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Assessment of *CXCL* 10 Gene Expression as Potential Biomarkers in Patient with Renal Failure Associated with BK Polyomavirus

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Abstract: BK polyomavirus, sometimes known as BKV infection, is a common and typically non-pathogenic virus that affects 80–90% of adults globally. BK virus (BKV) infection has become a significant side effect of immunosuppression. Chemokines are 8–12 kDa tiny chemotactic cytokines that form the chemokine fold by sharing four cysteines. A pro-inflammatory cytokine CXCL10 encourages immune cells to migrate and become activated at the sites of infection. The main objective of this research was to assess the expression levels of CXCL10 mRNA in urine and plasma in patient with renal failure detected as bk virus DNA positive by real-time as contrasted with controls who are healthy.

Both the polyomavirus BKinfected renal failure patient and the healthy control group were the subjects of this cross-sectional study. Using an in-house comparative real-time PCR, the mRNA levels of CXCL10 were assessed in the patient and control samples under study. The group of positive BKvirus DNA 16 cases were 8 males with mean age ±SD (41.0 ±15.58) and female 55.36 ± 18.65 vs. Control group 20 cases ,10 male mean age ±SD (38.1±10.6) and 10 female (47.8±10.6). The mRNA expression levels of CXCL10 in polyomavirus BK infected with renal failure patient increased compared with healthy controls. The $2^{-\Delta\Delta CT}$ gene fold level of CXCL10$2^{-\Delta\Delta CT}$of polyomavirus BK infected with renal failure patient with median 7.87 ranged (3.39 to 15.9) and $2^{-\Delta\Delta CT}$ of control with median 1.2 ranged (0.05 to 11.72). The p = 0.00 statistically significant. the mean rank 27.5 for patient and 11.3 for control. This upregulation was significant in polyomavirus BK infected vs. controls. These findings suggest that polyomavirus BK can cause renal problems by inducing inflammatory indicators such as chemokine. Further completed investigations with longer follow-up are required to confirm that individuals with polyomavirus BK infection who have renal failure also have increased expression and production of CXCL10, a pro-inflammatory chemokine.

Key words: Polyomavirus BK, renal failure, gene expression, CXCL10

1.Introduction

The as big protein family that makes up the chemokines can be further subdivided based on structural features. (1). Around 50 human genes encode chemokine ligands, and there are around 20 matching human chemokine receptor genes. The chemokine system evolved with vertebrates. (2). Chemokines can be classified generally by analyzing the structure of the two cysteine residues that are closest to the N terminus. These two residues might be adjacent (CC), separated by one amino acid (CXC), or separated by three amino acids (CX3C). XC chemokines are defined as chemokines that do not include the initial cysteine residue.(3). Interferon-γ-inducible protein 10 (IP-10), also known as chemokine C-X-C ligand 10 (CXCL10), typically binds to CXCR3 for performing biological functions.(4). CXCR3, which is expressed by T helper lymphocytes, natural killer (NK) cells, dendritic cells (DCs), macrophages, and B cells, gets bound by the chemokine CXCL10. (5). A pleiotropic molecule, CXCL10 has the ability to perform a wide range of powerful biological activities, such as stimulating CXCR3+ cells' chemotactic activity, triggering apoptosis, controlling cell division and growth, and boosting angiogenesis in cancer and other inflammatory and infectious illnesses.(6). BK polyomavirus (BKV) is essentially ubiquitous in all human populations worldwide. Asymptomatic infection with this virus occurs during early childhood, leading to life-long persistence in the kidney(7). BK polyomavirus is a non-enveloped, circular double-stranded DNA virus that may exceed 80% seroprevalence in adults(8). The cellular histones H2A, H2B, H3, and H4 are surrounded by the circular, double-stranded DNA molecule that makes up the BKPyV genome, which is roughly 5000 base pairs long and forms an average minichromosome. Each genome has roughly 20 nucleosomes.(9). The virus can reactivate in immunocompromised hosts, although it seldom affects kidney function—that is, unless renal grafts are involved, in which case it induces an inflammatory response in the tubulointerstitial space that resembles acute rejection. The mainstay of treatment is restoring host protection against the infectious agent.(10). Previous studies have highlighted the significance of CXCL10 up-regulation during viral infection, according to the fact that CXCL10 is a determinative chemokine involved in the immune cell's chemotaxis and activation. However, it's possible that polyomavirus BK might cause BKVAN by changing the CXCL10 expression levels. Thus, the objective of this study was to compare the expression levels of CXCL10 chemokine at mRNA in plasma and urine between renal failure patients with nephropathy who were infected with polyomavirus BK and healthy individuals with a view to characterize the function of CXCL10 in the pathogenesis of BKVAN.

