World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers © All Rights Reserved
Available online at: http://www.wjpsonline.org/
Original Article

Macrophage migration inhibitory factor and some cytokine gene polymorphism in patient with nephrotic syndrome

Manal M. Khadhim and Shaimaa Audah

Department of Medical Microbiology, College of Medicine, Al-Qadisiya University, Diwaniya, Iraq

Received: 02-11-2014 / Revised: 17-11-2014 / Accepted: 23-11-2014

ABSTRACT

Nephrotic syndrome is considered as a multi-factorial clinical condition characterized by increased glomerular permeability with massive consequent proteinuria. There is a variable tendency towards developing edema, hypoalbuminemia and hyperlipidaemia. The present study was conducted to evaluate the role of MIF G173C and TNF-α G308Agenes gene polymorphism in the nephrotic syndrome. We have investigated single nucleotide polymorphisms of MIF G173C and TNF-α G308Agenes in71 subjects. Forty-six were nephrotic patients while others were apparently healthy individuals used as a controls, then the serum level of TNF-α and IL-13 was detected by ELISA technique. The frequencies of MIF C-173C (13.04 vs 4.00%) genotypes and C allele (29.35vs 22.00%)were higher in nephrotic patients than control group while TNF-α A308A(21.74vs 0)genotypes and A allele(38.88vs10%) were significantly higher in patient than control groups and associated with higher mean serum concentration of TNF-α(668.33±27.60) versus (45.64±2.38) and IL-13(36.70±0.55) versus (2.72±0.22),in nephrotic patients than apparently healthy subjects. AA genotype with TNF- α -G308A allele polymorphism and CC genotype with MIF 173C allele are mainly expressed among nephrotic syndrome patients and susceptibility with disease might be prospected.

Key words: Nephrotic syndrome, TNF-α, MIF, IL-13, Allele, Genotype, RFLP

INTRODUCTION

Nephrotic syndrome (NS) is considered as a multi-factorial clinical condition characterized by increased glomerular permeability with massive consequent proteinuria. There is a variable tendency towards developing edema, hypoalbuminemia and hyperlipidaemia [1].The syndrome arises as a result of a failure of the glomerular filtration barrier to restrict the passage of proteins into Bowman's space. It implies structural abnormalities within the glomerular filter [2].

Primary nephrotic syndrome is a glomerulopathy of unknown etiology, there are five distinct histological variants of PNS: minimal change nephrotic syndrome (MCNS); focal segmental glomerulosclerosis (FSGS); membranous nephropathy; immunoglobulin A (IgA) nephropathy; and membrano proliferative glomerulo-nephritis (MPGN) [3]. Secondary nephrotic syndrome is present when the disease has extrarenal manifestations not dependent on the renal abnormality or has a specific etiology [4]. Congenital nephrotic syndrome is a rare kidney disorder characterized by heavy proteinuria, hypoproteinemia, and edema starting soon after birth, mostly within the first three month of life [5].

The prevalence of NS in childhood is approximately 2-5 cases per 100,000children. The cumulative prevalence rate is approximately15.5 cases per 100,000 [6]. In children below eight years, the ratio of males to females varies from 2:1–3:2 in various studies [7]. In older children, adolescents and adults, the sex ratio is approximately equal [8]. Complications in NS may occur as a part of the disease itself or as a consequence of drug treatment, and the most common complication are infections, thromboembolism, anemia, cardiovascular disease and acute renal failure [9].

Although its pathogenesis remains to be elucidated, there are some evidences which suggest that idiopathic nephrotic syndrome (INS) is primary immune disease associated with immunoregulatory imbalance between T helper subtype 1 (Th1) and T helper subtype 2 (Th2) cytokines [10]. Cytokines

***Corresponding Author Address:** Manal M. Khadhim and Shaimaa Audah, Department of Medical Microbiology, College of Medicine, Al-Qadisiya University, Diwaniya, Iraq; E-mail: shaima_awadh@yahoo.com, mic_drmanal@yahoo.in*

play a critical role as mediators of inflammation and as progressive factors in INS, various cytokines are considered as prime candidates for mediating INS progression. The genetic basis of childhood NS remains uncertain. Many authors have suggested that activated T-cells are involved in the pathogenesis of this syndrome. This is supported by the detection of increased levels of interleukin 4 (IL4) and IL13 in the serum and urine of children with acute relapse of NS [11]. Studies on several cytokine gene polymorphisms like, interleukin-1b (IL-1b), interleukin-1receptor antagonist (IL-1ra), and tumor necrosis factor-α (TNF-α), have shown an association with various inflammatory diseases including glomerulonephritis [12].

