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ASSOCIATION OF GENE POLYMORPHISM OF MIF, TLR4 AND CD14 AS A POTENTIAL BIOMARKER FOR ENDOMETRIOSIS

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ABSTRACT Endometriosis is the most frequent gynecologic disorder associated with pelvic pain and infertility. Numerous genetic polymorphisms in genes enclose the relationship among the polymorphisms and endometriosis. Present study was designed to know the effect of genetic polymorphism in immune related genes i.e.: CD14, MIF and TRL4 in endometriosis pathogenesis. From all women involved in the research, a 2mL of peripheral blood sample were collected to perform genotyping for CD14 -159C>T, MIF-173G>C and TRL4-299A>G. Variance in allelic and genotype frequencies was statistically evaluated between the control group and the group of patients. The analyzed group consisted of total 337 individuals of which 136 women were suffering from endometriosis and 201 were in the control group. C/T genotype of CD14, G/C genotype of MIF and A/G genotype of TLR4 gene was found to be predominant in patients when compare to control and which shows statistically significant. Homozygous variant for CD14 and MIF were also shown significant difference but small sample size was not taken into the consideration. This study shows genetic and functional polymorphisms in immune related genes might affect the disease pathogenesis. These Genes are also considered as biomarkers for diagnosis of endometriosis.

KEYWORDS : Endometriosis, MIF, TLR4, CD14, biomarker, polymorphism

INTRODUCTION

Endometriosis is one of the most important gynecological diseases of women of reproductive age and is collectively responsible for significant morbidity. It affects 10–20 % of women during their reproductive age Lin W et al., (2010), Govindan S et al., (2007), Giudice LC et al. (2004). Endometriosis described as the occurrence of endometrium-like tissue external to the uterine cavity. Genetics refers to characteristics that are heritable through genes and helps to explain familial clustering of disease. However, the etiology of endometriosis remains uncertain .It is a multifactorial disorder with unknown causes, but may be determined by combination of multiple genes, environmental factors, hormonal factors, and alterations in the endocrine or immune system Buck Louis GM et al. (2007), Bellelis P et al. (1992)

According to Sampson's implantation theory, endometrial cells pierce the abdominal cavity ensuing in retrograde menstruation, implants on peritoneal surfaces and develop there. Even though retrograde menstruation is observed in the majority of women, merely a few women develop endometriosis. Due to this effect molecular and/or genetic defects or changes in endometrial cells are considered to be a prerequisite for a triumphant implantation and growth of endometriosis lesions.

There are numerous factors which are involved which make it difficult to determine the mode of inheritance for endometriosis. The gold standard to diagnose the endometriosis is surgical assessment, invasively by laparotomy or laparoscopy, and a scoring method has been developed to evaluate degree of disease. The classic diagnosis of the disease, laparoscopy is allied with risks and cost. The vaginal ultra sound and the biomarker such as CA-125 are not diagnostically helpful in early stages of the disease. There is rising indication that before time non-invasive test for the diagnosis of endometriosis is required. This establishment of non-invasive diagnostic methods for endometriosis requires sensitive and disease specific biomarkers.

Reproductive characteristics associated with increased risk of endometrial cancer include nulliparity, infertility, early age of menarche, and late age of menopause McPherson CP et al. (1996). Long-term uncontrolled estrogen usage is related with type I endometrial cancers. Estrogen substitute therapy specified to control menopausal symptoms increases the hazard of emergence of endometrial cancer by 2- to 20-fold, by rising risk correlating with the duration of use.