2.Materials and Methods

2.1Study design

This was a laboratory-based cross-sectional study on renal failure patients who admitted to dialysis unit, at AL Hussein hospital. kerbala province who had been on dialysis since 2015 to 2023, and all patients were under medical observation. The study was accomplished from Feb 2023 to Dec 2023 at Medical Research Laboratory of pharmacy Collage at Kerbala University.

2.2clinical sample

A total of 114 out of 323 (37%) dialysis patients who were admitted to the dialysis unit enrolled in this study. the subjects consist of 73 male (64%) with mean age ±SD 49.13±14.07 and 41 female (36%) with

mean age ±SD 48.07± 15.61 with ranged 19 to 85years. The clinical data were obtained from each patient on file which contain: demographic information (sex, age), first dialysis, frequency of sessions and monthly laboratory tests.

2.3 Ethics approval

The limitations of this study include working in a laboratory and not having direct patient interaction. The general manager's office, the training and human development center number 606, the Kerbala Health department, and the ethics committee all gave their approval on April 24, 2023. The Al-Hussein Teaching Hospital received all discarded supplies and equipment, which were gathered, wrapped, and burned for a good and secure disposal.

2. 4 Sample Size Determination

After reviewing for all data of patients diagnosed with renal failure were collected 114 from Al-Hussein Teaching Hospital/ dialysis unit. The 114 cases out of 323 patient diagnosed as renal failure in duration period of this study. The percentage of cases studied 35% of total cases diagnosed with renal failure from Apr 2023 to Dec 2023in study site.

2.5 Sample Collection and nucleic acid Extraction

the urine specimens (41out of 114) were collected in sterile universal containers and the blood specimens (73 out of 114) were collected in a sterile EDTA vacutainer tube and both specimens were subjected to DNA extraction. Extraction of DNA from clinical specimens were carried out with the DNA extraction kit (Geneaid Biotech Ltd. Korea) following the manufacturers' instructions. Urine samples and blood were stored in a sterile Eppendorf at −20°C for further usage.

the positive result for bk virus DNA (16 out of 114) by real- time qPCR was following steps to assessment the CXCL10 by Total RNA extraction from urine and blood samples by using (TRIzol® reagent kit) and done in compliance with industry guidelines and Estimation RNA yield and quality The extracted RNA was examined using a Nanodrop spectrophotometer (THERMO. USA), which measures absorbance at (260 /280 nm) to estimate RNA purity and determine RNA concentration. Using samples (a DNase I enzyme kit) and following the Promega company's USA instructions, the extracted RNA was processed with DNase I enzyme to eliminate any remaining traces of genomic DNA from the eluted total RNA.. DNase-I treated RNA samples were used in cDNA synthesis step for CXCL10 and GAPDH genes by using M-MLV Reverse Transcriptase kit and done according to company instructions. CXCL10 and Housekeeping gene (GAPDH) (NM_001565.4 and NM_001256799.3) were design in this study by using NCBI-Database and Primer3 plus design online. These primers were provided by (Macrogen company, Korea) as following table1. qPCR master mix was prepared by using GoTaq® qPCR Master Mix kit that dependent on SYBER Green dye detection of gene amplification in Real-Time PCR system.

Table (1): The primer design used in this study

qPCR primer		SEQUENCE (5'-3')
CXCL10	F	ATCGATGCAGTGCTTCCAAG
	R	GTAGCAGCTGATTTGGTGACC
GAPDH	F	TCTGACTTCAACAGCGACAC
	R	TGACAAAGTGGTCGTTGAGG

2.6 Data analysis of data and qPCR

Statistical analyses were performed with excel software (Microsoft, version 16.0, USA). The results were shown as (means ± SE) for continuous variables normal distribution and median (ranged) to non-normal distribution. The means of the two groups were compared using the student t test. If the probability value was not <0.05, it was not regarded to be statistically significant.(11)

