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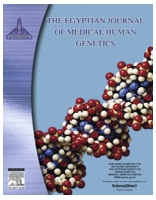

Original article

Association of variable number tandem repeats polymorphism in the IL-4 gene with end-stage renal disease in children

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ABSTRACT

Background: End stage renal disease (ESRD) is a common cause of morbidity and mortality among children. Interleukin 4 is a cytokine that might influence the progression of chronic kidney disease (CKD) to end stage renal disease. There are variable number of tandem repeats (VNTRs) in IL4 gene that could play major roles in genetic predisposition to some diseases. Aim of the study: The purpose of this study is to detect the association of allelic variant in intron 3 VNTR-IL4 gene with the end stage renal disease and if these variants could be considered as risk markers for this disease.

Subjects and methods: The study was conducted on fifty-five children with CKD and fifty healthy children served as controls. All participants were genotyped for intron 3 VNTR by Polymerase Chain Reaction.

Results: The frequency of intron 3 VNTR-IL4 P1P2 + P2P2 genotypes was significantly higher in ESRD-children than those with P1P1 genotype (88.7% vs. 15.4%, OR 43; 95% CI 13–134, P value < 0.001). Also, the frequency of P2 allele was significantly higher in ESRD-children compared with healthy controls (70.9% vs. 8%, OR 28; 95% CI 12–64, P value < 0.001). Furthermore, a significantly higher frequencies of P1P1 genotype and P1 allele among the control group were demonstrated (84.6% vs. 11.3%, P < 0.001 and 92% vs. 29.1%, P < 0.001, respectively).

Conclusion: we concluded that the P2 allele is an allelic variant predisposing to ESRD in children with CKD and it could be considered a risk factor for the development of ESRD.

1. Introduction

End stage renal disease is the irrecoverable decline of renal function, resulting from various factors including, hypertension, autoimmune diseases, diabetes mellitus, inherited disorders and congenital abnormalities, with heterogeneous etiology whether genetic or environmental. Inflammation is an important pathophysiological factor in primary renal disease and leads to the development of ESRD [1]. The affected kidney is struggling by increasing filtration capacity until only 10–15% of kidney function remains. The kidneys lose their regulatory and excretory function resulting in uremic syndromes. Whether renal disease is acute or chronic and regardless of the underlying pathological immune response (innate or adaptive), it is clear that inflammatory cytokines have crucial role not only as mediators of immune response and initiators of renal injury but also as immune modulators that can abrogate the development of renal disease [2].

One of these cytokines is T-helper 2 Interleukin-4 (IL4) with its dual role in the pathogenesis of renal disease, by both promoting and limiting renal disease progression [3]. IL4 is the principal mediator of immediate hypersensitivity reactions also it is the main stimulator of B cell Ig heavy chain switching to the IgE isotype [4]. In addition, the evolution of Th2 cells from naïve CD4+ T cells is stimulated by IL-4 which also served as an autocrine growth factor for these cells. At the same time, IL-4 suppresses the Th1 cell evolution from its mother naïve CD4+ T cells, that is to say, enhancing humoral immune response through antibody production and inhibiting cell mediated immunity. Furthermore, IL-4 inhibits the production of the proinflammatory cytokines such as Tumor necrosis factor-α, Interleukin-6 and Interleukin-1α and destructive enzymes by monocytes and this highlights its potent anti-inflammatory effect [5]. With the same respect IL-4 may control the inflammation induced by Th17, which plays a fundamental role in the pathogenesis of many autoimmune diseases [6].

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IL-4 gene has been mapped to the q arm (q23–31) of chromosome 5 [7]. There are variable number of tandem repeats (VNTRs) in cytokine genes and several specific single nucleotide polymorphisms (SNPs) that could play major roles in genetic predisposition to some diseases and cancers. A variable number of tandem repeat (VNTR) polymorphism is located in the third intron of IL-4 gene [8]. Also IL-4-590 promoter polymorphism, representing a C-to-T base substitution at 589 base pair (bp) upstream of the transcriptional site, has been identified [9]. Recent investigations have revealed that some of these polymorphisms could alter the cytokines production levels for instance, the 70-base-pair (bp) VNTR polymorphism in the third intron of the IL4 gene may alter the expression level of this gene. Three alleles for the IL4 gene VNTR polymorphism have been reported: RP*1 allele, three repeats; RP*2 allele, two repeats; and RP*3 allele, four repeats. The RP*1 allele is more frequent than RP*2 allele and RP*3 allele is the rarest one, which has been observed in few populations [10].

