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SERUM SIALIC ACID (TOTAL SIALIC ACID AND LIPID ASSOCIATED SIALIC ACID), B-CAROTENE AND SUPER OXIDE DISMUTASE (SOD) LEVELS IN IRAQI PATIENTS WITH KNEE OSTEOARTHRITIS

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ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease, occurring primarily in older persons, characterized by erosion of the articular cartilage, hypertrophy of bone at the margins (i.e., osteophytes), subchondral sclerosis, and a range of biochemical and morphological alterations of the synovial membrane and joint capsule. In osteoarthritis free radicals may act as triggering factor for degenerative changes seen in cartilage. Oxidative stress leads to increased risk for osteoarthritis but the precise mechanism remains unclear. Sialic acid concentration varies physiologically with age, but its level may also be influenced by such condition as inflammation. The aim of this study was to investigate the changes in the serum sialic acid levels (TSA and LSA), β-Carotene and super oxide dismutase (SOD) in patients with knee osteoarthritis. In this study 96 subjects divided into four groups, 33 patients with obese Knee OA and 25 patients non obese Knee OA, their age range (32-78). The other 38 subjects age and sex matched healthy subjects were studied as controls include 23 obese and 15 non obese. This study was conducted in AL-Kadhemiya Teaching Hospital during the period from October 2011 to April 2012. Serum total sialic acid level, lipid sialic acid, serum β-Carotene and serum SOD were determined. A significant increase in serum sialic acid (TSA & LSA) levels were observed, while there were a significant decrease in serum β-Carotene and SOD levels in patients with knee osteoarthritis when compared to healthy controls. The results of our study suggest higher oxygen free radical production, evidenced by decreased SOD and β-Carotene levels support to the oxidative stress in knee osteoarthritis. The increase of serum TSA and LSA levels is associated positively with the presence of inflammation and could be suggested as one of the many markers for Knee osteoarthritis.

Keywords: Sialic acid, super oxide dismutase (SOD), knee osteoarthritis (KOA).

INTRODUCTION

Osteoarthritis (OA) is a chronic degenerative disorder of multifactorial etiology, characterized by a gradual loss of articular cartilage, thickening of the subchondral bone, bony outgrowths (osteophytes) at the joint margins, and mild, chronic nonspecific synovial inflammation (Berenbaum *et al*., 2001; Morehead, 2003). Sialic acid (N-acetyl neuraminic acid NANA) is acetylated derivative of neuraminic acid. It is attached to non-reducing residues of the carbohydrate chains of glycoproteins and glycolipids. The suggested biological functions of sialic acid are as following: (a) stabilizing the conformation of glycoproteins and cellular membranes, (b) assisting in cell to cell recognition and interaction, (c) contributing to membrane transport, (d) affecting the function of membrane receptors by providing binding sites for ligand, (e) influencing the function stability and survival of blood glycoproteins, and (f) regulating the permeability of the basement membrane of glomeruli (Schauer *et al*., 1995). Sialic acid concentration varies physiologically with age, but its level may also be influenced by such a condition as inflammation (Novak *et al*., 2005). Neoplastic tumors or inborn genetic disorder which cause abnormal sialic acid metabolism (Fang-Kirsher, 1997). Sialic acid and its derivatives were used in the treatments of several diseases, including neuropathic and inflammatory diseases as well as certain tumors (Witczak and Nieforth, 1997). Lipid-associated sialic acid (LSA) is a useful adjunct in the management of a variety of malignancies (Mahmood and Ahmed, 2008). Elevation in blood LSA level was reported in patients with mammary (63%), gastroenteric (65%), pulmonary (79%) and ovarian (94%) neoplasms as well as those with leukemia (91%), lymphoma (87%), melanoma (84%), and Hodgkin disease (91%) (Bhargava *et al*., 1984; Dinstrian and Schwartz, 1983; Erbil *et al*., 1985; Katopodis *et al*., 1982; Khanderia *et al*., 1983; Dwivedi *et al*., 1990). Sialic acid levels do not appear to be a good marker for discriminating malignant from nonmalignant disease of the Lung (Turgut *et al*., 2001). Higher levels of total sialic acid were found in women with metabolic gestation syndrome (Srihana *et al*., 2002) and during periodontal disease.

