplant is suggested when kidney function is impaired for any reason, and this disorder is not reversible[15]. Chronic and acute are two types of kidney failure. In chronic kidney failure, kidney function decreases over time, which is irreversible. ESRD occurs when chronic renal failure reaches its peak and kidney function ceases[16].The most effective common method to improve ESRD kidney transplant patients[17]. An often-encountered complication after solid organ transplantation is organ rejection. Acute graft rejection is formed following a strong immune system response by T cells, macrophages, and antibodies[11]. Chronic graft rejection occurs following repeated uncontrolled acute graft rejection or slow progression of tissue inflammation. On the other hand, immunosuppressive drugs and infections, also play a role in transplant rejection[13]. For durability and survival of the transplanted kidney, the

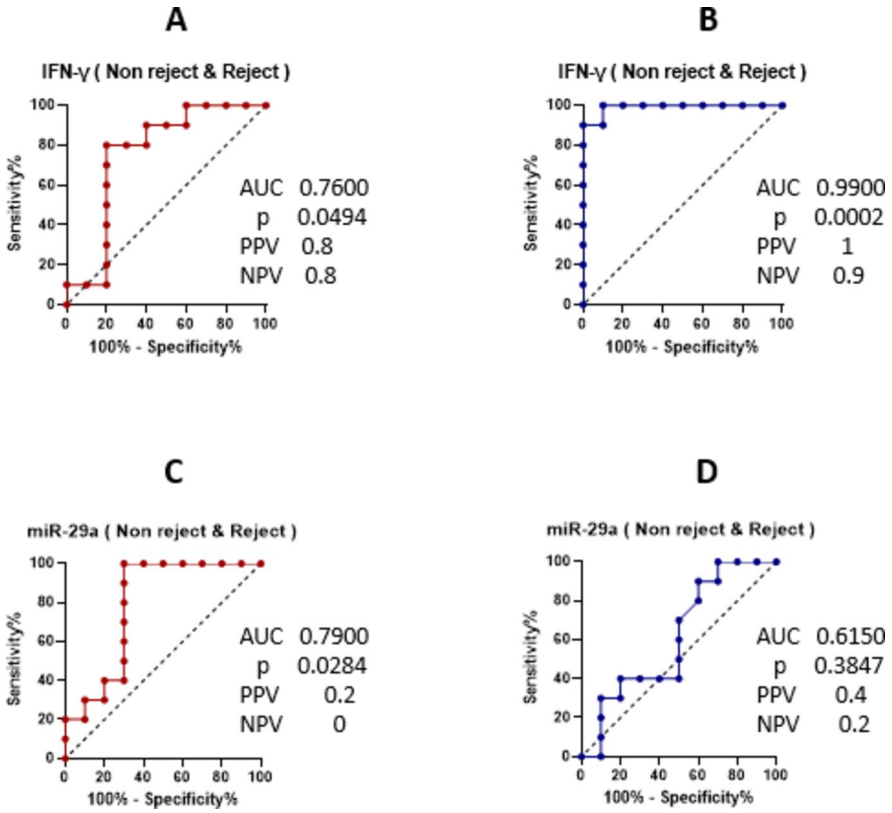

Fig. 17. ROC curve of IFN-γ and miR-29a. (**A**) IFN-γ in blood of KTR (Reject) and KTR (non-reject). (**B**) IFN-γ in the urine of KTR (Reject) and KTR (non-reject). (**C**) miR-29a in blood of KTR (Reject) and KTR (non-reject). (**D**) miR-29a in the urine of KTR (Reject) and KTR (non-reject).