Association studies of VNTR polymorphism of the IL-4 gene were carried out in many studies among different populations with multiple different diseases with conflicting results [11,12,4,13]. This study was conducted to determine the relevance of the 70-bp VNTR polymorphism of intron-3 of the IL-4 gene to the development and progression of ESRD in children and whether these allelic variants could be used as risk biomarkers of this disease.

2. Patients and methods

Fifty-five pediatric patients with advanced chronic kidney disease (CKD) [stage 5] based on estimated glomerular filtration rate (eGFR) according to the National Kidney Foundation classification [14] were included in the study, selected from the hemodialysis unit of the Center of Pediatric Nephrology and Transplantation (CPNT), Children's Hospital, Cairo University and 50 healthy children attended the pediatric clinic of the National Research Centre (NRC) with no clinical signs of renal disease and no family history of renal disease served as controls. The study was done from 2013 to 2016. The inclusion criteria for hemodialyzed patients (HD) includes: onset of hemodialysis below 18 years of age with at least 6 months duration on maintenance hemodialysis (MHD), they were treated with hemodialysis for 3–4 h three times weekly with a polysulfone membrane using bicarbonate-buffered dialysate. The exclusion criteria: children with ESRD on hemodialysis less than 6 months. An informed consent for genetic studies was obtained from parents of all participants. The protocol of the study was read and approved by the Ethics Committee of NRC in Egypt. The work has been carried out in accordance with The Code of Ethics of The World Medical Association (Declaration of Helsinki) for experiments in humans.

A peripheral blood sample was obtained from HD and healthy children. An immediate centrifugation was done for 10 min at 5000 rpm at 4 °C. The centrifuged serum was transferred into sterile tubes. All samples were stored at −20 °C until assay. One ml of venous blood sample was collected in EDTA vials for the extraction of genomic DNA. The following parameters were measured: creatinine, urea, calcium, phosphorus and albumin by routine methods using (Olympas AU 400: Olympus diagnostic, Japan).

2.1. Determination of intron 3 VNTR-IL4 gene polymorphism

Genomic DNA was extracted from EDTA–anticoagulated whole blood samples using the QIAamp DNA Mini isolation kit (QIAGEN, #51304) following manufacturer's instructions and was stored at −20 °C until the analysis.

2.2. PCR-genotyping of intron 3 VNTR-IL4 gene polymorphism

DNA concentration was determined by Nano Drop 2000c Spectrophotometer (Thermo Fisher). Genomic DNA was amplified using polymerase chain reaction (PCR). Amplification was carried out on Veriti thermal cycler Applied Biosystems (USA). A 25 μl reaction mixture in 0.2 ml thin-wall PCR strip tubes (Axygen Scientific, Inc., CA) containing 200 ng genomic DNA, 12.5 μl master mix using Hot-Star-Taq Plus DNA Polymerase (250 units) Catalog No. 203603. (PE Applied Biosystems), 5 pmol each forward and reverse primers. PCR was performed using:

forward primer 5′ TAGGCTGAAAGGGGGAAAGC-3′ and
reverse primer 5′-CTGTTCACCTCAACTGCTCC-3′.

The following reaction conditions were used: 95 °C for 10 min; 32 cycles of 94 °C for 30 s, 60° C for 30 s and 72 °C for 30 s followed by one cycle of 72 °C for 5 min. The PCR products were analyzed directly by electrophoresis on 2% agarose gels stained with ethidium bromide. Alleles of 183 and 253 base pairs (bp) in lengths were recognized as allele P1 and P2, respectively [15].

2.3. Statistical analysis

Data were processed and analyzed using the Statistical Package of Social Science (SPSS) for windows, version 17.0 (SPSS Inc., Chicago, IL). Nominal data were summarized as frequencies (percentages). Associations between Nominal data were investigated using

Table 1
Clinical and biochemical characteristics of the studied group.