In osteoarthritis free radicals may act as triggering factor for degenerative changes seen in cartilage. Oxidative

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stress leads to increased risk for osteoarthritis but the precise mechanism remains unclear. Body has a host of protective mechanism to prevent the tissue damage caused by reactive oxygen species (ROS). These include both enzymatic and non enzymatic mechanism. Enzymatic mechanism includes Super oxide dismutase (SOD), catalase and glutathione peroxidase (GPX). Vitamins A (β-carotene), vitamin C (Ascorbic acid), vitamin E (α- tocopherol) and glutathione are some of the major non enzymatic antioxidant in the body (18). Although an area much less studied, free radicals may also play a role in the pathogenesis of Osteoarthritis and in particular, via the effects upon lipids and cartilage. Observational and epidemiological studies suggest that diets deficient in antioxidants may be associated with an increased incidence of Osteoarthritis or faster disease progression. Antioxidant supplements and diets have long been advocated for the treatment of oesteoarthritis (OA) and other inflammatory arthritis. Some investigators support the hypothesis that free radicals from oxygen metabolism destroy antioxidant system (Ascorbic acid and sulphydryl group) (Situnayake, 1991). The present study aims to assess serum levels of sialic acid (TSA and LSA), super oxide dismutase (SOD) and β-Carotene, levels in Iraqi patients with knee osteoarthritis.

MATERIALS AND METHODS

The present study was conducted in AL-Kadhemiya Teaching Hospital, Baghdad during the period from October 2011 to April 2012. Study population consisted of 96 subjects divided into four groups, 58 subjects with age range (32-78) 33 were obese KOA (O-KOA) and25 patients non obese KOA (NO-KOA). The other 38 age and sex matched healthy subjects were included as a controls consisted of 23 obese and 15 non obese individuals. Complete clinical and personal histories of the subjects were also recorded. Inclusion criteria for knee osteoarthritis were on the basis of clinical and radiological evidence. Patients with asymptomatic knee osteoarthritis or association of any other chronic debilitating disease were excluded from the study; 5 ml venous blood was aspirated from a suitable vein. Samples were collected between 8:00-9:00 am after 12 hours fast. Serum total sialic acid level was determined using the assay method described by Svenerholm methods (1957). Estimation of serum lipid sialic acid level was determined using the assay method described by Katopodis *et al*. (1982) in mg/dl. Estimation of SOD was done by method described by Varley, Expressed in U/g Hemoglobin, estimation of Serum Beta-carotene was done by Carr and Price (1988) and expressed as μg/dl.

STATISTICAL ANALYSIS

All data were expressed as mean ± SD. The statistical significance was evaluated by Student's t- test using Statistical Package for the Social Sciences (SPSS Cary, NC, USA) version 15.0.p value were expressed as significant if the values of $p < 0.005$.

RESULTS AND DISCUSSION

Serum sialic acid (TSA & LSA), Oxidative stress marker (SOD) and serum β-Carotene levels were estimated in 96 patients with knee osteoarthritis patients, (33 O-KOA&25 NO-KOA) 38 patients were females and 20 were males in knee osteoarthritis group, compared with 38 healthy control group, age and sex matched, include 24 obese and 14 non obese. Control group consist of 20 females and 18 males. Mean studied age of Knee osteoarthritis patients was range (32-78 years) and that of control was also of same range. By using students t test there was no significant difference seen in mean age, between control group and knee osteoarthritis group. A significant difference was seen according to body mass index (BMI) between the two groups (obese and non obese) in KOA and control as shown in tables 1 and 2. A significant statistical difference was observed in LSA, SOD and β-Carotene in KOA patients between two groups (O-KOA and NO-KOA) as shown in table 2. The levels of serum total sialic acid (TSA) and lipid sialic acid (LSA) indicate significant increase in the Knee osteoarthritis patients group compared with healthy control group as shown in table 3. The levels of antioxidant enzymes SOD and βcarotene were significantly decrease in patients with knee osteoarthritis compared to controls as shown in table 3. The mean ± SD of sialic acid (TSA & LSA), Oxidative stress (SOD) and β-Carotene in healthy control and knee osteoarthritis patients are depicted in tables 1, 2 and 3.

Osteoarthritis is a mechanically induced disorder in which the consequences of abnormal joint mechanics provoke biological effects that are mediated biochemically through local or systemic factors (Brandt *et al*., 2006). Many studies on KOA have focused more on evaluation of biochemical markers in serum and/or synovial fluid of knee joint; such as, adipokines, MMPs, TIMPs, toxic oxygen radicals, and others (Crofford *et al*., 2004; Dumond *et al*., 2003; Hurter *et al*., 2005; Pottie *et al*., 2006). The present data show that TSA and LSA are significantly higher in patients with knee osteoarthritis (KOA). KOA causes inflammation of joints and surrounding tissues, but also it can affect other organs, TSA and LSA may, thus be able to differentiate inflammatory knee osteoarthritis from adehydrative, non-inflammatory osteoarthritis. Our results are supported with previously published work (Alturfan *et al*., 2007). These results can suggest that increased levels of total sialic acid and lipid sialic acid levels might be considered as a defense molecule against the increased oxidative stress in KOA. Antioxidant property of Sialic acid as a H_2O_2 scavenger has been reported by Tanaka *et al*. (1997). In this study, different antioxidant levels were

Table 1. Statistical Data of Control Group.

