Original paper

SUSCEPTIBILITY TO ULCERATIVE COLITIS AND GENETIC POLYMORPHISMS OF A251G SOD1 AND C-262T CAT

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Summary

Background: Reactive oxygen species can attack and damage almost every molecule found in living cells, including proteins, carbohydrates, lipids, and DNA. For this reason, their production is normally tightly controlled. Among the most important defenses against these radicals are the superoxide dismutase (SOD) enzymes and catalase (CAT). Increasing attention has been given to the role of reactive oxygen metabolites in the pathogenesis of ulcerative colitis (UC), which is defined as an idiopathic and chronic intestinal inflammation. Accordingly, we hypothesized a relation between genetic polymorphisms in the two antioxidant enzymes SOD1 A251G (rs2070424) and CAT C-262T (rs1001179) and the risk of UC.

Methods: The present case-control study included 109 UC patients (46 males and 50 females) and 186 (67 males and 119 females) gender-matched healthy controls. Genotyping was done by the PCR-RFLP method.

Results: After adjusting for age and gender, a significant association was observed between the AG+GG genotypes of SOD1 A251G polymorphism (vs. AA genotype) and risk of UC (OR=0.29, 95% CI: 0.10–0.86, P=0.025) after adjusting for age and gender. Our statistical analysis revealed that the CAT C-262T polymorphism did not associate with the risk of UC before and/or after adjusting for age and gender.

Conclusions: Based on the present statistical analysis, the G allele of the SOD1 A251G polymorphism decreases the risk of UC, thus it might be assumed that the G allele has a protective role.

Keywords: CAT, inflammatory bowel disease, SOD1, ulcerative colitis

Kratak sadržaj

Uvod: Reaktivne kiseonične vrste mogu napasti i oštetiti gotovo svaki molekul u živim celijama, uključujući proteine, ugljene hidrate, lipide i DNK. Zbog toga je njihova produkcija obično strogom kontrolom. Međutim, najvažnije odbrane protiv ovih radača predstavljaju enzimi superoksid-dismutaze (SOD) i katalaze (CAT). Sve veća pažnja pridaje se i ulozi reaktivnih kiseoničnih metabolita u patogenezi ulceroznog kolitisa (UK), koji se definise kao idiopatska hronična inflamacija creva. U skladu sa ovim, iznosimo hipotezu o odnosu između genskih polimorfizama u dva antioksidantna enzima SOD1 A251G (rs2070424) i CAT C-262T (rs1001179) i rizika od UK.

Metode: Ova anamnestička studija obuhvatila je 109 obolelih od UK (46 muškaraca i 50 žena) i 186 (67 muškaraca i 119 žena) zdravih kontrolnih subjekata odgovarajućeg pola. Genotipizacija je izvršena metodom PCR-RFLP.

Rezultati: Posle prilagođavanja za starost i pol, uočena je značajna povezanost između genotipova AG+GG genotipa SOD1 A251G (vs. AA genotip) i rizika od UC (OR=0.29, 95% CI: 0.10–0.86, P=0.025) posle prilagođavanja za starost i pol. Naša statistička analiza je pokazala da polimorfizam CAT C-262T nije bio povezan sa rizikom od UK pre i/ili posle prilagođavanja za starost i pol.

Zaključak: Na osnovu statističke analize, G alel polimorfizma SOD1 A251G smanjuje rizik od ulceroznog kolitisa, zbog čega se može pretpostaviti da G alel ima zaštitnu ulogu.

Ključne reči: CAT, zapaljenska bolest creva, SOD1, ulcerozni kolitis

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Introduction

Inflammatory bowel disease (IBD) is defined as an idiopathic and chronic intestinal inflammation. Studies show that IBD is a multifactorial disease (1, 2). It develops in genetically susceptible individuals due to the influence of environmental factors (3, 4). IBD has two main forms, ulcerative colitis (UC) and Crohn's disease (CD). UC is characterized by inflammation that is limited to the colon. By contrast, CD involves any part of the gastrointestinal tract from the mouth to the anus—most commonly the terminal ileum. Reactive oxygen species (ROS) are free radicals that can attack and damage almost every molecule found in living cells, including proteins, carbohydrates, lipids, and DNA. For this reason, their production is normally tightly controlled. In recent years, increasing attention has been given to the role of reactive oxygen metabolites (ROMs) in the pathogenesis of IBD (5–8).

Among the most important defenses against ROS are the superoxide dismutase (SOD) and catalase (CAT) enzymes. Superoxide dismutase 1 (cytosolic Cu/ZnSOD; SOD1; OMIM: 147450) is the major intracellular SOD (9). It catalyzes the dismutation of the toxic superoxide to hydrogen peroxide and molecular oxygen. The human SOD1 is 9,309 bp in length on chromosome 21q22.11 and consists of five exons interrupted by four introns (10). A genetic polymorphism of A251G (rs2070424) is located in intron 3 of the SOD1 gene. The association between the SOD1 A251G polymorphism and several multifactorial diseases has been reported (11–16).

Catalase (CAT; OMIM: 115500) is an antioxidant enzyme responsible for hydrogen peroxide (H2O2) conversion into oxygen and water. Its gene is located on chromosome 11p13, 34 kb long and is split into 13 exons (17). A C/T polymorphism located 262 bp upstream to the CAT transcription site (C-262T, rs1001179) has been described (18). The AG+GG genotypes (vs. AA genotype) and risk of UC (OR=0.45, 95% CI: 0.17–1.14, P=0.093). However, after adjusting for age and gender, a significant association was observed (OR=0.29, 95% CI: 0.10–0.86, P=0.025). This means that the G allele decreases the risk of UC and thus has a protective role.