application of immunosuppressive medicine is necessary. However, the use of these drugs makes the patient susceptible to infections and increases the risk of malignancy[18]. Infectious agents, including bacteria, viruses, and fungi, can endanger kidney transplantation[19]. Polyomavirus BK is one of the most prominent viral infections in KTR. This virus is very common in KTR. and if it is not correctly diagnosed at the right time, it will lead to nephropathy[20]. Cytokines, as regulatory molecules of the immune system, have a significant impact on immunological responses and inflammation and can regulate the immune response towards transplant rejection or acceptance[21]. The important function of IFN-γ is in identifying and removing infectious agents and inhibiting the replication of viruses, which is possible by regulating and stimulating the host's immune system. IFN-γ via activation of Jak-Stat directly and indirectly, leads to MHC-I and II expression increasing antigenic molecules and triggers antiviral states in infected cells[22]. Interferon gamma can inhibit BKV genes expression at the transcription and translation level. Interferon-gamma inhibits the expression of viral LTAg and VP1 genes, thereby inhibiting BK polyomavirus duplication and preventing the production of new viral particles[23]. We in this research observed IFN-γ in the urine and blood of control subjects. IFN-γ can force BK polyomavirus to enter the latent phase. The findings indicate that BK polyomavirus replication continues in healthy people despite a certain amount of IFN-γ gene expression and virus particles can also be observed in the urine of healthy people, but the reduction of IFN-γ expression in people with system defects. Immunity can cause the virus to reactivate and eventually BK polyomavirus-related diseases. Our results showed that IFN-γ performs significant job in BKV infectivity and reactivation[9]. Studies show that IFN-γ profoundly up-regulates miR-29a in a STAT1-dependent manner[24]. There are several hundreds of conserved miRNAs in the mammalian genome. These miRNAs can affect T cell differentiation and their subsets as well as immune regulation[25]. The IFN-γ and miR-29a gene expression levels in our studied groups have an inverse relationship with each other, and miR-29a can inhibit IFN-γ. In a study conducted on 110 candidate miRNAs to investigate their function in suppressing interferon-gamma production, it was found that miR-29a and b act as the key players in suppressing the IFN-γ production. miR-29a is a potent represses of IFN-γ that inhibits IFN-γ production by indirectly suppressing TBET and EOMES (Transcription factors essential for IFN-γ production). On the other hand, inhibition of miR-29a leads to increased production of IFN-γ and augment resistor to infection. Furthermore, miR-29a is influenced by IFN-γ, which represents a negative response ring[25]. It is also known that immune control depends on T-CD4 + and T-CD8 + cells. In healthy persons and KTRs, BKV-specific CD4 + and CD8 + cells can be identified and detected. T-CD4 + play a significant responsibility for regulating BKV infection due to IFN-γ, TNF, and granzyme B. Immunosuppressants (IS) are given to KTRs to stop transplant rejection. They can prevent the production of interferon gamma and TNF which are made by BKV-specific T cells[26–28]. Acute organ rejection is the main reason of death in KTR and can progress to chronic rejection. As the main mediators of