IL-13 gene expression is up regulated in both CD4+ and CD8+ T cells in children with steroid-sensitive nephrotic syndrome in relapse [13]. This was associated with increased intracytoplasmic IL-13 production by CD-3+ cells studies [14]. As well as down regulation of gene expression on the monocyte proinflammatory cytokines IL-8 and IL-12 [15].

The functional proinflammatory role of TNF-α has been shown in experimental models of immune complex-mediated glomerulonephritis, in which TNF-α deficiency or TNF-α blockade treatments clearly offer protection against glomerular injury. For instance, in the well described accelerated nephrotoxic serum nephritis model, systemic administration of TNF-α exacerbated glomerular injury in rats [16]. The association between MIF and autoimmune kidney disease was further investigated by Bruchfeld*et al.,*in which they were suggested that high MIF levels in patients with kidney diseases are closely linked to endothelial activation and that MIF might play a role in the vascular disease associated with chronic kidney impairment [17]. In this study, therefore, the genotypes of the MIF and TNF-α SNPs were identified in subjects with NS to determine the correlation between the genotypes/allotypes and cytokine serum concentration/ coclinico-pathological features.

MATERIALS AND METHODS

Patients: A total of Forty – six Iraqi patient clinically diagnosed as nephrotic syndrome by the Department of Pediatrics in AL-Diwaniya Maternity and Children Teaching Hospital and 25 age and sex matched healthy children as controls, between December 2013 to May 2014 were included in the study. Patient had features of systemic illness causing NS were excluded from the study were included in this study. All children were subjected to a detailed history and physical examination. In addition, the following biochemical tests were done to confirm the diagnosis of nephrotic syndrome-serum creatinine, total protein, albumin, cholesterol. Informed consent for the genetic analysis was obtained from all patients and/or their parents.

Genotyping: The genotypes of the MIF G-173C and TNF-α G308Ageneswere determined by PCR‑restriction fragment length polymorphism (RFLP) Table (1). The PCR products were purified using a AccuPower^{TM} PCR PreMix (Bioneer),then the PCR products were visualized in an ethidium bromide-stained 1.5% agarose gel using a UV Transilluminator. Following which they were digested with the Nco1 restriction enzymes for TNF-α &AluI restriction enzymes for MIF. The digested PCR products were visualized in an ethidium bromide-stained 2.5% agarose gel using a UV Transilluminator.

Serum cytokine assay: Serum concentrations of TNF- α IL-13were measure by using ELISA Kit (RayBio) Following the manufacturer's instructions.

Statistical analysis: The Hardy–Weinberg equilibrium (HWE) assumption was assessed for both the patient and control groups by comparing the observed numbers of each genotype with those expected under the HWE for the estimated allele frequency. Data were presented, summarized and analyzed using two software programs. These were the Statistical Package for Social Science (SPSS) version 20 and Microsoft Office Excel 2010.Logistic regression analysis was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) for the association between the genotypes, alleles or haplotypes and the risk of NS. The results are presented as the mean values ± 1 standard deviation (SD), and a P value of ≤ 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Demographic and Biochemical Profile: The demographic and biochemical profiles of both NS patient and control groups are shown in Table (2). In which there is a significant difference between the NS patients and controls at all the biochemical parameters except age, and male to female ratio.