	ESRD-patients (n = 55)	Controls (n = 50)	P-value
Age(years)	13.7 ± 3.4	13.0 ± 3.4	0.3
Sex (male/female)	28 (50.9%)/27 (49.1%)	30 (60%)/20 (40%)	0.2
Duration of dialysis (years)	3.3 ± 2.4		
Kt/v	1.8 ± 0.2		
Predialysis – SBP (mmHg)	126 ± 16.0	114 ± 4.9	<0.001
Predialysis – DBP (mmHg)	80 ± 9.7	74 ± 4.9	<0.001
Postdialysis – SBP (mmHg)	115 ± 12.9	NA	
Postdialysis – DBP (mmHg)	74 ± 8.7	NA	
Predialysis-urea (mg/dl)	113.2 ± 37.2	24.0 ± 5.0	<0.001
Postdialysis-urea (mg/dl)	23.3 ± 9.2	NA	
Calcium (mg/dl)	8.7 ± 1.1	9.4 ± 0.8	<0.001
Phosphorus (mg/dl)	4.6 ± 1.5	3.9 ± 0.6	0.08
Albumin (g/dl)	3.5 ± 0.5	4.0 ± 0.2	<0.001

Values are presented as mean ± SD or percentage as applicable. P-value < 0.05 is statistically significant. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, NA: Non applicable. Kt/v: Adequacy of dialysis.

Fisher's exact, chi square tests and odds ratio (OR) with a 95% confidence interval (95% CI). Numerical data were represented as mean and standard deviation. Differences between groups were detected using Student's *t*-test. p Value of <0.05 was considered statistically significant. The distributions of the allelic and genotypic frequencies of the studied SNP respected the Hardy–Weinberg equilibrium.

3. Results

Clinical and biochemical characteristics of the studied groups were summarized in table 1. The mean age of the 55 ESRD- children (28 males& 27 females) included in this study was 13.7 ± 3.4 years with mean duration of dialysis was 3.3 ± 2.4 years. The mean age of the 50 healthy children (30 males & 20 females) was 13.0 ± 3.4 years.

There were no significant differences between the groups with respect to age, sex ratio, urea and phosphorus serum levels. There were statistically significant differences between ESRD- patients and controls regarding predialysis systolic and diastolic blood pressure, calcium and albumin levels (P-value < 0.001).

The distribution of intron 3 VNTR-IL4 genotype frequencies, allele frequencies and risk association were compared between ESRD-children and controls and summarized in Table 2. Statistically significant differences were demonstrated regarding the intron 3 VNTR-IL4 genotypes between the two groups, where the P1P2 + P2P2 genotypes frequencies were higher in the ESRD-patients while the P1P1 genotype frequency was higher in the control subjects (88.7% vs. 15.4%, OR 43; 95% CI 13–134, P value < 0.001). Similarly, there were statistically significant differences regarding P1 and P2 alleles frequencies, where P2 allele display a higher frequency among the ESRD-patient group (70.9% vs. 8%, P value < 0.001). Furthermore, we demonstrated a significantly higher frequency of P1P1 genotype and P1 allele among the control group (84.6% vs. 11.3%, P < 0.001 & 92% vs. 29.1%, P < 0.001, respectively). Demonstration of the risk association of the different intron 3 VNTR-IL4 genotypes, alleles and ESRD among studied groups showed that children with [P1P2 and P2P2 genotypes] have 43 times more risk to develop ESRD than those with P1P1 genotype (OR 43; 95% CI 13–134). Moreover, carriers of mutant P2 allele have 28 times more risk to develop ESRD than those with the wild P1 allele (OR 28; 95% CI 12–64).

Comparison of clinical and biochemical characteristics between the carriers of the P2 allele (P1P2 + P2P2 genotypes) and non carriers (P1P1 genotype) is shown in Table 3. There were statistically significant differences between the two groups regarding Pre-dialysis systolic blood pressure and urea (P 0.03 & <0.001 respectively) where carriers of P2 allele exhibit higher levels of these parameters than the non carriers.

4. Discussion

End-stage renal disease (ESRD) is associated with high rates of morbidity and mortality, and increased health care use [16]. Substantial evidence suggests that ESRD could be considered a chronic systemic inflammatory state, and inflammation may be an important pathophysiological factor in primary renal disease and its progression towards ESRD. Although many etiological factors

Table 2
The frequency distribution and risk association of intron 3 VNTR-IL4 genotypes and alleles among the studied groups.