The ΔCT Method (using a reference gene) was utilized to examine the relative quantitative gene expression levels (fold change) of the q RT-PCR data for both the target and housekeeping genes. (12)as following equation:

$$\Delta CT\ (Test) = CT\ (target\ gene, test) - CT\ (HKG\ gene, test)$$
$$\Delta CT\ (Control) = CT\ (target\ gene, control) - CT\ (HKG\ gene, control)$$
$$\Delta\Delta CT = \Delta CT\ (Test) - \Delta CT\ (Control)$$
$$Fold\ change\ (target / HKG) = 2^{-CT}\ \Delta\Delta CT$$

3.Result

From the 114 sample,16 BK virus DNA were detected by real-time qPCR. the 16 cases were 8 males with mean age ±SD (41.0 ±15.58) and female 55.36 ± 18.65 (table 2 and figure 1) and 20 cases of healthy as control table 3 figure 2

Table (2): Descriptive statistic data for age to positive BK virus DNA

Subject	All	Female	Male
Mean	48.31	55.63	41.00
Standard Error	4.56	6.59	5.51
Median	48.00	55.50	46.50
Mode	76.00	76.00	Not Available
Standard Deviation	18.24	18.65	15.58
Sample Variance	332.63	347.70	242.86
Kurtosis	-0.79	-1.55	-1.24
Skewness	-0.01	-0.23	-0.48
Range	57.00	48.00	42.00
Minimum	19	28	19
Maximum	76	76	61
Count	16	8	8
Confidence Level (95.0%)	9.72	15.59	13.03

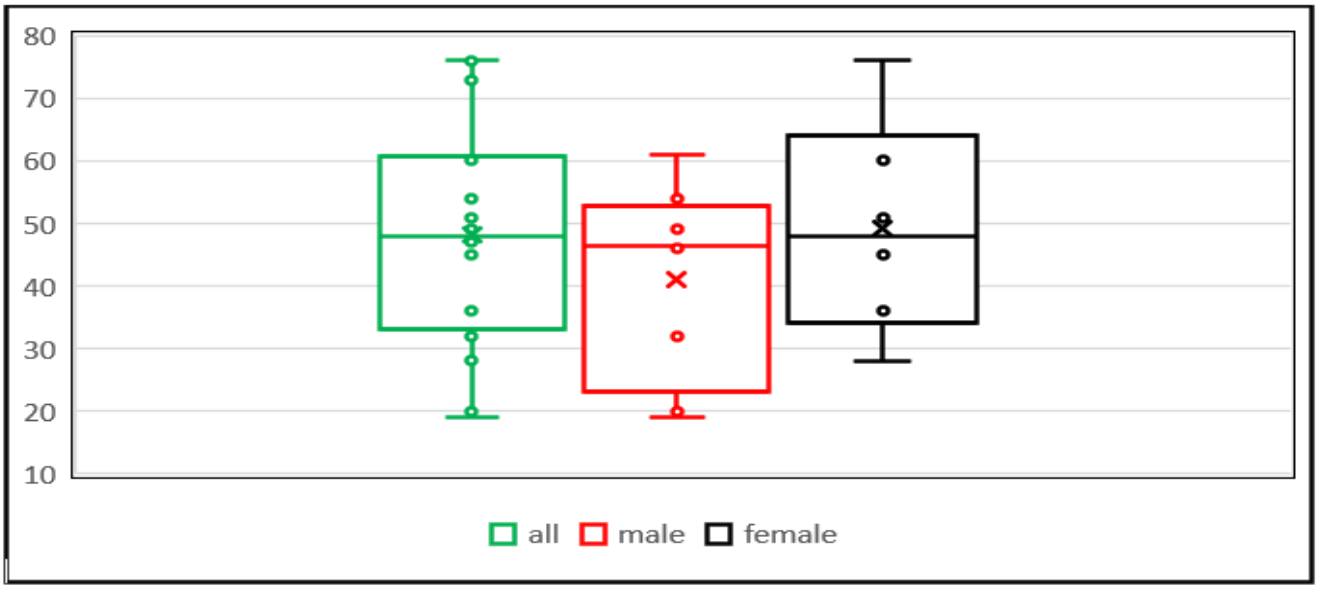

Figure (1): distribution of age based on sex in positive BKvirus DNA group

Table (3): Descriptive statistics for age of healthy (control) include in gene expression assay