The estimated growing risk of developing endometrial cancer by age 70 is 16% for MSH6, 21% for MSH2 and 54% for MLH1 mutations Bonadona V et al. (2011). This threat of endometrial cancer rises after the age of 40, with a mean age of diagnosis of 46 years. Somatic mutations in the PTEN gene are normal in sporadic endometrial cancer (Zhou XP et al. (2002). A germ line PTEN mutation can be found in patients with Cowden syndrome, and patients who comprise this a typical autosomal dominant familial syndrome are at greater risk for breast, thyroid, and endometrial cancer Eng C. (2003). The link between germ line mutations in BRCA genes and the possibility of endometrial cancer remains controversial Levine DA et al. (2001). The cytokine macrophage migration inhibitory factor (MIF), CD14, and Toll-like receptor 4 (TLR4) are linked with each other which obviously defined roles in immunologic and inflammatory pathways Ding L et al., (2017), Roger T David J et al.(2001) suggest that subgroup analyses sorted by ethnicity, only polymorphism of rs4986791 had a significant influence on decrease of cancer risk among both Caucasian and Asian populations. Some findings suggested that TLR4 polymorphisms may serve as a genetic risk factor for cancers Francisco D et al. (2017) suggest that the rs2294021 (CCDC22 gene) polymorphism could be associated with increased susceptibility to endometriosis in Brazilian women when the allele C is present. Numerous studies reported that genetic polymorphisms in CD14, TLR4 and MIF have increase susceptibility to various diseases. MIF, CD14 AND TLR4 genes are Essential Role in the Immune System and Cell Growth. These genes participate in inflammatory and immune response.

MIF plays an important role in endothelial cell proliferation and differentiation which is released by ectopic endometrial cells (Yang Y Degranpre P et al. (2000), Nishihira J et al. (2003).Increased expression of MIF in women leads to endometrial lesions particularly in those women with infertility and pelvic pain as it regulates the host

immune system which promotes pro inflammatory functions of immune systemKats R et al. (2002), Morin M et al.(2005).

Toll-like receptors (TLRs) recognize specific pathogen associated molecular patterns and serve an essential role in the innate immune system by initiating and directing immune response to microbial pathogens. Human immune system detect Toll like receptors (TLRs) present on mucosal surfaces of the respiratory, gastrointestinal, urinary tracts, endothelial, and endometrial cells Takeda K et al. (2003). TLR4 gene is expressed in the human endometrium and their regulation might be crucial for the pathogenesis of endometrial diseases. It is involved in altered gene expression levels leading to endometriosis and endometrial cancer.

CD14 plays an important role as a marker for endothelial cells and activated macrophages The present study was designed to investigate whether the genetic and functional polymorphism in MIF, TLR4, and CD14 genes affect susceptibility to endometriosis. These genes play an important l role in the innate immunity by initiating and directing immune response to pathogens

MATERIALS & METHODS

Study population

A Total of 136 endometriosis patients and 201 controls were recruited from Owaisi Hospital & Research Centre and MHRT Hyderabad, India in the present study. Written consent was taken from the entire subject. The study was approved Institutional Ethics Committee, MHRT, and Hyderabad. Study duration was for 2 years (Jan 2016- Nov 2017). Detailed information on clinical was recorded through Performa. Our sample size of 337 is large enough and exceeds the estimated number of samples (~200 cases + controls) required to obtain a 90 % statistical power.

DNA Extraction:

Genomic DNA was extracted from 2ml of peripheral blood sample was collected from each participant. DNA was extracted from peripheral blood samples using salting out method Jawdat N. Gaaib et al. (2011) and DNA was gel checked using 0.8% Gel.

Genotyping:

Genotyping were done for CD14, TLR4 and MIF genes. Primers were taking from the published paper Fei BY et al. (2008), Morange PE et al. (2004) Karhukorpi J et al. (2002) Genotyping of CD14 -159C>T and MIF-173G>C polymorphism were performed using the tetra primer amplification refractory mutation system polymerase chain reaction. TLR4 (299A>G) Polymorphism was performed by allele-specific amplification as described previously. All amplifications were repeated thrice and were analyzed using agarose gel electrophoresis system

Statistical analysis

The genotypic distribution of CD14, TLR4 and MIF gene was performed using ^{2}-test to determine significant differences in allele/genotype frequencies between patients and controls by comparing the different percentages. Distribution of genotypes and alleles between Endometriosis and control groups were tested using Fisher's exact test. Since differences between conditional logistical regression and unconditional logistical regression were small, unconditional logistical regression was used to estimate odds ratio (OR) and 95% confidence interval (CI). The above statistical analysis was performed using Graph pad prism version 5.0 (GraphPad Software, Inc., San Diego, California, USA).