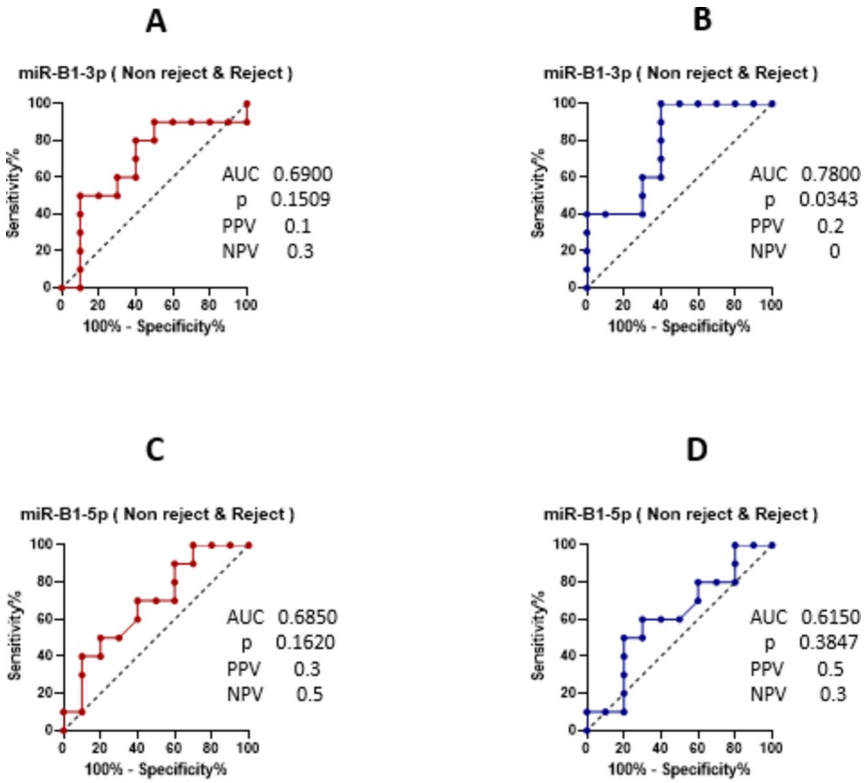

Fig. 18. ROC curve of miR-B1-3p and miR-B1-5p expression (**A**) miR-B1-3p in the blood of KTR (Reject) and KTR (non-reject). (**B**) miR-B1-3p in the urine of KTR (Reject) and KTR (non-reject). (**C**) miR-B1-5p in blood of KTR (Reject) and KTR (Non reject). (**D**) miR-B1-5p in the urine of KTR (Reject) and KTR (Non reject).

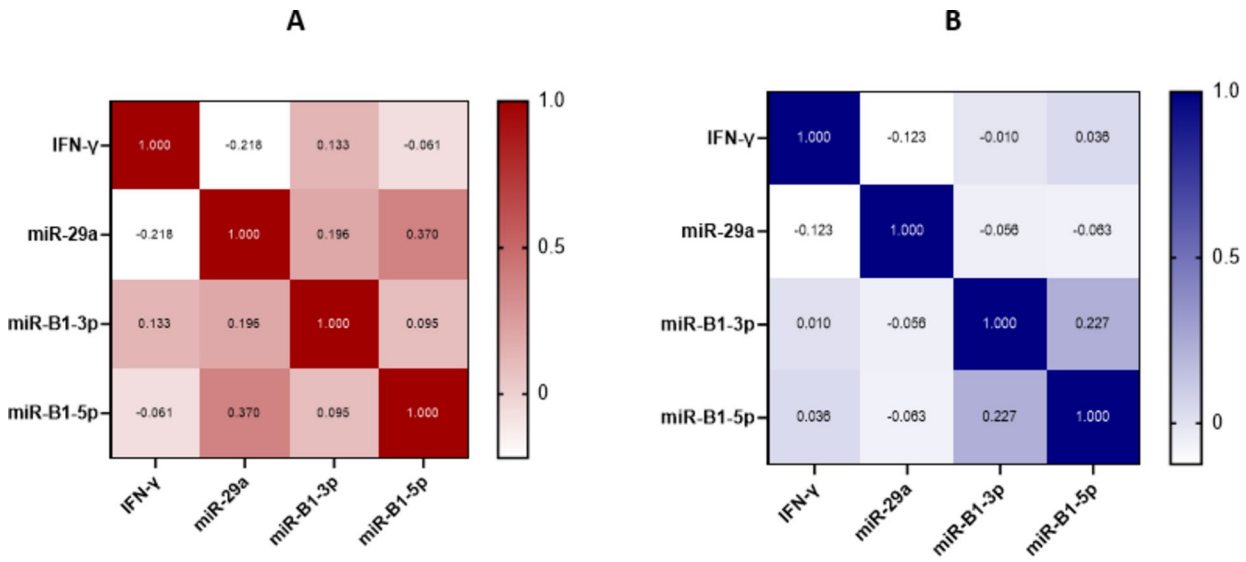

Fig. 19. Heat map. (**A**) for genes and miRNAs expressed in the blood of KTR (Reject) and KTR (Non reject). Correlations with lower intensity are shown in white, and correlations with higher intensity are shown in bold red. (**B**) for the IFN-γ gene expression and the three microRNAs expressed in the urine of KTR (Reject) and KTR (Non reject). Correlations with lower intensity are shown in white color and correlations with higher intensity are shown in bold blue color the dark sections show the spontaneous correlation between our study's gene and miRNA, and the range of coefficient is from 0 to + 1.

