Association between vascular cell adhesion molecule 1 and radiographic hand osteoarthritis

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ABSTRACT

Background. *A recent study showed that the level of soluble Vascular Cell Adhesion Molecule 1 (VCAM-1) emerged as a strong and independent predictor of the risk of hip and knee joint replacement due to severe osteoarthritis (OA). Therefore, we hypothesized that soluble VCAM-1 level can be associated with prevalence and severity of radiographic hand OA.*

Objective. *To evaluate the association between soluble VCAM-1, and radiographic hand OA in a large community-based sample.*

Methods. *The study population comprised Chuvashians (473 males age 45.90±17.16; and 451 females, age 46.18±16.70 years). OA was evaluated for 14 joints of each hand using Kellgren and Lawrence (K-L). OA was characterised by the number of affected joints and by the presence of at least one affected joint. The VCAM-1 level was determined by a sandwich enzyme immunoassay technique using ELISA-OSTEO kit. Statistical analyses included multiple linear and logistic regressions.*

Results. *The linear regression model showed a significant association of a number of affected joints with VCAM-1 level (p=0.004) after adjustment for age, sex, BMI. In the logistic regression model the level of association between the presence of at least one affected joint and soluble VCAM-1 level was p=0.070 (OR(95%CI): 1.003 (1.000, 1.007)).*

Conclusions. *In this cross-sectional population-based study, we found that the serum level of soluble VCAM-1 level is positively associated with the number of affected joints of hand OA.*

Introduction

Osteoarthritis (OA) is the most common form of arthritis and the leading cause of musculoskeletal morbidity in the elderly (1). The hands are the most frequent site of OA development (2). OA is not considered a classic inflammatory arthropathy; however, it is frequently associated with signs and symptoms of inflammation, including joint pain, swelling and stiffness leading to significant functional impairment. Evidence from *in vivo* and *in vitro* studies indicates that chondrocytes can produce and/or respond to a number of pro-inflammatory cytokines and chemokines that are present in OA joint tissues and fluids (3, 4). Cytokine activation alters the phenotype of quiescent endothelial cells, resulting in a protein synthesis-dependent hyper adhesive state for leukocytes (5). The vascular cell adhesion molecule 1 (VCAM-1) is one of the adhesion proteins, which mediate the adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium and play a role in the development of inflammation (5). The results from the marker staining patterns show four-fold increase of VCAM-1 positive cells in OA cartilage (6). VCAM-1 also has been shown to mediate the interaction of chondrocytes with immune cells and could thus by itself contribute to immune-mediated cartilage damage in OA (7).

Recent study (8) showed that the level of soluble VCAM-1 emerged as a strong and independent predictor of the risk of hip and knee joint replacement due to severe OA. Therefore, we hypothesized that soluble VCAM-1 level can be associated with prevalence and severity of radiographic hand OA. In the present cross-sectional, population-based study, we evaluated the association between soluble VCAM-1 level and radiographic hand OA.

Methods

Sample

The population studied included 924 apparently healthy individuals (473 men, 451 women) ranging in age from 18 to 86 years. The samples were collected randomly from 206 nuclear families living in Chuvashia and the Bashkortostan Republics, Russian Federation (for details see Kalichman *et al*. (9)). The data were collected during two studies undertaken during May/June of 1999 and September 2002 by the Anuchin Research Institute and Museum of Anthropology, Moscow State University (Russia) and by the Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel-Aviv University (Israel). The families were all ethnically Chuvashians, have lived at least for the last three generations un-

Table I. Descriptive statistics of the studied sample.

Variables	Males (n=473)	Females (n=451)	Total (n=924)
Age (yeas)	45.90 (SD=17.16)	46.18 (SD=16.70)	46.04 (SD=16.93)
BMI (kg/m²)	23.28 (SD=3.38)	25.13 (SD=4.85)	24.18 (SD=4.26)
VCAM-1 (ng/ml)	231.18 (SD=54.67)	239.28 (SD=64.11)	235.14 (SD=59.57)
Num-KL	2.74 (SD=3.96)	2.90 (SD=4.16)	2.82 (SD=4.06)
Dich-KL (N(%) affected)	262 (55.39%)	225 (49.89%)	487 (52.71%)

Table II. Results of logistic and multiple linear regression analyses.