Subject	All Control	Male	Female
Mean	42.95	38.1	47.8
Standard Error	2.56	3.34	3.36
Median	41.5	37	48
Mode	48	Not Available	48
Standard Deviation	11.5	10.6	10.6
Sample Variance	131.1	111.9	11
Kurtosis	-1.3	-0.2	-0.3
Skewness	0.0	0.7	-0.7
Range	35	31	32
Minimum	25	25	28
Maximum	60	56	60
Count	20	10	10
Confidence Level (95.0%)	5.36	7.57	7.59

***Not Available: Not Available**

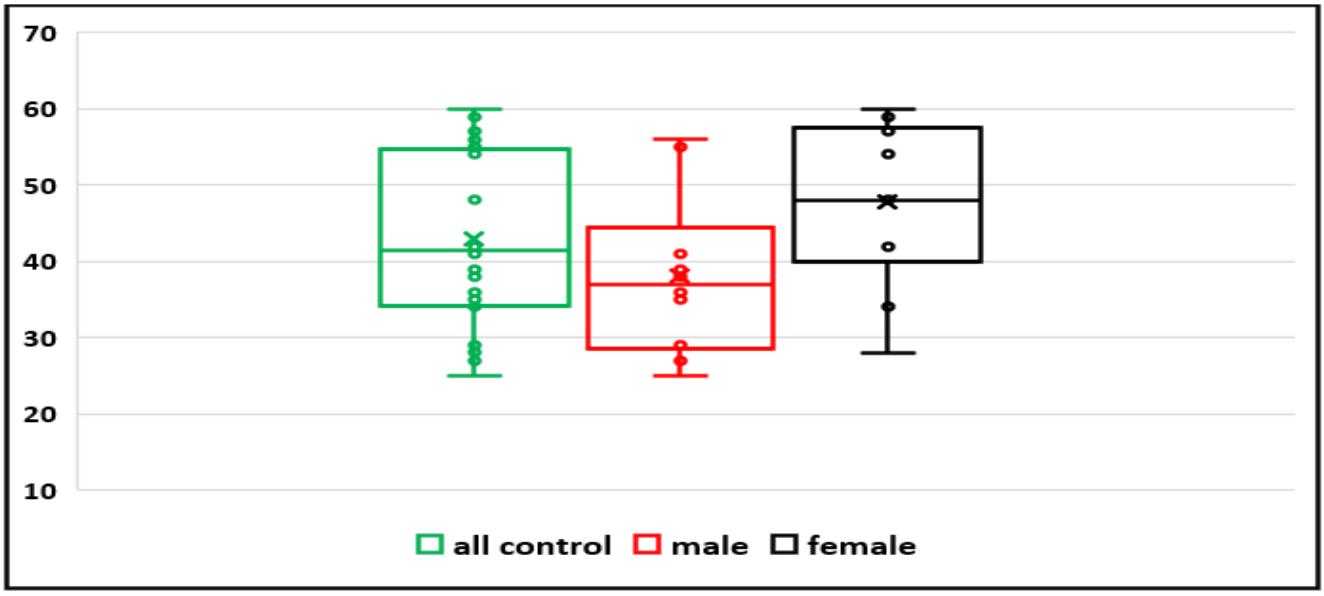

Figure (2): distribution of age based on sex in healthy (control) include in gene expression assay

The CXCL10 gene expression level (based on ΔCt and $2^{-\Delta\Delta CT}$ analysis) in positive BK virus DNA patients and controls were presented in (figure 4,5and 6) and table 3 and 4 respectively. The findings have been shown that CXCL10 gene expression level was upregulated in polyomavirus BK infected patients compared with controls.

Table (3): Descriptive statistics data for Ct real -time qPCR result of patient and healthy (control) include in gene expression assay

Subject	Ct CXCL10 Target Gene Patient	Ct GAPDH Patient	Ct CXCL10 Control	Ct GAPDH Control
Mean	30.89	27.29	33.83	27.32
Standard Error	0.24	0.26	0.29	0.30
Median	31.11	27.37	33.96	27.60
Mode	29.48	27.30	33.61	27.53
Standard Deviation	0.97	1.02	1.29	1.33
Sample Variance	0.94	1.05	1.66	1.76
Kurtosis	-1.00	2.28	-0.88	0.11
Skewness	-0.69	-1.51	-0.11	-0.82
Range	2.90	3.84	4.47	5.06
Minimum	29.10	24.63	31.27	24.42
Maximum	32.00	28.47	35.74	29.48
Sum	494.17	436.69	676.56	546.33
Count	16	16	20	20
Confidence Level (95.0%)	0.52	0.55	0.60	0.62