immune responses to transplants, T cells can cause acute rejection (ACR) through various cytokines. However, IFN-γ can help generate lymphocyte of T cells and increase half-life of patients with allograft. However, in a study, researchers reported that the serum concentration of IFN-γ and IL-12 p70 in early ACR patients in comparison with the control group was significantly lower. Th1-like Treg cells can secrete IFN-γ and this IFN-γ protect the graft in primary ACR, and these findings show a decrease in IFN-γ production, that can help to reject the graft[12]. T17 increasing in the transplanted kidney is related to chronic rejection. In a study in the tubular epithelial cells of KTRs, the expression of IL-17 with ACR was identified. In other words, types of transplant rejection including chronic rejection, acute rejection, and subacute rejection are related to Th17[29]. This can prove a significant reduction of IFN-γ in patients with rejection's history[30]. BKV encodes miR-B1-5p and miR-B1-3p can cause persistent infection. These miRNAs could be identified in the different part of the body and fluids[31]. BKV miRNAs might be considered as a sign of latent or hidden infection[32]. We witnessed this discrepancy from BKV miRNAs in the blood and urine of the studied groups. In one study, it was found that miRNAs can be produced and released in cells infected with viruses that are inactive and do not replicate, such as herpes simplex virus. This explains why no BKV's DNA is found in the plasma of healthy individuals, but a large number of viral miRNAs is observed in these individuals. Overall, small amount of circulating viral miRNAs is considered biomarkers for latent infection, and higher levels may indicate increased viral activity. Furthermore, due to the same sequence of miR-3p in BKV and JCV, we also observed this miRNA in the urine and blood of individuals who are in good health[3]. Also, we investigated BKV-miR-B1-5p and 3p in KTRs, control group, and BKVN patients. The results showed that there is a significant increase in KTR (BK+), as well as KTR (Reject). Our results are agreed with Li et al. who observed BKV-miR-B1-5p increasing in BKVN and KTR (BK+)[33]. Our limitation study was that we did not specifically screen for EBV and post-transplant follow-up; however, we accept that it could be a potential co-infection in transplant recipients. Despite this, the findings of our study may serve as a valuable strategy to address viral infections and the pathogenesis associated with BK polyomavirus in kidney transplantation.

Materials and methods

Subjects

Ten KTR (reject) complications (50% female and male), ten KTR (BK+) (20% female and 80% male), ten KTR (BK-)(Non reject) (40% female and 60% male), ten BKVN tissue samples (20% female and 80% male), 20 people who were not transplanted as a control group (85% female and 15% male), the study subjects were KTRs hospitalized in the nephrology department of Abu Ali Sina Hospital in Shiraz. These patients were evaluated for the presence or absence of active BKPyV infection using the Taq-man PCR method using the Sina Gene DNA synthesis kit. The Reject patients studied in our research were all found to be BK negative after the Taq-man PCR test. All kidney transplant recipients (KTRs) involved in the study had experienced their first transplantation at least six months previous to the study, and we specifically excluded those who had received a retransplant. The transplantations were verified by medical reports. The KTRs who were included in the study were all adults (20–60 years old) and BKV seropositive, although those with adenovirus, CMV, HIV, HBV, HCV infections were excluded. For immunization data, panel-reactive antibody (PRA) levels and donor-specific antibodies (DSA) to determine the immunological profile of the patients[34], were not accessible in our study. Regarding viral co-infections, we omitted participants with active infections from adenovirus, CMV, HIV, HBV, and HCV. We did not specifically screen for EBV and post-transplant follow-up. Organ rejection was approved via clinical evaluation, comprising histopathological assessment of biopsy samples when accessible, together with raised serum creatinine levels and specific alterations in kidney performance. However, we didn't access to the data. Informed consent was also obtained from all of them in writing. This work was authorized by the University of Zabol's Research Ethic Committee (IR.UOZ.REC.1402.017). This research was determined in Iran according to national norms and standards and ethical principles.