Genotyping

Blood samples were obtained from patients and controls. Immediately after collection, whole blood was stored at −20 °C until use. Genomic DNA for PCR was isolated from whole blood using the thawed blood samples. Genotypic analysis for the CAT C-262T and SOD1 A251G polymorphisms was determined as described previously (11, 22).

Statistical analysis

For the control group, the observed frequencies of the genotypes in both polymorphisms were assessed for Hardy–Weinberg equilibrium using the χ2 statistic. The odds ratio (OR) and 95% confidence intervals (CIs) were calculated to estimate the strength of the association between polymorphisms and disease. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) (version 11.5). A probability of P<0.05 was considered statistically significant.

Results

Table I shows the prevalence of the genotypes of SOD1 A251G polymorphism in IBD patients and healthy control subjects. Hardy–Weinberg equilibrium was determined. The distribution of genotypic frequencies of SOD1 A251G polymorphism was in Hardy–Weinberg equilibrium in the control group (χ2=0.03 df=1, P=0.851). There was no significant association between the AG+GG genotypes (vs. AA genotype) and risk of UC (OR=0.45, 95% CI: 0.17–1.14, P=0.093). However, after adjusting for age and gender, a significant association was observed (OR=0.29, 95% CI: 0.10–0.86, P=0.025). This means that the G allele decreases the risk of UC and thus has a protective role.

Materials and Methods

Participants

A total of 96 UC patients (46 males and 50 females) and 186 healthy controls (67 males and 119 females) were included in this case-control study. The controls and patients were sex matched (P=0.71), but there was a significant difference in mean age between the patients (SD: 36.4±12.8) and controls (SD: 49.9±9.0) so they were not matched (P<0.001). Based on the several studies investigating numerous genetic polymorphisms, it is concluded that the Iranian population is a heterogeneous population (26–28).

It has been reported that the allelic frequency of the CAT C-262T and SOD1 A251G polymorphisms is influenced by ethnicity (21, 25, 28, also see: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs =2070424, and http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs= 1001179). Taken together, our patients and controls were selected among Persian (Caucasian) Muslims living in Shiraz (Fars province, south-west Iran). Informed consent was obtained from each subject before the study. This study was approved by the Shiraz University ethics committee.
Table I also shows the frequency of the genotypes of \(\text{CAT}C-262T\) polymorphism in the patients and healthy control subjects. The distribution of genotypic frequencies of \(\text{CAT}C-262T\) polymorphism was in Hardy–Weinberg equilibrium in the control group (\(\chi^2=1.97, \text{df}=1, P=0.158\)). Our statistical analysis revealed that this polymorphism did not associate with the risk of UC before and/or after adjusting for age and gender (Table I).

### Discussion

Our present study has indicated that there is a significant association between the AG+GG genotypes of \(\text{SOD1}\) gene and the risk of UC (Table I). The AG+GG genotypes, therefore, reduce the risk of UC. Further, the AG+GG genotypes seem to play a protective role and thus decrease the risk of UC. To our best knowledge, no studies have investigated the association of \(\text{SOD1}A251G\) polymorphism and the risk of UC. The \(\text{SOD1}A251G\) polymorphism has been found to be associated with several diseases either as a risk factor (11) or as a protective factor (12).

As mentioned in the Introduction section, studies have shown that oxidative stress plays an important role in the development of IBD (6–8). The genetic variation of C-262T in the \(\text{CAT}\) gene is a functional polymorphism (18). There is evidence that the T allele is associated with a lower expression level of the gene (18, 19). Taken together, it is hypothesized that this allele may increase the risk of UC. However, our present findings do not support the hypothesis (Table I). Only one published study investigated the association between the \(\text{CAT}C-262T\) polymorphism and susceptibility to UC (29). In that study, the authors reported that the CT genotype of the \(\text{CAT}C-262T\) polymorphism increased the risk of IBD. Our present findings are not consistent with the abovementioned report. As strongly recommended by STrengthening the REporting of Genetic Association studies (STREGA), the observed frequencies of the genotypes in control group should show no significant deviation from the expected values based on the Hardy-Weinberg equilibrium (30, 31). However, in the study of Khodayari et al. (29), although the CT genotype was observed in 69.2 percent of control persons, there was no healthy control subject having the TT genotype; therefore, it is worthwhile to consider that there is significant difference between the observed genotypic frequencies and the expected ones based on the Hardy-Weinberg equilibrium (\(P<0.001\)). It is suggested that errors in the genotyping procedures may have occurred in these situations. Taken together, the results of previous study should be interpreted with caution.

The three main limitations of our current study were the limited number of samples, the non-matching of age between cases and controls, and the fact that we studied only one polymorphism for \(\text{CAT}\) and \(\text{SOD1}\) genes. There are several other single nucleotide polymorphisms for these genes in humans, which were not studied in the present work. Simultaneous study of these polymorphisms (including the haplotype analysis) should be further researched. Further studies are recommended for more defensive conclusions.

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**Conflict of interest statement**

The authors stated that they have no conflicts of interest regarding the publication of this article.
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