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Table (4): Descriptive statistics data for real -time qPCR result of patient and healthy (control) include in gene expression assay

Subject	Δ Ct Patient	Δ Ct Control	Fold Change Patient	Fold Change Control
Mean	3.59	6.51	8.46	1.90
Standard Error	0.18	0.40	0.99	0.58
Median	3.54	6.26	7.87	1.20
Mode	Not Available	Not Available	Not Available	Not Available
Standard Deviation	0.72	1.77	3.96	2.59
Sample Variance	0.52	3.13	15.71	6.69
Kurtosis	-1.18	0.54	-1.08	11.68
Skewness	0.20	0.33	0.40	3.17
Range	2.16	7.76	11.80	11.67
Minimum	2.59	2.96	3.39	0.05
Maximum	4.75	10.72	15.19	11.72
Count	16	20	16	20
Confidence Level (95.0%)	0.38	0.83	2.11	1.21

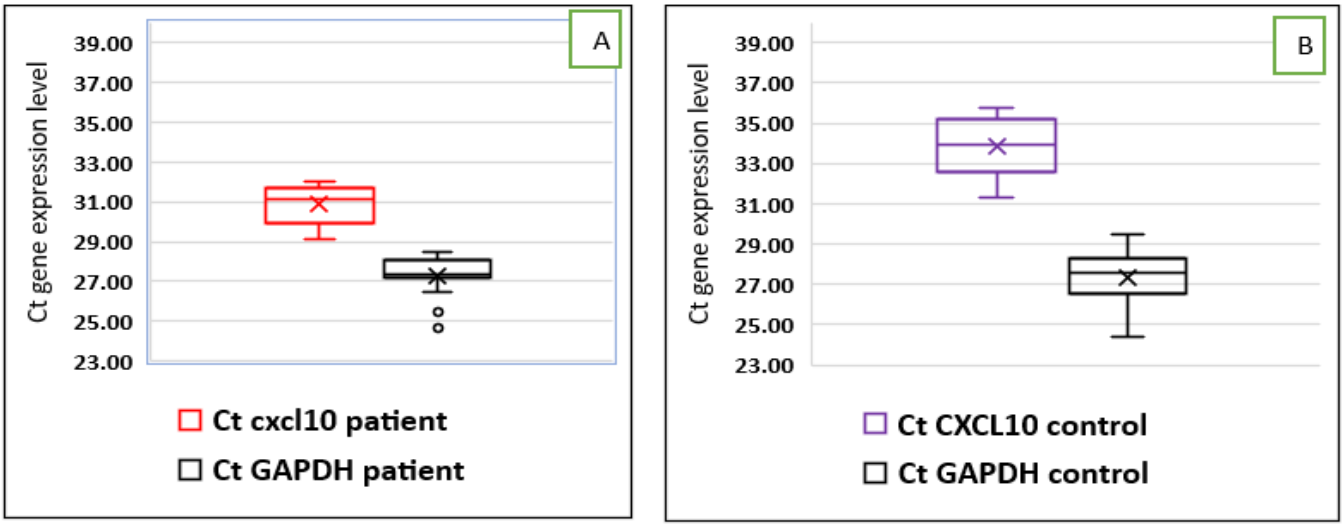

Figure (3): Ct CXCL10gene expression level compared with Ct GAPDH (housekeeping gene) as an internal control in the studied groups. The figure A shows Ct CXCL10 of patient (Red color) with mean ±SD (27.29 ± 1.02) and Ct GAPDH of patient (black color) with mean ± SD (27.32 ± 1.33) and B A shows Ct CXCL10 of control (green color) with mean ±SD (33.83 ± 1.33) and Ct GAPDH of control (black color) with mean ± SD (27.53 ± 1.29)