Sampling

After obtaining consent and completing the consent form, 10 ml of blood containing EDTA (Hamburg, Germany), 50 ml of urine, and a 10–15 µm paraffinized section from the biopsy tissue of BK positive patients were taken from all KTR and the healthy control group. The serum and buffy coat from each blood sample were separated using a Ficoll (Nycomed, Nederland) gradient. Urinary cells were separated by centrifugation.

Isolation of peripheral blood and plasma mononuclear cells using Ficoll

EDTA tubes containing blood were centrifuged (Hettich Universal, Germany) (3000 RPM, 5 min).

The samples were spun in a centrifuge at 12,000 rpm for 30 min. The liquid portion was then moved to an additional microtube. The next step was to dissolve the silt in 1 cc of PBS and then to store it at -80.

Deparaffinization of the tissue

A 10–15 µm section is placed in a sterile test tube, and 1 ml of xylene is added, vortexed, shaken, and centrifuged at 13,500 rpm for 15 min. Then, xylene and vortex were added and centrifuged three times. In two steps, 1 ml of 100% ethanol is added, centrifuged for 15 min, and the supernatant solution is removed. 30 ml of acetone is added. After deparaffinization, 10 ml of proteinase K enzyme buffer are added, and the test tube is placed at 37 °C overnight.

cDNA synthesis and extraction of RNA from Buffy coat and urinary cells and tissue cells

750 µl of Trizol (Asa gene, Iran) solution was added to 250 µl of buffy coat samples, urinary cells, and tissue cells, and incubated for 5 min at room temperature. 200 µl of cold chloroform (Merck, Germany) was added. The sample was centrifuged for 20 min at 13,500 rpm. 1000 µl of cold 100% ethanol was added to the upper layer.

Gene and miRNA	Primer sequence (5′→3′)	Condition	Product size (bp)
GAPDH	F - GGACTCATGACCACAGTCCA R - CCAGTAGAGGCAGGGATGAT	95℃ for 10 min 40 Cycles: 95℃ for 15 s, 57℃ for 20 s, 72℃ for 30 s Melt curve: 95℃ for 15 s, 57℃ for 1 min, 95℃ for 15 s	119 bp
IFN-γ	F - CAGCTCTGCATCGTTTTGGG R - TCCGCTACATCTGAATGACCTG		110 bp
hsa-miR-29a	TAGCACCATCTGAAATCGGTTA	95℃ for 2 min 40 Cycles: 95℃ for 15 s, 62℃ for 35 s Melt curve: 95℃ for 15 s, 62℃ for 1 min, 95℃ for 15 s	70 bp
hsa-miR-U6	CTCGCTTCGGCAGCACA		70 bp
BKV-miR-B1-5p	ATCTGAGACTTGGGAAGAGC		75 bp
BKV-miR-B1-3p	TGCTTGATCCATGTCCAGAGTC		75 bp

Table 2. Experimental conditions.

It was kept at -20 for one night. Then, the sample was centrifuged at 13,500 rpm for 20 min. 1 ml of cold 75% ethanol (Merck, Germany) was added to the precipitate and Centrifuge for 8 min at 8000 rpm. The final sediment was dissolved in 25 ml of DEPC water (Cinna gen, Iran) and placed in Dri-Block (Techne, England) for 10 min. RNAs' quantity and quality were evaluated with Nanodrop's help (Thermofisher, USA). The concentration and purity were measured at the optical density of 280/260 nm, and their cDNA synthesis was performed according to the kit protocol (Eurox, UK) and PCR Thermocycler (Eppendorf, Germany).

Real time PCR

Syber green real-time PCR (Applied Biosystems, USA) was used to assess the IFN-γ gene, miR-29a, miR-B1-3p, and miR-B1-5p in the isolated RNA from buffy coat, urine cells, and tissue cells. GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) and microRNA-U6 were used as internal control. We used the Livak approach[35] to assess the gene expressions. The experimental conditions are shown in Table 2.