Distribution of MIF G173C, TNF-α G308A Genotypes and Alleles in Patient and Control Group: Distribution of MIF G-173C , TNF-α G308A polymorphism was detected by PCR-RFLP technique, at this locus there're three genotype; for MIF G-173C GG, GC and CC with band sizes 100

pb, 100/263 pb and 363 pb respectively Figure (1),and for TNF-αG308A GG, GA and AA with band sizes 87 pb, 87/107 pb and 107 pb respectively, figure (2).The frequency distribution of genotypes and alleles of MIF G-173C , TNF-α G308A in patient and control groups are summarized in Table (3).In this study, we genotyped SNPs in the MIF and TNF-α genes in a group of patients with NS to determine the correlation between the genotypes/allotypes and their clinical features. Vivarelli et al. [18]. genotyped MIF G-173C in 257 Italian children with NS and found that the frequency of the C allele (high producer) was higher in patients than in controls. Similarly, Berdeli et al. [19]. investigated the MIF G-173C SNP in 214 children with idiopathic NS and found that the frequencies of the GC genotype and C allele were higher in the patients than in the control subjects. They also found an increased CC genotype frequency in patients with SRNS compared to those with SSNS. Although, in our study there was no statistical significant in the MIF G-173C genotypes/allotypes ,but the CC genotype has obviously suggests as an etiological factor for NS, as it had an OR of 3.6 and Etiologic Fraction (EF) of 0.619 , In contrast, the GG genotype had rather preventive role as it had Protective Fraction (PF) of 0.140 and low OR (0.794). With The possibility of G allele may be protective, whereas the C allele may increase susceptibility to NS. This may be due to the potential effects of macrophage migration inhibitory factor on the natural immune response due to the inhibition of immune cell activation, which is regulated by glucocorticoids [19] ,as MIF is expressed in glomerular parietal and visceral epithelial cells and in tubular epithelial cells in the kidney [20], which plays important physiological roles in the regulation of macrophage function, and lymphocyte immunity, as well as a pathogenetic role in some immunologically induced kidney diseases [21]. The low frequency of the 173C allele in our study may be one of the causes of the lack of a statistically significant association between the MIFG –173C allele and the INS in children. Because it limits the power of the study. TNF-α showed a strong association at genotypic level (OR = 14.671), as well as at allelic level (OR = 2.002), which demonstrates that this may be one of the risk factors for INS and may be affect the steroid response. Polymorphism at position 308 of the TNF-a promoter, representing G to A base transitions, has been linked to increased TNF transcription [22]. Earlier studies have shown increase of TNF-a synthesis and gene expression in patients with idiopathic NS and focal glomerular sclerosis [23].

Serum cytokine: Mean serum concentration of the IL-13(36.70±0.55) versus (2.72±0.22) and TNF-α (668.33±27.60) versus (45.64±2.38); was significantly higher in the patient group in comparison to control group, with a p-value of (<0.001) table (4).These results observe a significant association between concentration of TNF-α and NS , this may be due to its important role in pathophysiology of NS as the TNF-α expression in renal disease has been found in both resident cells and infiltrating monocytes-macrophages [24], and It acts in a paracrine way to recruit monocytes and macrophages to the glomerular region ,besides acting with other mediators to increase vascular permeability [25]. This would cause alteration of the barrier function of the capillary wall, leading to protein urea. Other opinion revealed that the TNF-α is down regulated by corticosteroids [24] and also by the shift of CD4-T cell differentiation to TH2 instead of TH1 cells with a consequent reduction of TH1 cytokine production, especially TNF [26]. Regarding the mean concentration of the IL-13 in NS, Several studies have suggested a potentially important role for IL-13.As it expression was associated with the elevation of CD4+ and CD8+ T cells in children with SSNS during a relapse [27], and overexpression led to renal injury that resembled MCNS, thus providing further support for potentially causative role of IL-13 in nephrotic syndrome [28]. Printza et al., reported that serum IL-13 levels were significantly higher in the active stage of NS compared with the two remission phases and that although IL-13 levels were even lower than in the active stage, they remained elevated during both remission phases compared with the controls [29]

CONCLUSION

There is significantly higher Concentration of IL-13 and TNF-α in NS patients in comparison to control group. This provides strong evidence that pro-inflammatory cytokines play a major role in the pathogenesis of NS. TNF- α -G308A polymorphism with AA genotype and A allele are mainly expressed among nephrotic syndrome patients and susceptibility with disease might be prospected whereas G allele might serve as protective factors for the disease.