RESULTS

Genotyping of the CD14 Gene polymorphism showing three genotypes CC, CT and TT in the **image 1**, MIF gene polymorphism showing different genotypes CC, GG and GC in the **image 2** and TLR4 gene polymorphism : M 100bp ladder different genotypes AA, AG and GG genotypes and **image 3**

The average age of the women with diagnosed endometriosis (32.39 ± 6.31 years) was higher in comparison with average age of women in the control group (28.62 ± 5.12 years).A frequency in individual alleles and genotypes in the patients and the check group are summarized in Tables 1 and 2 for both studied polymorphism In polymorphism of CD14 gene the most frequent occurring allele in both the groups was T with a frequency of 0.37 in the patients and 0.26 in the controls. (**Table1**). The C/T genotype of CD14 gene was found to statistically significant in endometriosis patients when compare to control which shows 1.80 fold increase risk of disease (OR 1.80, 95% CI 1.14-2.85, p=0.0025). (**Table 2**).T/T genotype of CD14 gene was also revealed significant difference but due to small number in patients and control significant was not taken for consideration (OR 4.77, 95% CI 1.57-14.52)

In polymorphism of MIF gene the most frequent occurring allele in both the groups was C with a frequency of 0.41 in the patients and 0.21 in the controls. (**Table3**). The G/C genotype of MIF gene was found to be predominant in endometriosis patients with 3.98 folds increased risk of disease when compared to control (OR 3.98, 95% CI 2.45– 6.46, p <0.0001) (**Table 4**). Homozygous variant (C/C) was observed in both the group. C/C genotype was found 11% in endometriosis patients and 5% in control.

In polymorphism of TLR4 gene the most frequent occurring allele in both the groups was G with a frequency of 0.33 in the patients and 0.24 in the controls. (**Table5**). The A/G genotype of TLR4 gene was found to predominant in endometriosis patients which was statistically significant when compare to control which shows 1.81 fold increase risk of disease (OR 1.81, 95% CI 1.15-2.85, p=0.02). (**Table6**). G/G genotype of TLR4 did not reveal any significant difference between endometriosis patients and control (OR 2.29, 95% CI 0.84-6.26)

DISCUSSION

Endometriosis is an immune related disease with polygenic heredity which reduces the quality of life in the reproductive age women Acien, P et al. (2013).The etiology of endometriosis still remains unclear. Genetic studies help us to understand the basic concept of the disease**.** The present study, revealed that the association of candidate CD14, TLR4 and MIF genes with susceptibility to endometriosis. It demonstrate that statistically significant associations with risk of endometriosis were observed for the candidate CD14 -159C>T, MIF-173G>C and TLR4 (299A>G) genes. Immunological changes in the body are the result of endometriosis MIF regulates innate immune responses by modulating the of pattern recognition receptors like TLR4. TLR4, a signal-transducing receptor, is solely responsible recognizing bacterial lipopolysaccharide. In brief, TLR4 particularly binds with lymphocyte antigen 96 (MD2) and, along with CD14 it forms a lipopolysaccharide complex which helps in lipopolysaccharide recognition and nuclear factor kappa B activation Calvano JE et al. (2004). Various reports strongly reiterate that genetic polymorphisms in CD14-159C > T specifically affect CD14 regulation and enhances susceptibility to endometriosis Lin J et al., (2007), Wang F et al. (2007), Obana N et al. (2002) On the other hand, the TLR4-299A > G polymorphism might trigger the inflammatory cascade in a similar fashion, as reported earlier Torok HP et al.(2004), Franchimont D et al.(2004) and therefore might play a selective role in determining susceptibility to endometriosis.