Statistical analysis

Data obtained from the patients' clinical and laboratory information were collected and also statistically analyzed using the relevant tests available in SPSS software. Parametric or non-parametric data were analyzed using relevant statistical tests. The normality of the data was determined using the Kolmogorov-Smirnov test (SPSS 28). Mann-Whitney U test or Student's t test was used to compare continuous variables, while ANOVA was used to compare independent variables[36]. Spearman's two-way correlation analysis was used to determine the relationship between variables. The power analysis produced an outcome approximately 0.24. Present sample size should be defended by providing a justification that captures the limits and environment of the research. Small sample sizes are unavoidable in some biomedical research contexts, for instance, because of ethical considerations or limited access of subjects[37]. The Livak method was used to analyze the rate of change in the expression of the studied genes. In addition, the ΔΔCT (ROC) surface was plotted for significant variables. To examine the significance of the data, 128 receiver operating characteristic curves were used. The statistical significance level method was used based on $p < 0.05$. Graphs were drawn using Prism GraphPad version 9 software (GraphPad Software, Inc., San Diego, USA). Each measurement was based on the average of three separate replicates. Statistical significance was indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Conclusions

Given the critical importance of transplantation of kidney and transplant rejection, understanding the myriad factors contributing to this rejection is crucial. Viral agents, particularly the polyomavirus BK, play a significant role in post-transplant complications such as BK virus-associated nephropathy. Investigating the mechanisms behind these complications is, therefore, of great value. Our study focused on IFN-γ and miR-29a genes in kidney transplant patients. miR-29a is known to inhibit IFN-γ through the TBET and EOMES transcription factors, and we observed a noteworthy increment in miR-29a went with a diminish in IFN-γ. This dysregulation of the IFN-γ/miR-29a pathway could weaken the immune response, making kidney transplant patients more susceptible to viral infections. Additionally, we examined BKV miRNAs essential for virus replication. Our findings revealed miR-B1-5p and miR-B1-5p increased in KTR (BK+) and BKVN, as well as KTR (Reject), in comparison with other groups studied. The results of this study can be used as a strategy to combat viral infections and pathogenesis caused by BK polyomavirus in kidney transplantation.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Received: 28 August 2024; Accepted: 7 March 2025

