Tumor necrosis factor alpha –C850T polymorphism is significantly associated with endometriosis in Asian Indian women

Kodati Vijaya Lakshmi, Ph.D., a Preetha Shetty, M.Sc., a Kiran Vottam, M.Sc., b Sujatha Govindhan, M.Sc., a,c Shaik Noor Ahmad, M.Sc., a,d and Qurratulain Hasan, Ph.D., a

a Department of Genetics and Molecular Medicine, Vasavi Medical and Research Centre, Khairatabad; b Department of Genetics and Molecular Medicine, Kamineni Hospitals, L.B. Nagar; c Centre for Cellular & Molecular Biology, Tarnaka; and d Department of Genetics, Osmania University, Hyderabad, India

Objective: To establish the association of tumor necrosis factor alpha (TNFA) promoter C850T polymorphism in Asian Indian women with endometriosis.

Design: Case control study, from multiple gynecological centers from Hyderabad, a cosmopolitan city in southern India. The study included 245 women who comprised 110 surgically confirmed cases of endometriosis, 50 ultrasoundographically confirmed cases of fibroid tumors, and 85 healthy female volunteers.

Setting: Academic hospital.

Patient(s): The cases were 245 women, 160 patients and 85 controls.

Intervention(s): None.

Main Outcome Measure(s): Tumor necrosis factor alpha –C850T polymorphism may be used as a molecular marker for endometriosis in our population.

Result(s): In this study we have demonstrated an association between TNFA –C850T polymorphism and endometriosis. The T allele is significantly associated with endometriosis when compared to women with fibroids as well as healthy controls. Our data imply that the T allele is associated with endometriosis (OR = 1.9594; 95% CI, 1.3833–2.7753; in our population. The TT genotype increases the risk of endometriosis by fourfold (4.5542: 95% CI, 2.0388–10.1701).

Conclusion(s): This study suggests that –C850T TNFA gene polymorphism could be used as a relevant molecular marker to identify women with risk of developing endometriosis in our population. (Fertil Steril® 2010;94:453–6. ©2010 by American Society for Reproductive Medicine.)

Key Words: endometriosis, gene polymorphism, TNF, –C850T, Asian Indian women

Endometriosis is the presence of endometrial cells and stroma at ectopic sites outside the uterine cavity. The natural history of endometriosis is uncertain, its etiology unknown, clinical presentation inconsistent, diagnosis difficult, and treatment poorly standardized. It causes significant morbidity owing to pelvic pain and infertility among 15%–25% of women during their reproductive age (1, 2). This benign disease is characterized by peritoneal inflammation, fibrosis, adhesions, and ovarian cysts, but displays features of malignancy, such as neovascularization, local invasion, and distant metastasis. Mechanical, hormonal, immunological, environmental, and genetic factors have been implicated in its etiology but provide inconclusive explanations. More recently, infectious factors have been proposed to be associated with the initiation of endometriosis by our group (2).

The inflammatory response observed in endometriosis may be mediated by proinflammatory cytokines such as tumor necrosis factor alpha (TNFA). Several lines of evidence support the involvement of TNFA as an important factor in the development of the following inflammatory pathology: [1] levels of tumor necrosis factor (TNF) are increased in the peritoneal fluid of patients with endometriosis and have been correlated with disease severity (3, 4), [2] TNF is known to stimulate the proliferation of endometriotic stromal cells (5), [3] anti-TNF therapy has been reported to inhibit the development of experimentally induced endometriosis in an animal model and to relieve pain in humans (6, 7), and [4] TNFA gene polymorphisms have been associated with endometriosis in some ethnic groups (8, 9).

Tumor necrosis factor alpha is a potent immunomodulator and proinflammatory cytokine that has been implicated in the pathogenesis of autoimmune and infectious diseases (10, 11). It also has a role in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase inflammatory reaction. Tumor necrosis factor supports

Received October 4, 2008; revised February 10, 2009; accepted March 9, 2009; published online April 25, 2009.

Supported by grants from the Indian Council of Medical Research (ICMR 5/7/26/02-RHN) and Department of Biotechnology (BT/PR5516/MED/14/646/2004), and the Ministry of Science and Technology, India.

K.V.L. has nothing to disclose. P.S. has nothing to disclose. K.V. has nothing to disclose. S.G. has nothing to disclose. S.N.A. has nothing to disclose.

Reprint requests: Kodati Vijaya Lakshmi, Ph.D., Gynaecologist, Geneticist and Research Coordinator, Vasavi Medical and Research Centre, 6-1-91, Khairatabad, Hyderabad, India, 500 004 (TEL: +91 40 2323 2729, +91 40 2321 0251; FAX: +91 40 2762 1762; E-mail: kovila@gmail.com).
cellular proliferation, differentiation, apoptosis, inflammation, tumorigenesis and viral replication. It is classically produced by monocytes/macrophages, although other cell types also produce significant amounts of this cytokine. It maps to chromosome 6p21.3, spans approximately 3 kb and has 4 exons. The last exon codes for more than 80% of the secreted protein.

Several polymorphisms in the promoter region of the TNFA gene have been described. They are -1032, -863, -857, -850, -755, -708, -274, -237, and -162 (12–14). The G308A promoter polymorphism has been widely studied in a number of inflammatory pathologies in different ethnic groups but the A allele was not identified in the cohort of individuals we studied (n = 110, unpublished data). The G308A and –C850T transition polymorphisms have been associated with chronic inflammatory diseases such as ulcerative colitis, rheumatoid arthritis, and Crohn’s disease (15, 16). In addition, TNFA –C850T polymorphism was reported to be significantly higher among pregnant women with eclampsia and preeclampsia (17, 18). Therefore, the aim of the present study was to assess the association of TNFA –C850T polymorphism with endometriosis—which is a chronic, progressive, inflammatory gynecological disease—in Asian Indian women.