Independent variables	Multiple linear regression NAJ		Logistic regression Dich-KL		
	Beta ± SE	p-level	B ± SE	Odds ratio for unit (95% CI)	p-level
Constant	–	**<0.001**	-6.640 ± 0.852	0.001	**<0.001**
Sample*	0.079 ± 0.025	**0.001**	0.267 ± 0.162	1.306 (0.951, 1.793)	*0.099*
Age	0.659 ± 0.026	**<0.001**	0.132 ± 0.008	1.142 (1.123, 1.160)	**<0.001**
Sex**	0.000 ± 0.025	0.997	-0.605 ± 0.207	0.546 (0.364, 0.819)	**0.003**
BMI	0.045 ± 0.026	*0.082*	0.022 ± 0.025	1.022 (0.973, 1.074)	0.383
VCAM-1	0.072 ± 0.025	**0.004**	0.003 ± 0.002	1.003 (1.000, 1.007)	*0.070*

*sample 1 collected in 1999, sample 2 collected in 2002; **1 male, 2 females. Statistically significant associations ($p<0.05$) marked in bold, associations with significance level $p<0.1$ marked in italics.

der the same environmental conditions, and have not been exposed to outside influences. The information that was gathered included sex, age, basic socioeconomic parameters, standard anthropometrical measurements, data on chronic morbidity and medical treatment, and finally x-ray radiographs of both hands, and blood samples. The individuals with known bone disease or with amenorrhoea were not included in the study, and no individuals received treatment with lipid-lowering drugs. There were no women using hormone replacement therapy in the sample. This study was conducted in agreement with the declaration of Helsinki, with the approval of the Tel-Aviv University Ethics Committee.

Measurement of VCAM-1 level
The method of measurement of adhesion molecules level in the same sample was previously described (10). Venous blood samples were collected by venipuncture after the patients' overnight fast, and were centrifuged within 2 hours after collection. Plasma samples were immediately separated and stored in aliquots at -80℃ until they were analysed. The VCAM-1 level was determined by a sandwich enzyme immunoassay technique us-

ing a set of specific antibodies and standards from R&D Systems (Minneapolis, MN, USA). This system uses a solid-phase monoclonal antibody and an enzyme-linked polyclonal antibody against recombinant human VCAM-1. Briefly, unbound capture antibodies were washed and blocked with bovine serum albumin in PBS. After the plate was washed, standard and samples were added and incubated for 2 h at room temperature. Next, the plate was washed and detecting biotinylated mouse anti-human VCAM-1 was added to each well. After 2 hours incubation, the wells were washed and horseradish peroxidase-conjugated streptavidin was added. At the final step, the substrate solution was added and the reaction was terminated by adding stop solution. The absorbance was measured immediately at 450 nm with a correction of 540 nm by using a microplate reader (Elx808, Bio-Tek Instruments, USA). The detection sensitivity was 15.625 pg/ml. The inter- and intra-assay coefficients of variation were 4.68 and 1.12%, respectively.

Body mass index
BMI was computed as the ratio of weight (in kg.) divided by height (in meters) squared.

Radiographic assessment of OA
Single plain radiographs of both hands were obtained from each study participant, in the postero-anterior position with the x-ray source located 60 cm above, using a standard radiographic technique, as described in detail by Pavlovsky (11). The hands were exposed for 5–10 sec at 100-150 mA, without intensifying screens, at 50kV. The same equipment was used in all studies and x-rays were taken according to the same standardised protocol. Each radiograph was read by an experienced and specially trained researcher. The development of OA was evaluated for each of the 14 joints (4 DIP, 4 PIP, 5 MP, and IP-1) according to the Kellgren and Lawrence (K-L) grading scheme (12). The extent of K-L index for each joint ranged from 0 to 4, with those scored K-L≥2 considered affected.

Reliability of the x-ray readings
First, two experienced researchers (an orthopaedic surgeon and a researcher experienced in reading x-rays) read a set of radiographs and decided on the protocol for evaluating the K-L scores. Thus, 12 x-rays were read using this protocol and then re-read by two investigators separately to estimate the intra- and inter-rater reliability of the readings. All discrepancies were reviewed for systematic errors. This procedure continued for ten different x-rays sets until high intra-rater and inter-rater reliability (kappa >0.80) was established. Then, one investigator read all the x-rays, blinded to the patient's name, sex and age. Before reading each set of x-rays, the investigator re-read five previously read x-rays to "calibrate" his readings to a standard. The intra-observer reliability (kappa statistics) for K-L scores was 0.84 ($p<0.01$), based on 20 repeated measurements.

Statistical analysis
All statistical computations were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA). We evaluated the associations between the hand OA indices (dependent variables) and VCAM-1 level age, sex, studied sample (collected in 1999 or in 2002) and BMI (independent variables). First, we

used multiple linear regression analyses (enter method) for the number of affected joints for each individual (NAJ) as a dependent variable. Then we performed the logistic regression analysis with dichotomous indices of presence of at least one affected joint (Dich-KL) as a dependent variable.