CD14, TLR4, and MIF genes are most crucial and intertwined determinants of innate immunity. A well-orchestrated and synchronized interaction among these genes is needed for an optimal first line of defense Admetlla AF et al.(2008). Alteration in this normal process notably dislodges the innate immunity network, leading to immunologic imbalance. Functional mutation in one of these genes is a vital factor that attenuates their corresponding protein expression levels and might also raise susceptibility to disease, may be immune-related disorders.

The polymorphism in the MIF gene (173 G > C), though did not influence disease susceptibility, but alters the expression pattern overall. These results are in exact conformity with many earlier studies supporting the roles of CD14-159C > T and TLR4- 299A > G polymorphisms in enhancing susceptibility to Chlamydia pneumoniae infection Eng HL et al.(2003)sub gingival period on to pathogens(Schulz S et al., 2008) and Behcet's disease in a Korean population is reported Horie Y et al.(2009)

Yet, a few complementary reports have established no association between these polymorphisms and the risk of developing endometriosis (Guo QS et al., 2005) and invasive meningococcal disease patients.

In recent years, there has been large influx of polymorphism studies on various genes that are either directly or indirectly take part in the disease process. MIF, CD14, and TLR4 genes represents a triad that regulates innate immunity; the present study attempted to demonstrate

the significance of SNPs in candidate genes (CD14, MIF and TLR4) of innate immunity in increasing the risk of endometriosis.

These three candidate genes CD14, TLR4 and MIF plays an important role in immune system thereby initiating and directing immune response to pathogens which are expressed in human endometrium which is crucial for pathogenesis of endometrial diseaseMorange PE et al.(2004) Karhukorpi J et al.(2002) Hoi AY et al. (2007)

On the basis of achieved results it is possible to state that polymorphism CD14 -159C>T, MIF-173G>C and TLR4 -99A>G genes plays an important role in the susceptibility of endometriosis.

In conclusion, this study demonstrated that polymorphisms in the CD14, MIF and TLR4 genes confer developing endometriosis as they are interlinked with each other. Further studies are required to gain insight on how SNPs in each of the genes alter the protein structure and interaction, resulting in perturbation of the delicate immunologic balance in endometriosis.

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Table 1: CD14 allele frequency distribution in controls and Endometriosis patients

	Controls		Patients	
Allele	**Number**	**Frequency**	**Number**	**Frequency**
C	298	0.74	172	0.63
T	104	0.26	100	0.37

Table 2: CD14 genotypic frequency distribution in controls and Endometriosis patients

Model	Genotype	Controls	Patients	OR (95% CI)	P-value
Co-dominant	C/C	102 (50.8%)	47 (34.6%)	1.00	0.0025
	C/T	94 (46.8%)	78 (57.4%)	1.80 (1.14-2.85)	
	T/T	5 (2.5%)	11 (8.1%)	4.77 (1.57-14.52)	

Table 3: MIF allele frequency distribution in controls and Endometriosis patients

	Controls		Patients	
Allele	**Number**	**Frequency**	**Number**	**Frequency**
G	316	0.79	160	0.59
C	86	0.21	112	0.41

Table 4: MIF genotypic frequency distribution in controls and Endometriosis patients

Model	**Genotype**	**Controls**	**Patients**	**OR (95% CI)**	**P-value**
Co-dominant	G/G	125 (62.2%)	39 (28.7%)	1.00	**<0.0001**
	G/C	66 (32.8%)	82 (60.3%)	3.98 (2.45-6.46)	
	C/C	10 (5%)	15 (11%)	4.81 (2.00-11.56)	

Table 5: TLR4 allele frequency distribution in controls and Endometriosis patients

	Controls		Patients	
Allele	**Number**	**Frequency**	**Number**	**Frequency**
A	305	0.76	182	0.67
G	97	0.24	90	0.33

Table 6: TLR4 genotypic frequency distribution in controls and Endometriosis patients

