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Association between butyrylcholinesterase activity and phenotypes, paraoxonase192 rs662 gene polymorphism and their enzymatic activity with severity of rheumatoid arthritis: Correlation with systemic inflammatory markers and oxidative stress, preliminary report

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ABSTRACT

Objectives: Evidences indicate that oxidative stress and inflammation are important processes in the development of destructive synovial tissue in rheumatoid arthritis (RA). The two major bioscavenger enzymes that are associated with inflammation and oxidative stress are human-butyrylcholinesterase (BuChE) and paraoxonase-1 (PON-1). Thus, the objective of this study was to determine the relation of BuChE phenotypes and PON-1 Q192R polymorphism with inflammatory markers such as anti-cytroline circulated peptide (CCP)-antibodies, CRP, neopterin, DAS28-CRP in RA patients.

Design and methods: In this study, we examined association of BuChE-phenotypes and activity, PON192rs662 (Q192R) polymorphism and its arylesterase activity (ARE) with systemic-inflammatory-markers and oxidative stress. The present case-control study consisted of 419-RA patients and 398 gender–age-matched unrelated healthy controls from west population of Iran. PON192rs662 polymorphism was detected by real-time-PCR. BuChE phenotype, TAC level, serum BuChE and ARE activities were determined spectrophotometrically. Anti-CCP-antibody and CRP were measured by ELISA and neopterin level was detected by HPLC. We used the EULAR activity criteria to measure DAS28-CRP.

Results: We found that PON-1-Q192R was associated with severity of RA [remission-to-low and moderate-to-high in dominant Q/Q + Q/R vs. R/R: OR = 2.27, $p < 0.001$; codominant Q/Q vs. R/R: OR = 1.65, $p < 0.001$ and Q/R vs. R/R: OR = 2.12, $p = 0.003$; recessive Q/Q vs. R/R + Q/R: OR = 1.79, $p = 0.032$; and allele Q vs. R: OR = 1.68, $p < 0.001$] and presence of anti-CCP-antibody (codominant model Q/Q vs. R/R: OR = 1.28, $p = 0.042$). The carriers of Q/Q genotype PON-1-Q192R and BuChE non-UU-phenotype had higher ARE activity, serum levels of neopterin, anti-CCP antibody titer and number of tender-joint and lower activity of BuChE and serum level of TAC than that of R/R genotype and BuChE-UU-phenotype.

Conclusions: The current findings demonstrate for the first time that there is a link between systemic inflammatory markers, oxidative stress, the PON192rs662-Q allele and BuChE-non-UU-phenotype and their corresponding enzymatic activity which may be considered as a risk factor for the severity of RA for a population in Iran.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation associated with massive influx of highly proliferative and invasive synovial fibroblasts and macrophages that adhere to extracellular matrix and increased reactive oxygen species (ROS) [1–3]. The etiology of RA is unknown, but evidences indicate that inflammation, oxidative stress, lipid peroxidation and increased ROS can affect progression and severity of RA [3,4]. Thus, we hypothesize that the level of activities of bioscavengers BuChE and PON-1 that are associated with inflammation and oxidative stress, are correlated with severity of RA.

Human butyrylcholinesterase (BuChE, EC.3.1.1.8), also known as pseudo or non-neuronal cholinesterase and paraoxonase-1 (PON-1, aryldialkylphosphatase, EC 3.1.8.1), the two serum major bioscavengers

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with antiatherogenic activities [5–7] have recently received a lot of attentions as antioxidants that are associated with inflammation. Association between BuChE activity with lipid and lipoprotein levels, type 2 diabetes mellitus (T2DM), stroke, preeclampsia, systemic lupus erythematosus (SLE) and cardiovascular disease is well studied [6–14]. We have recently found a high frequency of BuChE non-UU phenotypes associated with low BuChE activity in systemic lupus erythematosus (SLE) and stroke patients who had high levels of cholesterol, suggesting BuChE non-UU phenotypes increase susceptibility to SLE and stroke (7–9).

PON-1 also referred to as arylesterase (ARE) hydrolyzes organophosphate substrate paraoxon and aromatic esters, such as phenylacetate and has been shown to inhibit LDL oxidation. Low PON-1 activity has been associated with increased risk of coronary vascular disease (CVD) [15–21]. PON-1 activity is reduced in individuals with RA, SLE, hypercholesterolemia, non-insulin-dependent diabetes and in patients with vascular diseases, survivors of myocardial infarction and rheumatoid arthritis [5,7,16–18,20–23].