Table 1: The primer sets and restriction enzymes used for the PCR–RFLP analyses

Gene Variations	Restriction enzymes	Primers used for PCR analysis
MIF G173C	AluI	F: 5′-CTAAGAAAGACCCGAGGCGA-3′ R: 5′-GGCACGTTGGTGTTTACGAT-3′
TNF-α G308A	Nco1	F- 5AGGCAATAGGTTTTGAGGGCCAT-3 R-5 TCCTCCCTGCTCCGATTCCG-3

Table 2: Biochemical and demographic characteristics of nephrotic patients and controls

Parameters	Patients =46(mean ± SE)	Controls = 25 (mean ± SE)	P value
Age	10.30±0.78	10.36±0.66	0.957
Male/female	1.56:1	1.5:1	0.943
Serum cholesterol (mg/dl)	479.11±16.63	179.72±3.99	<0.001
Serum total protein (g/dl)	4.48±0.05	7.38±0.07	<0.001
Serum albumin (g/dl)	1.88±0.05	4.02±0.09	<0.001
Serum createnin (mg/dl)	0.86±0.02	0.59±0.04	<0.001

Table 3: Distribution of MIF G173C and TNF-a-G308A genotypes and alleles in patient and control group

Genotype	Patient's N = 46 (%)	Controls N = 25 (%)	P value	OR (95% CI)	EF	PF
MIF G-173C						
GG	25 (54.35)	54.35 (60.00)	0.803	0.794 (0.295-2.132)	---	0.140
GC	15 (32.61)	32.61 (36.00)	0.798	0.860 (0.309 -2.393)	---	0.092
CC	6 (13.04)	13.04 (4.00)	0.409	3.600 (0.408- 31.741)	0.619	---
G	65 (70.65)	70.65 (78)	0.429	0.679 (0.658.-3.296)	---	0.228
C	27 (29.35)	29.35 (22)	0.429	1.473 (0.303-1.520)	0.228	---
TNF-α-G308A						
GG	21 (45.65)	20 (80)	0.006	0.210 (0.067-o.656)	---	0.658
GA	15 (32.61)	5 (20)	0.287	1.935 (0.608-6.160)	0.363	---
AA	10 (21.74)	0 (0)	0.011	14.671* (0.822- 261.837	0.889	---
G	57 (63.33)	45 (90)	<0.001	0.181 (0.066-0.499)	---	0.717
A	35 (38.88)	5 (10)	<0.001	5.526 (2.002.-15.254)	0.717	---

Table 4 :Serum concentration of TNF-α and IL-13 in patients and control group

cytokine	Control		Patients		
	Mean	SE	Mean	SE	P-value
TNF-α	45.64	2.38	668.33	27.60	<0.001
IL-13	2.72	0.22	36.70	0.55	<0.001

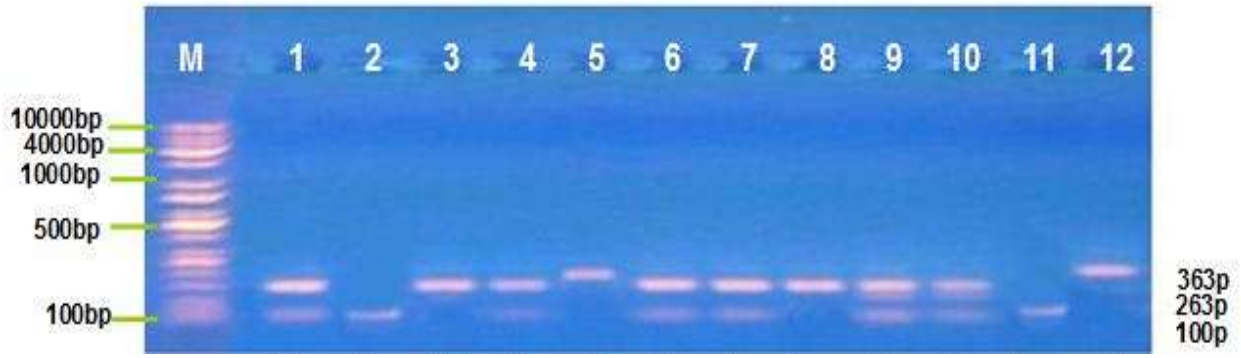

Figure (1):MIF G173C electrophoresis after restriction digestion with Alu1
Lane (M): DNA molecular size marker (KAPA Universal Ladder), 2,11= Homozygous for wild type genotype (100bp), Lane 3.5.12= Homozygous for mutant genotype (363bp), Lane 1,4,6,7,8,9,10=heterozygous genotype (100/263bp).