Published online: 14 March 2025

References

1. Sahragard, I. et al. Impact of BK polyomavirus NCCR variations in post kidney transplant outcomes. *Gene* **913**, 148376. https://doi.org/10.1016/j.gene.2024.148376 (2024).
2. Shen, C. L., Wu, B. S., Lien, T. J., Yang, A. H. & Yang, C. Y. BK polyomavirus nephropathy in kidney transplantation: balancing rejection and infection. *Viruses* **13** (3), 487. https://doi.org/10.3390/v13030487 (2021).
3. Lagatie, O., Van Loy, T., Tritsmans, L. & Stuyver, L. J. Viral MiRNAs in plasma and urine divulge JC polyomavirus infection. *Virol. J.* **11** (1). https://doi.org/10.1186/1743-422X-11-158 (2014).
4. Borriello, M. et al. BK virus infection and BK-Virus-Associated nephropathy in renal transplant recipients. *Genes* **13** (7), 1290. https://doi.org/10.3390/genes13071290 (2022).
5. Bennett, S. M., Broekema, N. M. & Imperiale, M. J. BK polyomavirus: emerging pathogen. *Microbes Infect.* **14** (9), 672–683. https://doi.org/10.1016/j.micinf.2012.02.002 (2012).
6. Delbue, S., Ferrante, P., Provenzano, M. & Polyomavirus BK and prostate cancer: an unworthy scientific effort? *Oncoscience* **1** (4), 296–303. https://doi.org/10.18632/oncoscience.32 (2014).
7. Jorgovanovic, D., Song, M., Wang, L. & Zhang, Y. Roles of IFN-γ in tumor progression and regression: a review. *Biomark. Res.* **8**, 49. https://doi.org/10.1186/s40364-020-00228-x (2020).
8. Vu, D. et al. Interferon-gamma gene polymorphism + 874 A/T is associated with an increased risk of cytomegalovirus infection among Hispanic renal transplant recipients. *Transpl. Infect. Dis.* **16** (5), 724–732. https://doi.org/10.1111/tid.12285 (2014).
9. Abend, J. R., Low, J. A. & Imperiale, M. J. Inhibitory effect of gamma interferon on BK virus gene expression and replication. *J. Virol.* **81** (1), 272–279. https://doi.org/10.1128/JVI.01571-06 (2007).
10. Yaghobi, R., Afshari, A. & Roozbeh, J. Host and viral RNA dysregulation during BK polyomavirus infection in kidney transplant recipients. *WIREs RNA*. **14** (4). https://doi.org/10.1002/wrna.1769 (2023).
11. Cozzi, E., Colpo, A. & De Silvestro, G. The mechanisms of rejection in solid organ transplantation. *Transfus. Apher Sci.* **56** (4), 498–505. https://doi.org/10.1016/j.transci.2017.07.005 (2017).
12. Xu, X. et al. IFN-γ-producing Th1-like regulatory T cells may limit acute cellular renal allograft rejection: Paradoxical post-transplantation effects of IFN-γ. *Immunobiol.* 222(2):280–290 (2017). https://doi.org/10.1016/j.imbio.2016.09.012. PMID: 27665996.
13. Chan, T. C., Ariizumi, T. & Asashima, M. A model system for organ engineering: transplantation of in vitro induced embryonic kidney. *Die Naturwiss.* **86** (5), 224–227. https://doi.org/10.1007/s001140050602 (1999).
14. Oweira, H. et al. Risk factors of rejection in renal transplant recipients: A narrative review. *J. Clin. Med.* **11** (5), 1392. https://doi.org/10.3390/jcm11051392 (2022).
15. Makris, K. & Spanou, L. Acute kidney injury: definition, pathophysiology and clinical phenotypes. *Clin. Biochem. Rev.* **37** (2), 85–98 (2016).
16. Hashiani, A. A. et al. Ten-year graft survival of deceased-donor kidney transplantation: A single-center experience. *Ren. Fail.* **32** (4), 440–447. https://doi.org/10.3109/08860221003650347 (2010).
17. Bond, M. et al. The effectiveness and cost-effectiveness of methods of storing donated kidneys from deceased donors: A systematic review and economic model. *Health Technol. Assess.* **13** (38), 1–156. https://doi.org/10.3310/hta13380 (2009).
18. Nickeleit, V. et al. BK-virus nephropathy in renal transplants - Tubular necrosis, MHC-class II expression and rejection in a puzzling game. *Nephrol. Dial Transpl.* **15** (3), 324–332. https://doi.org/10.1093/ndt/15.3.324 (2000).
19. Sia, I. G. & Paya, C. V. Infectious complications following renal transplantation. *Surg. Clin. North. Am.* **78** (1), 95–112. https://doi.org/10.1016/S0039-6109(05)70637-X (1998).
20. Abbott, K. C. et al. Y. Hospitalizations for bacterial septicemia after renal transplantation in the united States. *Am. J. Nephrol.* **21**(2). https://doi.org/10.1159/000046234
21. Karimi, M. H. et al. Association of IL-6 promoter and IFN-γ gene polymorphisms with acute rejection of liver transplantation. *Mol. Biol. Rep.* 38(7):4437–4443 (2011). (2011). https://doi.org/10.1007/s11033-010-0572-6