The inflammation, in general and in RA in particular, can be detected by erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level in the serum. Among various inflammatory makers rheumatoid factor (RF), detected in up to 70–80% of RA patients, and autoantibodies against citrullinated peptides (anti-CCP) found in around 98% of people with RA are most often used as serological markers in diganosis of RA (REF). In addition, induction of neopterin production by pro-inflammatory cytokine interferon-γ has been used as a marker of cellular immunity activation and a good correlation between neopterin concentrations and DAS-28 may facilitate assessing disease activity. The objective of the current work was to determine the relation of BuChE phenotypes and PON-1 Q192R polymorphism with inflammatory markers such as anti-CCP antibodies, CRP, neopterin, DAS28-CRP in RA patients.

Materials and methods

This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Iran and was in accordance with the principles of the Declaration of Helsinki II and written informed consent was obtained from all the participants.

Study design and patients

The study included 419 cases of RA patients (377 females and 42 males; ages 20–70 years) with disease duration of 5–13 years. RA patients were identified according to the 1997 ACR classification criteria for rheumatoid arthritis [24] by a rheumatologist. The ANA profile was used to confirm the absence of other autoimmune diseases such as systemic lupus erythematosus. We used the EULAR activity criteria (clinical remission values below 2.6, low activity between 2.6 and 3.2, moderate activity from 3.2 to 5.1 and high activity values over 5.1) to calculate disease activity score of 28 joints (DAS28-CRP) [24]. All of the patients were under treatment with corticosteroids and methotrexate. Patients receiving other drugs including therapeutic monoclonal antibodies (anti-TNF or anti-CD20) and smokers were excluded from the study.

The control group consisted of 397 healthy individuals (363 female and 36 male) with no history of autoimmune diseases. Baseline information including demographic and age of disease onset were collected by face-to-face interviewing.

Chemical analysis

Serum samples were obtained from patients and control groups, aliquoted and stored at −80 °C until use. One hour ESR was determined by the westergren method. IgG anti-CCP antibody was measured by Elisa (Genesis Diagnostics), according to manufacturer's instruction, the anti-CCP level at ≥6.25 RU/mL was considered as threshold for a positive result. The serum CRP (hs-CRP, mg/L), total RF, and RF isotypes were determined by ELISA (Monobine Inc., USA and Aesku, Wendelsheim, Germany), according to manufacturer's protocol, positive value was defined as a level of ≥24 U/mL for total RF and ≥18 U/mL for RF isotypes (RF-IgM, RF-IgA and RF-IgG). Neopterin was measured by HPLC [25].

Measurement of serum arylesterase activity (ARE) of paraoxonase

Serum ARE activity of paraoxonase was measured spectrophotometrically using phenylacetate as substrate according to protocol previously described [26,27].

Measurement of serum levels of total antioxidant capacity (TAC)

The serum levels of TAC were measured using commercially available kits (Randox Laboratories Ltd., Crumlin, Antrim, N. Ireland, Cat. no. NX2332). Briefly, ABTS® (2,2′-azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS®*+ with a relatively stable blue–green color measurable at 600 nm. Antioxidants in the added sample suppressed the color production proportional to their concentration.

Determination of serum butyrylcholinesterase activity (BuChE) and phenotypes

BuChE activity and phenotypes were determined spectrophotometrically using benzoylcholine chloride (50 μmol/L) as substrate in the presence or absence of the inhibitors, dibucaine hydrochloride (10 μmol/L) and sodium fluoride (50 μmol/L) as previously described [9,28].

To determine the precision of the assays, the variability (inter and intra assay coefficients) were measured for ARE activity, TAC, and BuChE activity by examining their reproducibility on eight samples containing different concentrations. The inter and intra assay coefficients were 4.6% and 3.3% for ARE activity, 4.9% and 4.1% for BuChE activity, and 3.6% and 3% for serum TAC concentration, respectively.

DNA analyses

Genomic DNA was extracted from peripheral blood leukocytes using phenol chloroform extraction method [8]. Genotyping of all individuals was done without knowledge of their groups or disease. Genotyping of PON192rs662 was performed using the TaqMan allelic discrimination assay as previously described [29].

Statistical analysis

The allelic frequencies were calculated by the gene counting method. The χ^2 test was used to verify the agreement of the observed genotype frequencies with those expected according to the Hardy–Weinberg equilibrium. The genotypes and allele frequencies of PON-192 rs662 and BuChE phenotype in RA patients were compared to control group and disease activity (remission-to-low and moderate-to-high) using χ^2 test in three different models: the genotype codominant model, the minor genotype dominant/recessive model, and the minor genotype heterozygous model.

Odds ratios (OR) were calculated as estimates of relative risk for disease and 95% confidence intervals obtained by SPSS logistic regression. The correlation of serum BuChE and arylesterase activities, neopterin, TAC, CRP, anti-CCP antibody and DAS28CRP with the PON192rs662 polymorphism between RA patients with and without anti-CCP were calculated using linear regression and an unpaired t test. A two-tailed Student's t test, ANOVA, and nonparametric independent sample Mann–Whitney analyses were used to compare quantitative data. Statistical significance was assumed at the $p < 0.05$. The calculations were