Introduction

Osteoarthritis (OA) is the most common joint disorder and the leading cause of disability worldwide. Major pathological features of the disease include degeneration of articular cartilage, bone changes and global articular dysfunction. The prevalence increases with aging and reaches more than 50% in people aged 60 years and older. Most commonly affected sites in primary OA are knee, hip, spine, hand (distal and proximal interphalangeal joints, first carpo-metacarpal joint), feet (first metatarsophalangeal joint). Despite the widespread occurrence and significant socio-economic impact of OA, the available pharmacological treatments mainly rely on non-steroidal anti-inflammatory drugs [1], and currently there are no approved drugs with disease-modifying effect in OA.

Among the various supplements employed in OA management, chondroitin sulfate (CS) stands out as one of the most commonly prescribed [2, 3]. Although CS is recommended in some guidelines as oral symptomatic slow-acting drug for treatment of knee, hip and hand OA [4-6], the Osteoarthritis Research Society International/OARSI guidelines for the non-surgical management of knee, hip, and polyarticular OA do not consider any of the dietary supplements [1]. The most common sources for CS used in dietary and pharmaceutical products are of animal origin, obtained by extraction from tissues and organs of several animals such as bovine and porcine, avian, cartilaginous and bone fishes. In healthy volunteers, the bioavailability of CS is 12-20% [7]. The absorption of CS occurs without lag-time and the time required to reach plasma CS peak concentration is around 1 h and in case of administration of gastro-resistant capsules in 4 hours, indicating that CS rate of absorption is rapid. In humans, the profile
of oral bovine CS plasma concentrations suggests that CS is absorbed in the proximal segments of the small intestine [8, 9]. The low bioavailability of CS does not appear to be associated with intestinal degradation. Possible saturation of the paracellular pathway has been suggested [9].

CS is a major type of glycosaminoglycan. Four subset glycosaminoglycans are recognized based on their chemical structure: CS and dermatan sulfate (i), the heparin and heparan sulfate (iii), keratan sulfate (iii), and hyaluronic acid (iv). Glycosaminoglycans are major components of proteoglycans that are anionic macromolecules, represent structural components of the cell surface and the extracellular matrix, which also take part in the regulation of cell proliferation, differentiation and migration. Proteoglycans contain a core protein and covalently attached glycosaminoglycans that are linear polysaccharides [10]. Commercially available CS is usually manufactured from animal sources such as shark and cow cartilage. It is well absorbed after oral administration with a half-life of 12-24 hours [11]. Proteoglycans may be associated with cellular membrane or to be a component of extracellular matrix that surrounds the cells and is composed of network of collagen, fibronectin, elastin as well as hyaluronan. Extracellular matrix is a dynamic structure that is modifiable during growth and repair. Continuous matrix remodeling is ongoing and is under regulation of processes of intracellular synthesis, post-translational modifications, secretion and extracellular degradation of different matrix components. The turnover time of extracellular matrix is tissue-dependent. Substances released in the process of enzyme-dependent extracellular matrix remodeling regulate cell functions i.e., cell proliferation, differentiation, adhesion, migration and cell death. Of note, disturbances in extracellular matrix remodeling may lead to initiation of tumor growth [12]. Increase in CS content has been observed in many solid tumors in humans i.e., breast cancer, prostate cancer, ovarian, gastric, colon cancer [13, 14, 15, 16, 17].

CS plays a crucial role in organizing the extracellular matrix and has implications in several diseases, including cancer. Currently, it is known that CS has complex dual role in tumor development and progression. The high prevalence of OA, wide use of CS, long therapeutic courses as well as the complex function of glycosaminoglycans raise the question about their long-term safety profile [12].

CS is a part of the tumor microenvironment. It has been suggested that CS may be involved in control of tumor cell phenotype. Experimental studies have proposed potential pro-tumor effects of CS. High CS expression in malignant cells in patients with breast cancer was associated with lymph node metastases, more aggressive phenotype, significantly shorter recurrence-free survival and overall survival, while a correlation with CS expression in tumor stroma was not present [18]. On the other hand, in mice with implanted breast cancer cells, the intra-tumor injections of the bacterial enzyme chondroitinase aiming to eliminate tumor-associated CS molecules has led to increased number of lung metastases without effect on tumor growth that suggests protective effect of CS [19].