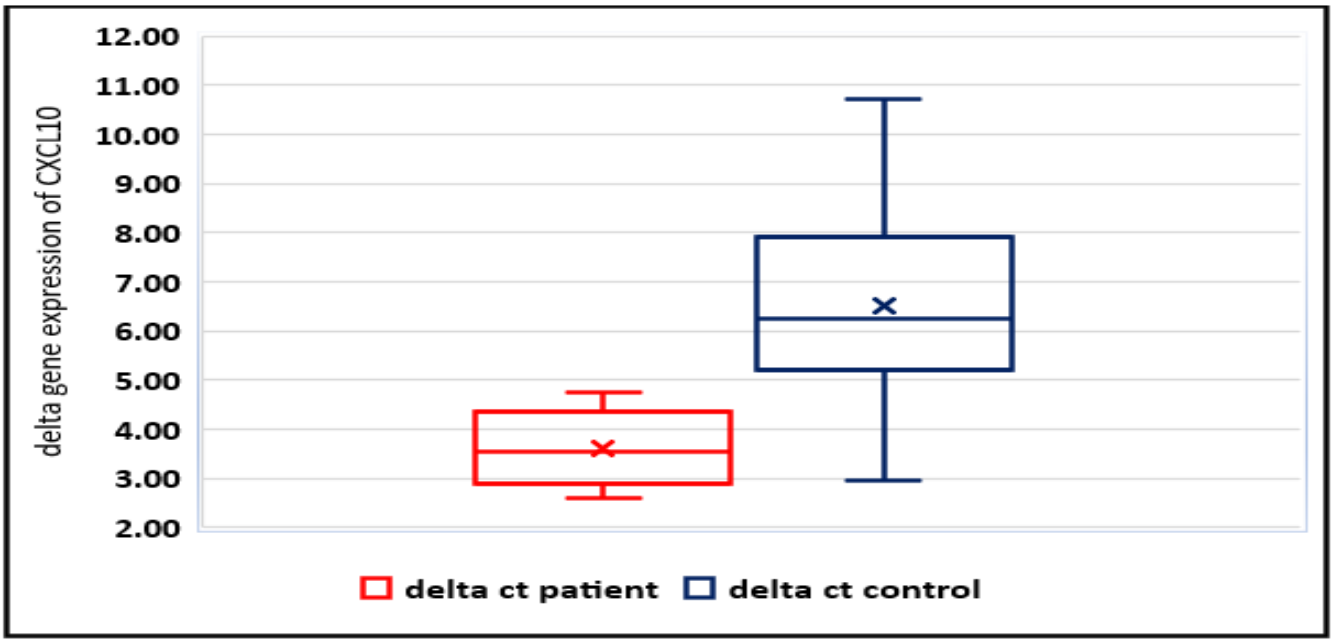

Figure (4): gene expression level of CXCL10 between two group include in study. The figure shows that Δ Ct CXCL10 of patient (Red color) with mean ±SD (3.59 ± 0.72) and Δ of control with mean ± SD (6.51± 1.77). The p = 0.00 statistically significant. confidence interval CI 95% = 3.21 to 3.98 for patient and 5.68 to 7.34 for control.