Gene	ESRD-patients (n = 55)	Controls (n = 50)	*OR (95% CI)	P-value
Genotypes				
P1P1	8 (14.5%)	44 (88%)		
P1P2	31 (56.4%)	2 (4%)		<0.001
P2P2	16 (29.1%)	4 (8%)		
P1P1 (n = 52)	8 (15.4%)	44 (84.6%)		
		43 (13–134)	<0.001	
P1P2 + P2P2 (n = 53)	47 (88.7%)	6 (11.3%)		
Alleles	(n = 110)	(n = 100)		
P1	32 (29.1%)	92 (92%)		
			28 (12–64)	<0.001
P2	78 (70.9%)	8 (8%)		

Data were evaluated by the gene counting method. Values are presented as percentage. P < 0.05 was statistically significant.
* Odd's ratio was used.

Table 3
Comparison of clinical and biochemical characteristics between carriers of P2 allele and non carriers.

	P1P2 + P2P2 (n = 53)	P1P1 (n = 52)	P-value
Age (years)	13.6 (5–18)	13.1 (6–18)	0.5
Sex (male/female)	29 (54.7%)/24 (45.3%)	29 (55.8%)/23 (44.2%)	1.0
Predialysis – SBP (mmHg)	123.3 (100–170)	117.6 (100–150)	0.03
Predialysis – DBP (mmHg)	78.7 (60–100)	75.8 (70–100)	0.07
Postdialysis – SBP (mmHg)	114.6 (90–150)	121.3 (100–150)	0.1
Postdialysis – DBP (mmHg)	73 (60–100)	78.8 (60–90)	0.1
Predialysis-urea (mg/dl)	103 (14–185)	37.7 (15–166)	<0.001
Postdialysis-urea (mg/dl)	24 (9–46)	19.4 (7–38)	0.2
Calcium (mg/dl)	8.9 (5.9–13.6)	9.3 (5–12)	0.09
Phosphorus (mg/dl)	4.6 (1.5–7)	4.7 (2.5–7)	0.8
Albumin (g/dl)	3.6 (2.5–4.6)	4.6 (3–4.5)	0.2

Data are presented as mean (minimum/maximum) or percentage as applicable. P was significant if <0.05.

predisposed to the development of ERSD, yet immune dysregulation and inflammatory cytokines have negotiable role in its pathogenesis acting not only as immune modulators initiating renal injury but also as mediators that ameliorate renal damage. IL-4 is a potent anti-inflammatory cytokine that reduces the production of destructive enzymes and proinflammatory cytokines [4].

Genetic factors that affect this cytokines may play fundamental roles in the development of ESRD. IL-4 VNTR located in intron 3 of IL-4 gene and could alter messenger ribonucleic acid splicing, which leads to different splice variants [8]. Some studies suggested the possible association between P1 allele and higher expression of the IL-4, other studies reported the association between P2/P2 genotype and lower expression of this cytokine [10]. In this study, the frequencies of the intron 3 VNTR-IL4 P1P2 + P2P2 genotypes were significantly higher among ESRD-children than those with P1P1 genotype. Also, the frequency of P2 allele was significantly higher in ESRD- patients compared with healthy controls indicating that the P2 allele may be considered as an allelic variant predisposing to ESRD in children with CKD and a risk factor for the development of ESRD. Moreover, we demonstrated a significantly higher frequency of P1P1 genotype and P1 allele among the control group. Although the frequency of P1 allele, which was supposed to lead to over expression of IL-4 observed in many inflammatory, autoimmune diseases and cancers [17,18], yet in this study we supposed that the wild allele P1 is protective against the development of ESRD by combating the inflammatory process by its high production. With the same respect, IL4 is claimed to be a potent anti- inflammatory cytokine with its critical role in the regulation of TH0 cell differentiation stimulating Th2 immunity and inhibiting Th1 responses. Furthermore IL-4 provokes a macrophage phenotype that has greater scavenger receptor activity and increased release of anti-inflammatory and fibrogenic factors, supporting its significant role in the clearance of cell debris and promotion of tissue repair [19].

In concordance with our result, Vasudevan et al. (2011) concluded that VNTR polymorphism of the IL-4 gene is a risk factor for the development of ESRD among Malaysians [11]. Similarly, Mittal and Manchanda (2007) reported that the frequency of P2P2 genotype was higher in ESRD- patients as compared to controls (62.7% vs 46.7%) [4].

Since diabetes mellitus is known to be one of the leading factors for the development of renal impairment, Tripathi 2015, studied the impact of VNTR IL4 genetic polymorphism on type 2 Diabetes Mellitus demonstrating that the genotype P2/P2 frequency was significantly higher in patients as compared to healthy controls (60.00% vs. 49.04%) [20]. Also, Bid et al. 2008 reported that IL-4 P2P2 genotype was higher in patients of diabetes type 2 with odds ratio 2.30 [21]. Moreover, murine model showed that a mice treated with IL4 had improved insulin sensitivity and glucose tolerance while lipid accumulation in adipose tissues was inhibited [22].