Parameters	Obese control	Non-obese control	P value
Number %	24	14	……
Age (years)	58.11±9.51	57.91±8.91	NS
BMI (kg/m^2)	34.64±4.35	23.68±1.19	<0.001
TSA(mg/dl)	65.53±14.31	61.88±11.23	NS
LSA(mg/dl)	17.64±1.62	18.86±1.02	NS
SOD (U/gm)	1458.66±117.33	1317.36±133.87	NS
β-Carotene (µg/dl)	90.36±3.81	86.14±2.82	NS

Table 2. Statistical Data of KOA Group.

Parameters	Obese KOA	Non-obese KOA	P value
Number %	33	25	…….
Age (years)	59.89±7.9	58.65±8.8	NS
BMI (kg/m^2)	34.26±4.55	22.82±2.15	<0.001
TSA(mg/dl)	87.22±12.23	80.87±13.15	NS
LSA(mg/dl)	43.95±2.31	29.83±2.18	<0.001
SOD (U/gm)	866.86±102.31	1053±111.46	<0.001
β-Carotene (µg/dl)	36.81±9.72	27.34±2.11	<0.001

Table 3. Comparison of mean S. Total sialic acid(TSA),lipid sialic acid (LSA) ,super oxide dismutase(SOD) and β Carotene in controls and patients with knee osteoarthritis studied by Students 't' test.

Parameters	Total control	Total KOA	P value
Number %	38	58	……
Age (years)	56.55±11.36	58.35±12.95	NS
BMI (kg/m^2)	24.16±2.82	26.84±2.68	□0.001
TSA(mg/dl)	64.39±9.58	83.46±14.95	□0.001
LSA(mg/dl)	17.46±1.35	36.87±2.86	□0.001
SOD (U/gm)	1439.71±127.18	922.4±123.84	□0.001
β-Carotene (µg/dl)	88.78±5.79	31.09±3.86	□0.001

compared between the control group and knee Osteoarthritis groups. One of the most important antioxidant is the enzyme superoxide dismutase, which in the present study, have been decreased significantly in patients with knee osteoarthritis compared with healthy control. Our findings were consistent with a study done by Surapaneni *et al*. (2007) and Surapaneni and Venkataramana (2007) as they demonstrated a significant increase in erythrocyte MDA levels; superoxide dismutase (SOD), glutathione peroxidase (GPX) and plasma glutathione - S - transferase (GST) activities; and a significant decrease in erythrocyte glutathione (GSH), ascorbic acid, plasma vitamin E levels and catalase activity in patients with osteoarthritis when compared to controls, indicating that oxidative stress in OA is much higher than the ability of protective reducing system to overcome (Surapaneni and Venkataramana, 2007). SOD is an important antioxidant enzyme having an antitoxic effect against superoxide anion. The body's overall vascular and neural functions are closely related (Motilal *et al*., 2012). Statistical analysis, of mean serum β-Carotene levels among the two groups (Table 3), indicate significant difference between control group and knee Osteoarthritis group ($p<0.001$). These results are in agreement with previous study done by Peter *et al*. (2001), who found that Serum β-carotene and α-tocopherol concentrations have different associations with diet, smoking, and general and central adiposity. BMI showed statistically difference between obese and non obese in healthy control and knee osteoarthritis patients, these result goes with study demonstrated by Hotamisligil (2006), who demonstrate that Metabolic inflammation associated with obesity is believed to exacerbate the condition by contributing to metabolic inflexibility and the sustained production of pro-inflammatory mediators, Metabolic inflammation also appears to increase osteoarthritis risk, although the mechanisms for this association are not yet well understood. Several recent studies suggest that metabolic inflammation and hyperlipidemia increase the susceptibility of chondrocytes to biomechanically-induced cellular stress, as occurs following joint injury (Aspden, 2011; Mooney *et al*.,

2011). The elucidation of the exact role of biochemical factors that regulate the behavior of the chondrocytes and other cells in the joint will lead to identification of new targets for osteoarthritis therapy (Van der Kraan, 2006). In conclusion, the increase of plasma TSA and LSA levels is associated positively with the presence of inflammation and appears to be a consequence of the disease itself, and could be suggested as one of the newly discovered marker for KOA. While the decrease in the levels of SOD and β-Carotene parameters may be due to the increased turnover for preventing oxidative damage in these patients, suggesting an increased defense against oxidant damage in knee osteoarthritis.

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