Model	**Genotype**	**Controls**	**Patients**	**OR (95% CI)**	**P-value**
Co-dominant	A/A	112 (55.7%)	55 (40.4%)	1.00	**0.02**
	A/G	81 (40.3%)	72 (52.9%)	1.81 (1.15-2.85)	
	G/G	8 (4%)	9 (6.6%)	2.29 (0.84-6.26)	

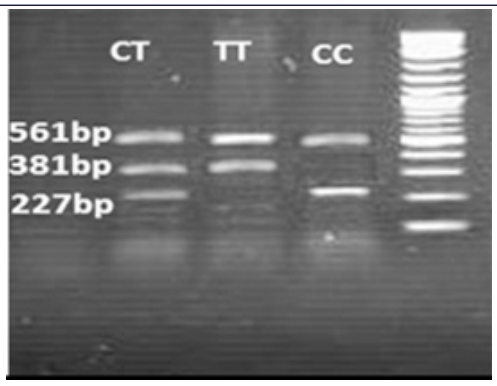

Image 1 depicting CD14 gene polymorphisms showing different genotypes CC, GG, GC and 100bp marker

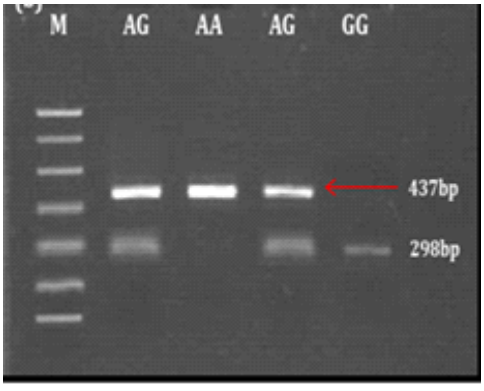

Image 2 depicting TLR4 gene polymorphisms showing different genotypes CC, GG, GC and M 100bp marker

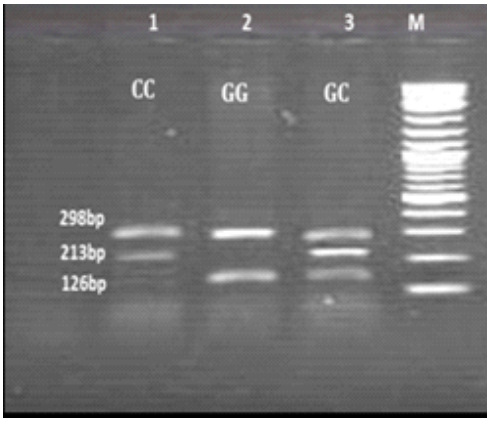

Image 3 depicting MIF gene polymorphisms showing different genotypes CC, GG , GC and M 100bp marker

REFERENCES

1. Acien, P & Irene Velasco .(2013). Endometriosis: a disease that remains enigmatic. ISRN Obstetrics Gynecology242149
2. Admetlla AF, Bosch E, Sikora M, Marques-Bonet T, Ramirez-Soriano A, Muntasell A, Navarro A, Lazarus R, Calafell F, Bertranpetit J & Ferran Casals. (2008).Balancing selection is the main force shaping the evolution of innate immunity genes. J Immunol, 1811315-1322.
3. Bellelis P, Podgaec S & Abrao MS. (1992).Environmental factors and endometriosis, Rev Assoc Med Bras. 2011, 57448-52.
4. Bonadona V, Bonaiti B, Olschwang S, Grandjouan S, Huiart L, Longy M Guimbaud R, Buecher B, Bignon YJ, Caron O, Colas C,Noguès C, Lejeune-Dumoulin S, Olivier-Faivre L, Polycarpe-Osaer F, Nguyen TD, Desseigne F, Saurin JC, Berthet P, Leroux D,Duffour J, Manouvrier S, Frébourg T, Sobol H, Lasset C & Bonaiti-Pellie C. (2011) .Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA, 3052304-2310.
5. Buck Louis GM, Hediger ML, Peterson CM, Croughan M, Sundaram R,Stanford J, Chen Z, Fujimoto VY, Varner MW, Trumble A, Giudice LC. (2011).Incidence of endometriosis by study population and diagnostic method: the ENDO study. Fertil Steril, 96360.
6. Calvano JE, Agnese DM, Um JY, Goshima M, Singhal R, Coyle SM, Reddell MT, Kumar A, Calvano SE & Lowry SF(2003) . Modulation of the lipopolysaccharide receptor complex (CD14, TLR4, MD-2) and Toll-like receptor 2 in systemic inflammatory response syndrome-positive patients with and without infection: relationship to tolerance. Shock, 20415.
7. Ding L, Jiang Q, Li G, Shen J, Du J, Lu X & Xiong X . (2017). Comprehensive assessment of association between TLR4 gene polymorphisms and cancer risk: a systematic meta-analysis. Oncotarget, 8100593-100602