Similar results were also reported in other diseases where, Salimi et al. (2014) declared that the P2 allele was found more prevalent in preeclampsia patients in comparison with healthy subjects [10].

With respect to IL4 impact on cancer development, progression and treatment, IL4 is advertised as a potent anti-apoptotic cytokine and survival factor for tumour cells helping these cells to evade the apoptotic effect of anti-tumour therapies with consequent resistance to cancer treatments. Conversely, IL-4 has been investigated as a therapeutic agent in cancer, through its perchance toxicity on some tumour cells. Subsequently, intron 3 VNTR-IL4 polymorphism could influence the risk of human cancer. Similar result to ours, Tsai et al. (2005) suggested that intron 3 VNTR- IL4 gene polymorphism was a potential genetic marker for the risk of bladder cancer [23]. On other hand, Duan et al. (2014) announced that P1 allele might be a risk factor for several malignancies [24]. Likewise, Ahmed et al. (2016) reported that the frequency of P1 allele was found higher in leukemic patients than control group and the carriers of P1 allele were at 1.24 times more risk to develop leukemic disease compared to P2 carriers [25].

Another contradictory findings were reported in Behcet disease (BD) where Inanir et al. (2013) suggest that possession of the P_1 allele of the IL4 gene 70 bp VNTR polymorphism may constitute a risk for developing BD [26].

In children, Intron 3 VNTR-IL4 gene polymorphism was studied in Japanese patients with IgA nephropathy to clarify the impact of this polymorphism on progression of this disease to ESRD and announced that although there was no difference between the IgA nephropathy and healthy control groups regarding the frequencies of both IL4 VNTR allele and genotype yet the frequencies of IL4 P1 allele and P1/P1 genotype in patients with progressive IgA nephropathy children (end-stage renal disease) were significantly greater than corresponding values in the non-progression group. This result could highlight the debate about the role of IL4 in the pathogenesis and progression to ESRD [27].

To conclude, a P2 allele of the intron 3 VNTR-IL4 gene polymorphism showed a significantly higher frequency in children with CKD and could be used as a risk marker for the development of ESRD. Meanwhile, the wild P1 allele is protective against the development of ESRD. However, The mechanisms that drive the development of ESRD among children with chronic kidney disease with the contribution of environmental and genetic factors require linking immune and inflammatory mechanisms with more attention and clarification of the role of cytokines and their genetic polymorphisms to outline some of the common therapies used to target renal disease with an emphasis on their potential to suppress inflammation and immune system function.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee at which the studies were conducted (IRB approval number 13 169) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all participants the study included.

Conflict of interest

The authors have declared that no conflict of interest exists.

References

[1] Desai N, Rahman M. Nephrology update: end-stage renal disease and renal replacement therapy. FP Essent 2016;444:23.
[2] Makusidi M, Liman H, Yakubu A, et al. Hemodialysis performance and outcomes among end stage renal disease patients from Sokoto, North-Western Nigeria. Indian J Nephrol 2014;24(2):82–5.
[3] Dimitrova PA, Skapenko ML, Herrmann R, et al. Restriction of de novo pyrimidine biosynthesis inhibits Th1 cell activation and promotes Th2 cell differentiation. J Immunol 2002;169:3392–9.
[4] Mittal RD, Manchanda PK. Association of interleukin (IL)-4 intron-3 and IL-6 -174 G/C gene polymorphism with susceptibility to end-stage renal disease. Immunogenetics 2007;59:159–65. doi: http://dx.doi.org/10.1007/s00251-006-0182-6.
[5] Genevay S, Di Giovine FS, Perneger TV, et al. Association of interleukin-4 and interleukin-1B gene variants with Larsen score progression in rheumatoid arthritis. Arthritis Rheum 2002;47(3):303–9.
[6] Tesmer LA, Lundy SK, Sarkar S, et al. Th17 cells in human disease. Immunol Rev 2008;223:87–113.
[7] Cantagrel A, Navaux F, Loubet-Lescouli P, et al. Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms relationship to occurrence and severity of rheumatoid arthritis. Arthritis Rheum 1999;42:1093–100.
[8] Mout R, Willemze R, Landegent JE. Repeat polymorphisms in the interleukin-4 gene. Nucleic Acid Res 1991;19:3763.
[9] Rosenwasser LJ, Klemm DJ, Dresback JK, et al. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allerg 1995;25:74–8.