22. Larkin, J., Ahmed, C. M., Wilson, T. D. & Johnson, H. M. Regulation of interferon gamma signaling by suppressors of cytokine signaling and regulatory T cells. *Front. Immunol.* 4:1664–3224 (2013). (2013). https://doi.org/10.3389/fimmu.2013.00469
23. Wilson, J. J. et al. Gamma Interferon Controls Mouse Polyomavirus Infection *In Vivo*. *J. Virol.* 85(19) (2011). https://doi.org/10.1128/jvi.00761-11
24. Schmitt, M. J. et al. Interferon-γ-induced activation of signal transducer and activator of transcription 1 (STAT1) up-regulates the tumor suppressing microRNA-29 family in melanoma cells. *Cell. Commun. Signal.* **10** (1), 41. https://doi.org/10.1186/1478-811X-10-41 (2012).
25. Jeker, L. T. & Bluestone, J. A. MicroRNA regulation of T-cell differentiation and function. *Immunol. Rev.* **253**, 65–81. https://doi.org/10.1111/imr.12061 (2013).
26. Zhou, W. et al. Functional characterization of BK virus-specific CD4 + T cells with cytotoxic potential in seropositive adults. *Viral Immunol.* **20**(3), 379–38892007). https://doi.org/10.1089/vim.2007.0030
27. Krueger, M. B. et al. *Vitro* profiling of commonly used Post-transplant immunosuppressants reveals distinct impact on antiviral T-cell immunity towards CMV. *Transpl. Int.* **37**, 12720. https://doi.org/10.3389/ti.2024.12720 (2024).
28. van Aalderen, M. C. et al. I.J.M. Phenotypic and functional characterization of Circulating polyomavirus BK VP1-Specific CD8 + T cells in healthy adults. *J. Virol.* 87. https://doi.org/10.1128/JVI.01540-13
29. Abadja, F., Sarraj, B. & Ansari, M. J. Significance of T helper 17 immunity in transplantation. *Curr. Opin. Organ Transplant.* 17(1):8–14 (2012). (2012). https://doi.org/10.1097/MOT.0b013e32834ef4e4
30. Kwan, T. et al. IL-17 deficiency attenuates allograft injury and prolongs survival in a murine model of fully MHC-mismatched renal allograft transplantation. *Am. J. Transpl.* **15** (6), 1555–1567. https://doi.org/10.1111/ajt.13140 (2015).
31. Pietilä, T., Nummi, M., Auvinen, P., Mannonen, L. & Auvinen, E. Expression of BKV and JCV encoded MicroRNA in human cerebrospinal fluid, plasma and urine. *J. Clin. Virol.* **65**, 1–5. https://doi.org/10.1016/j.jcv.2015.01.019 (2015).
32. Sullivan, C. S. & Ganem, D. MicroRNAs and viral infection. *Mol. Cell.* **20** (1), 3–7. https://doi.org/10.1016/j.molcel.2005.09.012 (2005).
33. Li, J. Y. Z. et al. M. BK virus encoded MicroRNAs are present in blood of renal transplant recipients with BK viral nephropathy. *Am. J. Transpl.* **14** (5), 1183–1190. https://doi.org/10.1111/ajt.12694 (2014).
34. Zielinski, M. et al. Impact of donor and recipient human cytomegalovirus status on kidney transplantation. *Inter Iimmunol.* **29** (12), 541–549. https://doi.org/10.1093/intimm/dxx062 (2017).
35. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods* **25** (4), 402–408. https://doi.org/10.1006/meth.2001.1262 (2001).
36. Foroudi, M. R. et al. The effect of the BK polyomavirus large T antigen on the function and maturity of the CD4 + T cell subsets in kidney transplant recipients. *Transpl. Immunol.* **80**, 101884. https://doi.org/10.1016/j.trim.2023.101884 (2023).
37. Yang, S. & Berdine, G. Small sample size. *Southwest. Respir Crit. Care Chron.* **11** (49), 52–55. https://doi.org/10.12746/swrccc.v11i49.1251 (2023).

Acknowledgements
AcknowledgementThis work was authorized by the University of Zabol's Research Ethic Committee (IR.UOZ.REC.1402.017). This research was determined in Iran according to national norms and standards and ethical principles. The University of Zabol, Iran, and Shiraz Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran is highly acknowledged.

Author contributions
Author's Contributions.M. C.: Investigation, Validation, Methodology, Writing- Original draft; G.M.: Conceptualization, Formal analysis, Validation, Data curation, Methodology, Resources, Writing- Original draft; R.Y.: Conceptualization, Formal analysis, Validation, Data curation, Methodology, Review and editing; A.A.: Formal analysis, Visualization, Validation, Review and editing, J.R.: Formal analysis, Visualization, Validation, Review and editing. All authors read and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-93503-6.

Correspondence and requests for materials should be addressed to G.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.