8. Eng C. (2003).PTEN one gene, many syndromes. Hum Mutat, 22183-198.
9. Eng HL, Chen CH, Kuo CC, Wu JS, Wang CH & Lin TM. (2003).Association of CD14 promoter gene polymorphism and Chlamydia pneumoniae infection. J Infect Dis,18890-97
10. Fei BY , LV HX , Yang JM & Ye ZY. (2008). Association of MIF-173 gene polymorphism with inflammatory bowel disease in Chinese Han population. Cytokine ;4144–7.
11. Francisco D, de Paula Andres M, Gueuvoghlanian-Silva BY, Podgaec S, & Fridman C ,. (2017). CCDC22 gene polymorphism is associated with advanced stages of endometriosis in a sample of Brazilian women. J Assist Reprod Genet 34939-44.
12. Franchimont D, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Devière J & Rutgeerts P. (2004).Deficient host–bacteria interactions in inflammatory bowel disease? The Toll like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. Gut, 53987-992.
13. Giudice LC, Kao LC. (2004)Endometriosis. Lancet, 364 1789-1799.
14. Govindan S, Ahmad SN, Vedicherla B, Kodati V, Jahan P, Rao KP Ahuja YR, Hasan Q.(2007). Association of progesterone receptor gene polymorphism (PROGINS) with endometriosis, uterine fibroids and breast cancer. Cancer Biomark, 373–78.
15. Guo QS, Xia B, Jiang Y, Morre SA, Cheng L, Li J, Crusius J & Pena A. (2005). Polymorphisms of CD14 gene and TLR4 gene are not associated with ulcerative colitis in Chinese patients. Postgrad Med J, 81526–529.
16. Hoi AY, Iskander MN & Morand EF. (2007).Macrophage migration inhibitory factor: A therapeutic target across inflammatory diseases. Inflamm Allergy Drug Targets, 6183-273
17. Horie Y, Meguro A, Ota M, Kitaichi N, Katsuyama Y, Takemoto Y, Namba K, Yoshida K, Song YW, Park KS, Lee EB, Inoko H, Mizuki N & Ohno S. (2009). Association of TLR4 polymorphisms with Behcet's disease in a Korean population. Rheumatology, 48638-642.
18. Jawdat N. Gaaib, Adnan F. Nassief & Akeel H. Al-Assi. (2011). Simple salting –out method for genomic DNA extraction from whole blood. Tikrit Journal of Pure Science 162
19. Karhukorpi J, Yan Y, Niemela S, Valtonen J, Koistinen P, Joensuu T, Saikku & Karttunen R. (2002). Effect of CD14 promoter polymorphism and H. pylori infection and its clinical outcomes on circulating CD14. Clin. Exp. Immunol, 128326-332.
20. Kats R, Collette T, Metz CN & Akoum A. (2002).Marked elevation of macrophage migration inhibitory factor in the peritoneal fluid of women with endometriosis. Fertil Steril, 7869-76.
21. Levine DA, Lin O, Barakat RR, Robson ME, McDermott D, Cohen L, Satagopan J , Offit K & Boyd J. (2001).Risk of endometrial carcinoma associated with BRCA mutation. Gynecol Oncol, 80395-8.
22. Lin J, Yao YM, Yu Y, Chai JK, Huang ZH, Dong N & Sheng ZY. (2007).Effects of CD14159 C/T polymorphism on CD14 expression and the balance between proinflammatory and anti-inflammatory cytokines in whole blood culture. Shock, 28148-153.