[10] Salimi S, Khorasani M, Namazi L, et al. Association between interleukin 4 gene seventy-base-pair variable number of tandem repeats polymorphism and uterine leiomyoma. Gene, Cell Tissue 2014;1(2):e19462.
[11] Vasudevan R, Norhasniza MN, Patimah I. Association of variable number of tandem repeats polymorphism in the IL-4 gene with end-stage renal disease in Malaysian patients. Genet Mol Res 2011;10(2):943–7.
[12] Hussein Yousri M, El-Shal Amal S, Rezk Noha A, et al. Influence of interleukin-4 gene polymorphisms and interleukin-4 serum level on susceptibility and severity of rheumatoid arthritis in Egyptian population. Cytokine 2013;61 (3):849–55.
[13] Konwar R, Bid HK. Location of the 70bp VNTR polymorphic site is in third intron of IL-4 gene. Ind J Clin Biochem 2008;23:204–5.
[14] Mula-Abed W, Rasadi Kh, Al-Riyami D. Estimated Glomerular Filtration Rate (eGFR): a serum creatinine-based test for the detection of chronic kidney disease and its impact on clinical practice. Oman Med J 2012;27(2):108–13.
[15] Gyan B, Goka B, Cvetkovic J, et al. Allelic polymorphisms in the repeat and promoter regions of the interleukin-4 gene and malaria severity in Ghanaian children. Clin Exp Immunol 2004;138:145–50.
[16] Elshamaa M, Sabry S, Badr A, et al. Endothelial nitric oxide synthase gene intron4 VNTR polymorphism in patients with chronic kidney disease. Blood Coagul Fibrinolysis 2011;22:487–92.
[17] Nakashima H, Miyake K, Inoue Y, et al. Association between IL4 genotype and IL-4 production in the Japanese population. Genes Immun 2002;3:107–9.
[18] Buchs N, Silvestri T, di Giovine FS, et al. IL-4 VNTR gene polymorphism in chronic polyarthritis. The rare allele is associated with protection against destruction. Rheumatology (Oxford) 2002;39:1126–31.
[19] Buharaliglu G, Bond M, Sala G, et al. Regulation of epigenetic modifiers, including KDM6B, by interferon-γ and interleukin-4 in human macrophages. Front Immunol 2017. doi: http://dx.doi.org/10.3389/00092.
[20] Tripathi AK, Shukla S, Tripathi JK, et al. Association of genetic polymorphism of inflammatory genes (IL-1β and IL-4) with diabetes type 2. Enliven: J Genet Mol Cell Biol 2015;1(1):004.
[21] Bid HK, Konwar R, Agrawal CG, Banerjee M, et al. Association of IL-4 and IL-1RN (receptor antagonist) gene variants and the risk of type 2 diabetes mellitus: a study in the north Indian population. Indian J Med Sci 2008;62:259–66.
[22] Kok Y, Ong H, Say Y. Interleukin-1 receptor antagonist and interleukin-4 genes variable number tandem repeats are associated with adiposity in Malaysian subjects. J Obesity 2017:8. Article ID 4104137.
[23] Tsai FJ, Chang CH, Chen CC, et al. Interleukin-4 gene intron-3 polymorphism is associated with transitional cell carcinoma of the urinary bladder. BJU Int 2005;95:432–5.
[24] Duan Chi Pan, Shi Jinan, et al. Association between interleukin-4 gene intron 3 VNTR polymorphism and cancer risk. Cancer Cell Int 2014;14:131.
[25] Ahmed A, Abdelgadir R, Muddathir A, et al. Interleukin-4 intron 3 VNTR polymorphism gene in leukemic patients. J Blood Disord Transfus 2016;7:3.
[26] Inanir A, Tural S, Yigit S, et al. Association of IL-4 gene VNTR variant with deep venous thrombosis in Behçet's disease and its effect on ocular involvement. Mol Vis 2013;19:675–83.
[27] Masutani K, Miyake K, Nakashima H, et al. Impact of interferon-γ and interleukin-4 gene polymorphisms on development and progression of IgA nephropathy in Japanese patients. Am J Kidney Dis 2003;41(2):371–9.