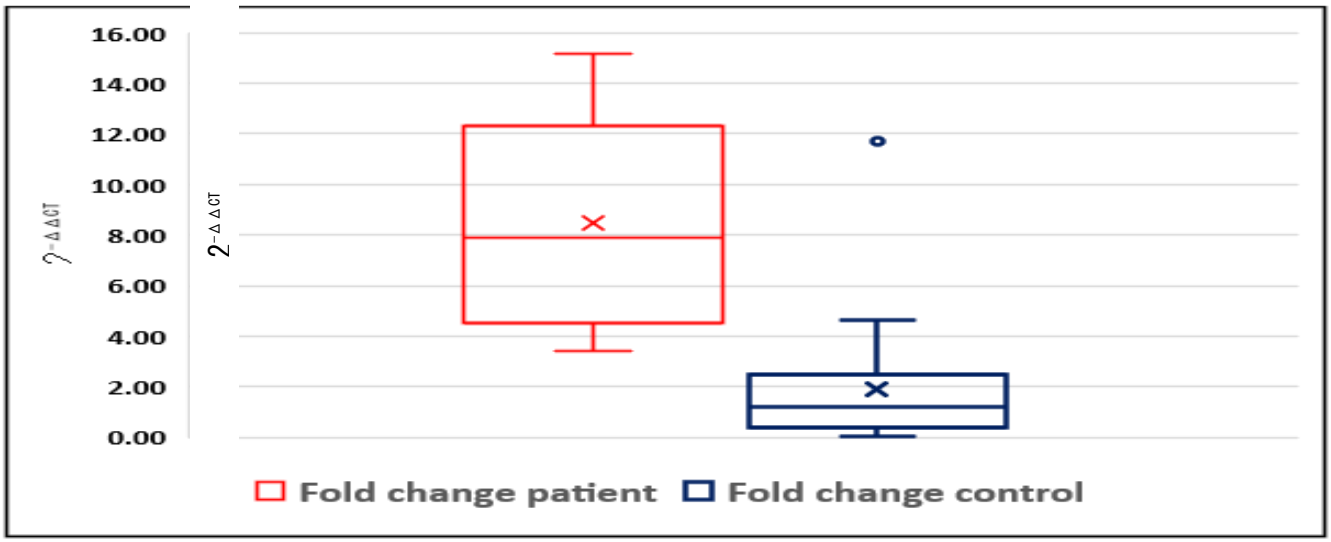

Figure (6): $2^{-\Delta\Delta CT}$ gene fold level of CXCL10 between two group include in study. The figure shows that $2^{-\Delta\Delta CT}$of patient (Red color) with median 7.87 ranged (3.39 to 15.9) and $2^{-\Delta\Delta CT}$ of control with median 1.2 ranged (0.05 to 11.72). The p = 0.00 statistically significant. By Mann-Whitney Test the mean rank 27.5 for patient and 11.3 for control.

Discussion :

Immune changes in immune status seem to be the consequence of hemodialysis (HD) treatments. The BKV polyomvirus infection has raised interest in immunosuppression.(13). After new and more effective immunosuppressive regimens were introduced, the incidence of NBKV increased. (14).the study present incidence of bkvirusDNA in age group with mean age 48.31±18.24 conduct with (15). C-X-C motif chemokine ligand 10 (CXCL10), also known as interferon-γ–induced protein 10 kDa, is a tiny cytokine that is a member of the CXC chemokine family. It is among the most encouraging indicators of BKPyV infection. (16). Determining the expression levels of CXCL10 in both mRNA in urine and plasma of dialysis patients with polyomavirus BK infection and healthy controls was the purpose of this study because CXCL10 is a pro-inflammatory chemokine and plays a significant role in inducing immune responses. The findings demonstrated that, when compared to healthy controls, dialysis patients with polyomavirus BK infection had higher levels of CXCL10 mRNA. BKPyV viremia and viruria have previously been linked in multiple studies to increased CXCL10(17) and(18) .

While there was no significant change in mRNA expression, patients infected with polyomavirus BK who had renal failure also had higher levels of CXCL10 than controls. This could be related to the possibility that the polyomavirus BK increased CXCL10 production through increased ribosome activity or stability of CXCL10 mRNA. A different situation would involve negative inhibitory, which restricts the rise in CXCL10 expression at the mRNA level. Furthermore, earlier studies showed that other cells, such as renal tubular epithelial cells, can produce CXCL10. (19, 20). All things considered, it seems that CXCL10, a pro-inflammatory chemokine, may be regarded as a significant risk factor in the pathogenesis of renal failure patients linked to polyomavirus BK reactivation. BK reactivation may also be a major inducer of CXCL10 expression, which would worsen the pathologic state and cause BKVAN in a manner that is dependent on CXCL10.. Stated differently, CXCL10 stimulates inflammatory biomarkers and infiltrates immune cells, causing nephropathy in polyomavirus BK infections. Previous studies confirmed the critical functions of CXCL10 in the pathophysiology of renal failure, including renal failure associated with BKV.

According to our findings, there was an increase in CXCL10 expression in polyomavirus BK-infected kidney cells when compared to controls. This suggests that polyomavirus BK has a significant direct and indirect role in kidney cell lysis and the upregulation of pro-inflammatory molecules like CXCL10. They proposed, that CXCL10 may induce renal injury by promoting inflammation and inhibiting the repair of tissue damage in patients with BKVan(21). On the other hand, although BKV replicated effectively in glomerular endothelial cells infected with it, no appreciable alterations in CXCL10 gene expression were detected in these cells. This could perhaps result from BKV replication in these cells inhibiting the expression of the CXCL10 gene.(22)

These results suggest that CXCL10, a significant immunoregulatory molecule, plays a role in the pathophysiology of BKVAN. To prove this crucial function in more concluded research, however, targeting CXCL10 with further biochemical information in renal failure patients infected with polyomavirus BK is required.

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