MATERIALS AND METHODS

Subjects

This case control study was performed with 245 individuals and included 110 surgically confirmed cases of endometriosis, 50 cases of fibroids detected by ultrasound scanning, and 85 healthy women who had come for normal delivery. Patients with fibroid tumors diagnosed by ultrasound examination had subsequent surgery for either myomectomy or hysterectomy. It was then confirmed that they did not have endometriosis. However, the women who had normal vaginal deliveries had no family history, clinical symptoms, or diagnostic evidence suggestive of endometriosis as ascertained by information obtained from the completed proforma from each of these women. The study was approved by the institutional ethical committee.

Sampling

Two milliliters of peripheral blood was collected from all of the patients and controls along with clinical data, personal history, and family history.

Isolation of DNA and Genotype Analysis

Genomic DNA was isolated from the peripheral blood of subjects according to the method routinely used in our laboratory (1, 19). The DNA was stored at −20°C until processed. Genotyping for the TNFA polymorphism was performed by polymerase chain reaction (PCR) with specific published primers (17) synthesized from Bioserve Biotechnology, Ltd. (Hyderabad, India), followed by restriction fragment length polymerization (RFLP) analysis. A three-step PCR by the method of Alluri et al. (20) was performed using XP thermal cycler (UV Gene, Cambridge, United Kingdom). Briefly the PCR conditions included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 53°C for 30 seconds and extension at 72°C for 45 seconds, final extension at 72°C for 7 minutes. The 140-bp amplified PCR product was digested with Hinc II (MBI Fermentas, Hanover, MD), in a total volume of 20 μL for 2 hours at 37°C, initially checked on 2% agarose gel that was stained with ethidium bromide, and subsequently analyzed on 12% polyacrylamide gel with silver nitrate staining showed bands of 120 bp in case of TT genotype, 140 bp and 120 bp in CT genotype, and an undigested 140 bp band in CC genotype (Fig. 1) that was imaged and analyzed by documentation in UV I Tech gel documentation system (UVI-Tech Ltd., Cambridge, United Kingdom).

Statistics

Data are reported as mean ± SD. Statistical comparisons between groups’ mean were done by chi squared test (χ²). The odds ratio (OR), together with the 95% confidence interval (CI), comparing the allele and genotype distributions was performed by MedCalc version 7.4.3.0 software (Windows 98/NT/Me/2000/XP; Microsoft, Renton, WA). Two-tailed P values less than 0.05 were considered significant.
RESULTS

A PCR product of 140 bp was obtained, which on digestion with Hinc II restriction enzyme gave fragments of 140 bp indicating TT genotype, 140/120 bp indicating CT genotype and 120 bp indicating CC genotype (Fig. 1). The homozygous TT genotype was seen in higher percentage of patients with endometriosis (24.54%) when compared to both individuals with fibroids (6%) and healthy women (7.05%). Results showed that the genotype percentage among the fibroid patients and controls was the same, hence these groups were combined before statistical analysis (Table 1). Data showed that TT vs. CT and TT vs. CT + CC genotypes exhibited a significant difference between patients and controls ($P < 0.05$) and the TT genotype was associated with endometriosis (OR, 4.5542; 95% CI, 2.0388–10.1701; Table 2).

Frequency of T allele was 0.60 and 0.40 among patients and controls respectively and was significantly associated with endometriosis (OR, 1.9594; 95% CI, 1.3833–2.7753; $P < 0.05$).

DISCUSSION

It is well known that several molecular entities play a role in establishing and maintaining endometriosis. The relationship between TNFA and endometriosis has been indicated in several studies making it a good candidate gene (21, 22). There are some studies that have assessed different TNFA polymorphisms in endometriosis patients (8, 9, 23–26). The present study analyzes for the first time the TNFA –C850T polymorphism in Asian Indian women with endometriosis. Our data showed a significantly increased frequency of the TNFA T allele with endometriosis and a fourfold increase in risk in women carrying TT genotype (Table 1). To the best of our knowledge, this is the first study in the Indian population, which examined the association of –C850T TNFA gene polymorphism with endometriosis.

In Korean and Japanese populations, TNF –T1031C polymorphism was associated with advanced stage endometriosis (8, 9). Other studies on Australian, Chinese, Taiwanese, and Austrian populations did not show any association this TNFA polymorphism with endometriosis (23–26). But none of these studies have assessed the –C850T polymorphism of TNFA with endometriosis. However, this SNP C850T of TNFA has been associated with several other inflammatory diseases such as ulcerative colitis, rheumatoid arthritis, Crohn’s disease, eclampsia and preeclampsia (15–17). To the best of our knowledge, this is the first study to establish the association of –C850T TNFA polymorphism with endometriosis.

Elevated TNFA levels in peritoneal fluid have been associated with up-regulated TNFA production in peritoneal macrophages and peripheral monocytes of women with endometriosis (27, 28). The functional role of TNFA in endometrial tissue is unknown. It has been postulated that a TNFA
polymorphism that alters its transcription/expression subsequently enhances its level, leading to increased proliferation and decreased apoptosis seen in the inflammatory cascade. However, the commonly studied -308 polymorphism did not show a correspondingly increased TNFA level in earlier studies (11). It is possible that the less studied C850T TNFA promoter polymorphism may be affecting TNFA levels more consistently. This finding requires further evaluation.

In conclusion, an association has been demonstrated between C850T TNFA polymorphism and endometriosis, indicating that it can be used as a relevant molecular marker to assess the risk of endometriosis in Asian Indian women.

Acknowledgments: We would like to acknowledge Mr. Sambasivan Venkatasubramanian for the statistical analysis of the data.

REFERENCES


