Urinary Cytokine Response In BK Virus Infection

BK Virus İnfeksiyonunda İdrar Sitokin Yanıtı

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ABSTRACT

Objective: Human BK virus (BKV) is a member of the polyomavirus family. In renal transplant recipients, reactivation of BKV may cause the renal-allograft dysfunction. Primary BKV infection usually occurs asymptomatically during childhood. After primer infection, BKV persists latently especially in the urogenital system. Cellular immunity plays an important role in the pathogenesis of BKV infection. The aim of this study was to determine the urinary cytokine responses in patients with BKV infection and associated levels of urinary cytokines and BK viruria.

Methods: Urine samples of 72 patients with BKV infection were included in this study. BKV DNAs were detected by using quantitative real-time polymerase chain reaction. The levels of cytokines in urine samples were determined by ELISA.

Results: The levels of urinary proinflammatory cytokines in BKV DNA positive patients were significantly higher than those of patients who were BKV DNA negative. In addition, urinary proinflammatory cytokines were also higher in BKV DNA positive patients with high viral load than in patients with low viral load.

Conclusion: According to our results, it is suggested that proinflammatory cytokines may play an important role in the pathogenesis of BKV infections. A better understanding of cytokine responses in BKV infections may help to provide new therapeutic approaches to the treatment of BKV infection especially in transplantation patients.

Key Words: BK Virus, urine, cytokine

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ÖZET

Amaç: İnsan BK virüs (BKV) polyomavirus ailesinin bir üyesidir. Böbrek transplantasyonu yapılmış hastalarda, BKV reaktivasyonu böbrek allograft disfonksiyonuna neden olabilir. Primer BKV infeksiyonu genellikle çocukluk döneminde asemptomatik olarak geçirilir. Primer infeksiyon sonrası, BKV özellikle ürogenital sistemde latent olarak kalır. Hücresel bağışıklık, BKV infeksiyonu patogenezinde önemli role sahiptir. Çalışmamızın amacı, BKV infeksiyonlu hastalarda idrar sitokin yanıtını belirlemek ve idrar sitokin düzeyleri ile BK virüri ilişkisini tespit etmektir.

Yöntemler: BKV infeksiyonlu 72 hastanın idrar örnekleri çalışma kapsamına alındı. BKV DNA'sı kantitatif Gerçek Zamanlı Polimeraz Zincir Reaksiyonu yöntemi kullanılarak belirlendi. İdrar örneklerinde sitokin düzeyleri ELISA yöntemi ile tespit edildi.

Bulgular: BKV DNA'sı pozitif hastalarda idrar proinflamatuvar sitokin düzeyleri, BKV DNA negatif hastalara göre istatistiksel olarak anlamlı düzeylerde daha yüksek olarak tespit edildi. Ayrıca, idrar proinflamatuvar sitokin düzeyleri, yüksek viral yüke sahip BKV DNA'sı pozitif hastalarda düşük viral yüke sahip hastalara göre daha yüksekti.

Sonuç: Çalışmamızın sonuçlarına göre, proinflamatuvar sitokinlerin, BKV infeksiyonu patogenezinde önemli rol oynayabileceği düşünülmüştür. BKV infeksiyonunda sitokin yanıtının tam olarak anlaşılması özellikle transplantasyon hastalarında BKV infeksiyonunun tedavisi için yeni yaklaşımların geliştirilmesine yardımcı olabilecektir.

Anahtar Sözcükler: BK virüs, idrar, sitokin

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INTRODUCTION

Human BK virus (BKV) is widespread human polyomaviruses. In 1971, BKV was isolated from the urine of a renal transplant patient (1). BKV causes ubiquitous infections in early childhood, with seroprevalence in adults ranging from 60% to 100%. Measuring BKV DNA in urine and serum is a useful and noninvasive tool for early detection and monitoring of BKV infections (2).

Primary infections with BKV are usually asymptomatic and may result in transient viruria (3). After primary infection, BKV becomes latent in renal tissues and the peripheral blood (1). The BKV reactivation with urinary shedding of infected urothelial cells occurs in 10% to 60% of renal transplant recipients (2). The reactivation of BKV has been associated with a haemorrhagic cystitis in bone marrow transplanted patients (4). It is known that the BKV reactivation is associated with a failure of cellular immune response in renal transplant patients (5). T cells may also play a negative role in the pathogenesis of Polyoma BK virus-associated nephropathy (6). For all that, the immune response to a BKV infection has not been widely studied.

Cytokines are a diverse group of secreted proteins that act as key communication molecules between virtually all pairs of cells in the body (7). They play a central role in regulating immune and inflammatory responses during infection with pathogens. Activated CD4+ T cells divide Th1 and Th2 cells on the basis of their cytokine secretion (8).

The aim of this study was to determine the urinary cytokine responses in patients with BKV infection and evaluate the relationship between urinary cytokines and urinary BK viral load in these patients.

METHODS

Patients

We assessed BKV-DNA viral load in urine samples of 72 patients. Fourty-eight urine samples of healthy individuals were used as a control group.

BKV DNA

The urine samples were tested to evaluate the BKV DNA. Urine BKV DNA load was measured by using the quantitative real-time polymerase chain reaction (real-time PCR) (Qiagen, Hamburg, Germany).

BKV DNA extraction

Viral DNAs were extracted using the MagAttract Virus Mini M48 kit (Qiagen, Hamburg, Germany) on the BioRobot M48 workstation (Qiagen, Hamburg, Germany) following the manufacturer's instructions.