Results

Descriptive statistics are shown in Table I. The studied sample included 924 individuals: 473 males with mean age 45.90 years, standard deviation (SD)=17.16; and 451 females, mean age 46.18 (SD=16.70) years. Mean measured level of VCAM-1 was 235.14 (SD=59.57) ng/ml. The percentage of studied individuals who had at least one finger joint affected at K-L$\geq$2 level was 52.71%.

The linear regression model (Table II) showed significant association of NAJ with age ($p<0.001$), sample ($p=0.001$) and VCAM-1 level ($p=0.004$). The logistic regression model showed significant association of prevalence of hand OA with age (OR (95%CI): 1.142 (1.123, 1.160), $p<0.001$) and sex (0.546 (0.364, 0.819), $p=0.003$), but association with VCAM-1 level did not reach the statistical significance (1.003 (1.000, 1.007)), $p=0.070$.

Discussion

In this cross-sectional population-based study, we found that serum level of soluble VCAM-1 level is positively associated with NAJ of hand OA. The significance of association between presence of at least one joint with K-L$\geq$2 and soluble VCAM-1 level was $p=0.070$ ((OR(95% CI): 1.003 (1.000,

1.007)). The previous study of Schett and colleagues (8) found that soluble VCAM-1 level is an independent predictor of severe OA of knee and hip, suggesting that VCAM-1 is mediating the interaction of chondrocytes with immune cells and could therefore, by itself contribute to immune-mediated cartilage damage in OA. In our study, we found the similar association of VCAM-1 with radiographic hand OA, as indicated by NAJ.

VCAM-1 is a cell surface sialoglycoprotein that promotes leukocyte adhesion and homing to sites of inflammation (5). This adhesion molecule is expressed by microvascular endothelial cells, synovial fibroblasts, and chondrocytes. In chondrocytes, expression of VCAM-1 is induced by inflammatory cytokines, such as tumour necrosis factor alpha, interleukin-1 and hyaluronic acid, a major components released during cartilage damage. Consequently, the VCAM-1 levels may reflect active cartilage damage or an inflammatory component in OA.

Our study has several strengths. The studied samples were collected especially to represent the general population in the studied region. NAJ is an index that presents all ranges of hand OA development and not only end-stage OA, as was previously studied (8). The major limitation of our study was its cross-sectional design.

References

1. LAWRENCE RC, HELMICK CG, ARNETT FC *et al.*: Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* 1998; 41: 778-99.
2. BUCKWALTER JA, MARTIN J, MANKIN HJ: Synovial joint degeneration and the syndrome of osteoarthritis. *Instr Course Lect* 2000; 49: 481-9.
3. GOLDRING MB, OTERO M, TSUCHIMOCHI K, IJIRI K, LI Y: Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism. *Ann Rheum Dis* 2008; 67 (Suppl. 3): iii75-82.
4. MARTEL-PELLETIER J, BOILEAU C, PELLETIER JP, ROUGHLEY PJ: Cartilage in normal and osteoarthritis conditions. *Best Pract Res Clin Rheumatol* 2008; 22: 351-84.
5. MULLER WA: Mechanisms of transendothelial migration of leukocytes. *Circ Res* 2009; 105: 223-30.
6. GROGAN SP, MIYAKI S, ASAHARA H, D'LIMA DD, LOTZ MK: Mesenchymal progenitor cell markers in human articular cartilage: normal distribution and changes in osteoarthritis. *Arthritis Res Ther* 2009; 11: R85.
7. KIENZLE G, VON KEMPIS J: Vascular cell adhesion molecule 1 (CD106) on primary human articular chondrocytes: functional regulation of expression by cytokines and comparison with intercellular adhesion molecule 1 (CD54) and very late activation antigen 2. *Arthritis Rheum* 1998; 41: 1296-305.
8. SCHETT G, KIECHL S, BONORA E *et al.*: Vascular cell adhesion molecule 1 as a predictor of severe osteoarthritis of the hip and knee joints. *Arthritis Rheum* 2009; 60: 2381-9.
9. KALICHMAN L, KOBYLIANSKY E: Hand osteoarthritis in Chuvashian population: prevalence and determinants. *Rheumatol Int* 2009; 30: 85-92.
10. VISTOROPSKY Y, TROFIMOV S, PANTSULAIA I, LIVSHITS G: Genetic and environmental determinants of variation of soluble adhesion molecules. *Ann Hum Genet* 2006; 70: 749-58.
11. PAVLOVSKY O: Methods of osseographic studies of the bone. *In*: Morpho-physiological researches in anthropology, PAVLOVSKY O Ed. Moscow: MGU Publishing House 1981: 44-61.
12. KELLGREN J, LAWRENCE J: Atlas of standard radiographs: The epidemiology of chronic rheumatism. Volume 2. Oxford, Blackwell Scientific Publications 1963.