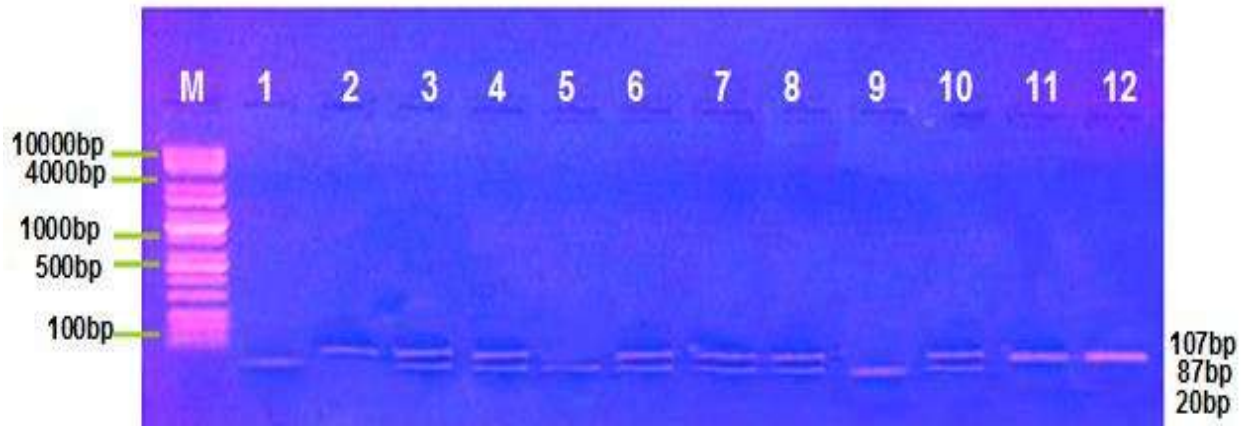

Figure (2):TNF-α G308A electrophoresis after restriction digestion with Nco1
Lane (M): DNA molecular size marker (KAPA Universal Ladder), Lane 1,5,9,= Homozygous for wild type genotype (87bp), Lane 2,11,12= Homozygous for mutant genotype (107bp), Lane 3,4,6,7,8, 10= heterozygous genotype (87/107bp).

REFERENCES

1- Krishnaswany D et al. Serum proteins, initial and follow-up lipid profile in children with nephrotic syndrome. IJABPT 2011;2:59-63.
2- Steddon S , Ashman N, Chesser A , Cunningham J. Oxford Handbook of Nephrology and Hypertension , 2nd ed, C&C Offset Printing Co. Ltd.;oxford, UK, 2014:pp.454-557
3- Tan Y et al. Elevated Levels of Immunoglobulin E May Indicate Steroid Resistance or Relapse in Adult Primary Nephrotic Syndrome, Especially in Minimal Change Nephrotic Syndrome. J. of Int. Med. Res 2011.; 39: 2307-:23
4- Robert C , Aileen B. Nephrotic Syndrome. Pediatrics in Review 1993;14;30-38
5- Tudorache E et al. Congenital nephrotic syndrome with acute renal failure. Pediatr. Nephrol.2012 :pp.27:49
6- Burgstein JM. Nephrotic syndrome. In: Nelson Textbook of pediatrics, 18th ed , Behrman RE, Kliegman R, Jenson H; Philadelphiaq, Saunders WB, 2008: 2430-2442
7- Hawkins P, McMillan J , Angelis C , Feigin R. Nephrotic syndrome In: Oski pediatrics principles and practice. 3rd ed. Philadelphia: Lippincott Williams and Wilkings,2000:pp.1590-1599.
8- Falk J , Jennette J, Nachman P. Primary glomerular disease. In: The Kidney, 7th ed, Brenner B , Levine S; Philadelphia: Saunders WB,2004:pp.1295-1307
9- Park S , Shin J. Complications of nephrotic syndrome. Korean J. Pediatr. 2001; 54: 322-328
10- Mathieson P. Cytokine polymorphisms and nephrotic syndrome. Clin. Sci. 2002;102:513–514.