Real-time quantative PCR: BKV DNA load was measured by quantitative real-time PCR. Quantification of viral DNA load in urine samples was performed using the Qiagen Artus BKV RG PCR kit (Qiagen, Hamburg). Amplification cycling was performed using the Rotor-Gene 6000 device (Corbett research Australia). Data analysis was performed with the Rotor-Gene software according to the manufacturer's instructions.

Cytokine measurement

Urine interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-23 (IL-23), interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), transforming growth factor (TGF-β) levels were determined by commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manifacturer's instructions (Biosource, California, USA).

Statistical methods

The results were analysed by using a one-way analysis of variance (ANOVA). The Bonferroni test was used as a Post Hoc analysis. $p<0.05$ was considered to be significant.

RESULTS

The levels of urinary proinflammatory cytokines such as IL-2, IL-23 and IFN-γ in BKV-DNA positive patients were significantly higher than those of healthy individuals who were BKV-DNA negative ($p<0.05$). On the other hand, BK viruria did not change the levels of IL-6, TNF-α as compared to the BKV DNA negative group ($p>0.05$).

In addition, urinary proinflammatory cytokines were also higher in the BKV DNA positive patients with a high viral load than in patients with a low viral load ($p<0.05$) (Figure 1, 2,3). In BKV DNA positive group, the levels of antiinflammatory cytokines such as IL-10, TGF-β did not differ significantly from those of BKV DNA negative group ($p>0.05$).

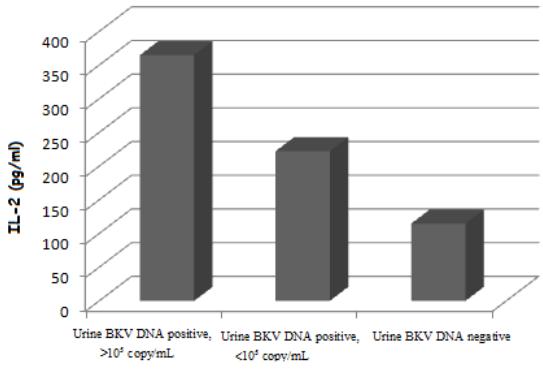

Figure 1. Urine IL-2 levels of BKV DNA positive and negative urine samples.

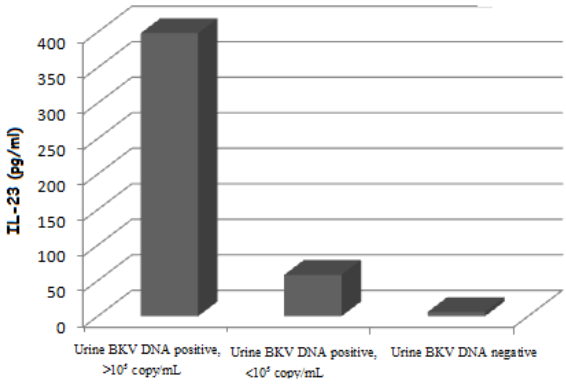

Figure 2. Urine IL-23 levels of BKV DNA positive and negative urine samples.

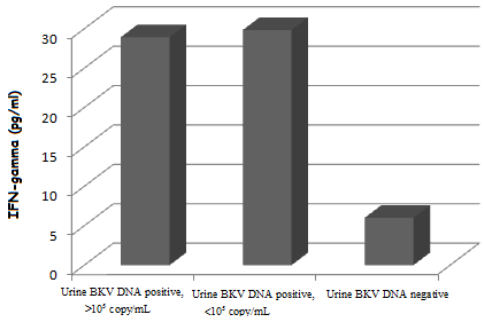

Figure 3. Urine IFN-γ levels of BKV DNA positive and negative urine samples.

DISCUSSION

Recently, several studies have shown PCR to be an effective method for detecting BKV in various clinical samples. So, PCR is now emerging as the "gold standard" for detection of polyomavirus (3). For this reason, in our study we determined the BKV infection in patients by using PCR methods.

BKV remains in a latent stage throughout life and can reactivate under immunosuppressive conditions (9). In our study, we investigated the immunological changes such as cytokines secretion in the urine of patients with BKV infection. Cellular immune response has been shown to play an important role in the pathogenesis of BKV infection (10).

Th1 effector cells produce largely proinflammatory cytokines. Th1 cytokines primarily activate cellular immune responses, whereas Th2 tip cytokines primarily stimulate humoral immune responses. However, while it is generally accepted that a critical balance of both Th1 and Th2 cytokines is necessary to develop protective immunity, IL-2, IFN-γ, IL-23 are members of a family of proinflammatory cytokines (11). In our study, urine proinflammatory cytokines were increased in patients who were BKV DNA positive. We did not observe any changes in the levels of Th2 type cytokines such as IL-10 and TGF-β between the groups. Our data indicated that patients with significant BK viruria show signs of proinflammatory responses with the induction of IL-2, IL-23, and IFN-γ, which results in increased urinary levels of these cytokines.

So we suggest that proinflammatory cytokines might be involved in the pathogenesis of BKV infection. Sadeghi et al. also informed that BK-positive renal transplant recipients, especially those with high viral load, showed strong inflammatory cytokine responses (2).

CONCLUSION

Our results suggest that proinflammatory cytokines may play an important role in BKV infections. A better understanding of cytokine responses in BKV infection may aid in the development of strategies for immune therapy of polyomavirus infections that attempt to limit the inflammation to activate an effective immune response. In addition, the monitoring of urine cytokines could be helpfull for the diagnosis of BKV infections and the polyoma BK virus-associated nephropathy.

Conflict of Interest

No conflict of interest was declared by the authors.

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