23. Lin W, Chen S, Li M, Wang B, Qu X, Zhang Y (2010).Expression of macrophage migration inhibitory factor in human endometriosis: relation to disease stage, menstrual cycle and infertility. J Obstet Gynaecol Res, 36344-5.
24. McPherson CP, Sellers TA, Potter JD, Bostick RM, Folsom AR.(1996). Reproductive factors and risk of endometrial cancer. The Iowa Women's Health Study. Am J Epidemiol, 1431195-1202.
25. Morange PE, Tiret L, Saut N, Luc G, Arveiler D, Ferrieres J Amouyel P, Evans A, Ducimetiere P, Cambien F & Juhan-Vague I. (2004). TLR4/Asp299Gly, CD14/C-260T, plasma levels of the soluble receptor CD14 and the risk of coronary heart disease. Euro. J. Human. Gene, 121041-1049.
26. Morin M, Bellehumeur C, Therriault MJ, Metz C, Maheux R & Akoum A. (2005). Elevated levels of macrophage migration inhibitory factor in the peripheral blood of women with endometriosis. Fertil Steril, 83865-72.
27. Nishihira J, Ishibashi T, Fukushima T, Sun B, Sato Y & Todo S. (2003). Macrophage migration inhibitory factor (MIF): its potential role in tumor growth and tumor-associated angiogenesis. Ann N Y Acad Sci; 995171-82.
28. Obana N, Takahashi S, Kinouchi Y, Negoro K, Takagi S, Hiwatashi N & Shimosegawa T. (2002).Ulcerative colitis is associated with a promoter polymorphism of lipopolysaccharide receptor gene, CD14. Scand J Gastroenterol, 37699-704.
29. Roger T David J, Glauser MP & Calandra T. (2001). MIF regulates innate immune responses through modulation of Toll-like receptor 4. Nature, 414920-924.
30. Schulz S, Zissler N, Altermann W, Klapproth J, Zimmermann U, Glaser C , Schaller HG & Reichert S. (2008). Impact of genetic variants of CD14 and TLR4 on subgingival periodontopathogens. Int J Immunogenet, 35457-464.
31. Takeda K, Tsuneyasu Kaisho & Shizuo Akira. (2003). Toll-like receptors. Annu Rev Immunol, 21335-376.
32. Torok HP, Glas J, Tonenchi L, Mussack T & Folwaczny C. (2004). Polymorphisms of the lipopolysaccharide-signaling complex in inflammatory bowel disease: association of a mutation in the Toll-like receptor 4 gene with ulcerative colitis. Clin Immunol, 11285-91.
33. Wang F, Tahara T, Arisawa T, Shibata T, Nakamura M, Fujita H, Iwata M, Kamiya Y, Nagasaka M, Takahama K, Watanabe M, Hirata I, & Nakano H. (2007). Genetic polymorphisms of CD14 and Toll-like receptor-2 (TLR2) in patients with ulcerative colitis. J Gastroenterol Hepatol, 22925-329.
34. Yang Y Degranpre P, Kharfi A & Akoum A. (2000).Identification of macrophage migration inhibitory factor as a potent endothelial cell growth promoting agent released by ectopic human endometrial cells. J Clin Endocrinol Metab , 854721-7.
35. Zhou XP, Kuismanen S, Nystrom-Lahti M, Peltomaki P & Eng C . (2002). Distinct PTEN mutational spectra in hereditary non-polyposis colon cancer syndrome-related endometrial carcinomas compared to sporadic micro satellite unstable tumors. Hum Mol Genet, 11445-450.