11- Tenbrock K et al. Type I IgE receptor, interleukin 4 receptor and interleukin 13 polymorphisms in children with nephrotic syndrome , Clin. Sci.2002;102:507–512
12- Mann C et al. Interleukin 1 genotypes in multiple sclerosis and relationship to disease severity. J. Neuroimmunol. 2002;129:197–204.
13- Ortega L , Fornoni A. Role of cytokines in the pathogenesis of acute and chronic kidney disease, glomerulonephritis, and end-stage kidney disease. Int. J. of Inter. Cyt. and Mediat. Res.2010;2:49-62
14- Cheung W et al. Atopy, serum IgE, and interleukin-13 in steroid responsive nephrotic syndrome. Pediatr. Nephrol.2004;19(6):627-632
15- Chen SP et al. Childhood nephrotic syndrome in relapse is associated with down-regulation of monocyte CD14 expression and lipopolysaccharide- induced tumor necrosis factor-alpha production. Clin. Exp. Immunol. 2003 ;134: 111–119.
16- Ernandez T , Mayadas N. Immunoregulatory role of TNFα in inflammatory kidney diseases. Int. Society of Nephrol. 2009; 76: 262–276
17- Bruchfeld A et al. Elevated serum macrophage migration inhibitory factor (MIF) concentrations in chronic kidney disease (CKD) are associated with markers of oxidative stress and endothelial activation. Mol. Med. 2009; 15(3-4):70
18- Vivarelli M et al. Association of the macrophage migration inhibitory factor −173*C allele with childhood nephrotic syndrome. Pediatr Nephrol. 2008; 23:743–748
19- Berdeli A et al. Association of macrophage migration inhibitory factor -173C allele polymorphism with steroid resistance in children withnephrotic syndrome. Pediatr Nephrol.2005; 20:1566–1571
20- Zhang W et al. Effect of MDR1 gene polymorphism on progression of end-stage renal disease. Acta. Pharmacol. Sin. 2007 ;28:579–583
21- Lolis E. Glucocorticoid counter regulation: macrophage migration inhibitory factor as a target for drug delivery. Curr Opin Pharmacol. 2001 ;1:662–668
22- Wilson A. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc. Natl. Acad. Sci. USA. 1997;1:3195–9.
23- Matsumoto K. Spontaneous and LPS-stimulated release of tumor necrosis factor-alpha by peripheral blood monocytes in patients with focal glomerular sclerosis. Nephron.1995;70:118–119.
24- Abott F et al. Interleukin-1beta stimulates human mesangial cells to synthesize and release interleukins-6 and -8. Kidney int. 1991; 40(4):597–605.
25- Gruss H. Molecular, structural and biological characteristics of the tumor necrosis factor ligand superfamily. Int. J. of clin. & lab. Res. 1996 ;26(3):143–59.
26- Daniel V et al. T-lymphocyte populations, cytokines and other growth factors in serum and urine of children with idiopathic nephrotic syndrome. Clin. Nephrol.1997;47(5):289–297.
27- Yap H et al. Th1 and Th2 cytokine mRNA profiles in childhood nephrotic syndrome: evidence for increasedIL-13 mRNA expression in relapse. J. Am. Soc. Nephrol.1999;10(3):529-537
28- Lai K et al. Overexpression of interleukin-13 induces minimal-change-like nephropathy in rats. J. Am. Soc. Nephrol.2007;18:1476–1485
29- Printza N et al. IL-18 is correlated with type-2immune response in children with steroid sensitive nephrotic syndrome. Cytokine 2008;44(2):262-8