Increased synthesis of CS has been observed in melanoma cells as compared with melanocytes. In vitro analysis of the effect of exogenous glycosaminoglycans has shown that CS-6 and CS-4 inhibited significantly migration and translocation murine melanoma cell (B16V) co-cultured with fibroblasts [20].

Migration of cancer cells is associated with proteolysis of extracellular matrix components. This process is mediated by different enzymes such as matrix metalloproteinases (MMP)-2 on cell surface. In an in vitro study it has been demonstrated that CS-4 facilitates the activation of pro-MMP-2 by membrane-type-3 MMP, while CS-6 or low-molecular-mass hyaluronan were ineffective [21].

Interestingly, double sulfated CS (4,6-O-disulfated disaccharides/CS-E) has been shown to reduce the invasiveness of breast cancer cells. In an in vitro study breast cancer cell cultures were treated with CS-E that negatively regulated the expression of the pro-metastatic extracellular matrix gene Col1a1 and interfered with the known pro-tumorigenic Wnt/beta-catenin signaling pathway. It has been concluded that exogenous CS-E inhibits breast cancer cell motility through interference with a pro-tumorigenic Wnt/beta-catenin – Collagen I axis. Culture treatment with CS-4 and CS-6 did not produce such effects [22].

However, Mizumoto et al. (2013) have suggested that CS-E expressed at the surface of Lewis lung carcinoma cells may also contribute the binding to the luminal side of the vascular endothelial cells in lungs, thus playing a crucial role for development of pulmonary metastasis [23]. Similarly, Vallen et al. (2012) have found significantly increased expression of CS-E in ovarian cancer cells as compared with benign tumors and tumors with low malignant potential [24].

As mentioned above, in a number of non-clinical, basic studies it has been suggested that CS may influence tumorigeneses. Thus, CS has been studied
as a potential biomarker in prostate cancer due to its influence on cell growth and differentiation and suggested potential to affect cancer progression. CS concentration in stromal tissue of patients with prostate cancers was a stronger prognostic feature than tumor grade that could predict negative outcome for patients with moderately differentiated tumors. Cases with a low CS concentration had significantly better progression-free survival after radical prostatectomy vs patients with high CS levels [14].

Lin et al. have found that oral gavage of CS-4 lead to increase in growth rates and sizes of tumors in mouse models of melanoma inoculated with human primary melanoma tissues expressing BRAF V600E. In addition, development of resistance to BRAF inhibitors has been observed. Oncogenic BRAF V600E mutant is expressed in more than 50% of melanomas and is a therapeutic target because of its pathogenic role. Interestingly, CS-4 administration did not influence the growth rates and sizes of melanoma tissues with other gene variant [11].

In an in vitro study, an increased expression of genes for CS-4 and its sulfation enzyme (chondroitin 4 sulfotransferase-11) in aggressive human breast cancer cell lines was detected, while in less aggressive breast cancer cell lines their expression was significantly lower. A significant positive correlation was observed between the expression levels of chondroitin 4 sulfotransferase-11 and P-selectin binding to cells. It has been demonstrated that CS glycosaminoglycans on the surface of breast cancer cells is involved in P-selectin binding of cancer cells to platelets and endothelial cells [25]. P-selectin is suggested to be involved in development of blood-borne metastases [25, 26].

Of note, CS is suggested to possess anti-inflammatory effects on articular cartilage and synovium. In articular cartilage, CS decreases the expression of phospholipase A2, cyclooxygenase-2 (COX-2), prostaglandin E2 concentration as well as the level of proinflammatory cytokines i.e., TNF-α, IL-1β. CS reduces the systemic and joint level of NO and reactive oxygen species and suppresses IL-1β – mediated increase in the level of MMP-2,3,9,13,14 [27].

Moreover, a link between chronic inflammation and cancer initiation and progression has been suggested via influence of inflammation on processes of mutagenesis, mitogenesis, angiogenesis, defective apoptosis. This additionally increases the interest in anti-inflammatory properties of different supplements [27, 28].

Considering the anti-inflammatory properties of CS, a protective role for development of different type of cancers in people taking chondroitin has been proposed. Epidemiological studies have assessed possible protective association of CS use with reduced risk of cancer. However, neither protective effect nor increased risk has been found in CS users [29, 30, 31, 32]. Of note, these studies were not controlled in populations with specific genetic backgrounds.

Analysis of the available data shows evidence for dual role of CS in cancer development and progression. However, studies evaluating associations between CS use and risk of cancer in humans are limited.

Notably, studies investigating the influence of CS use on the overall risk of cancer failed to reveal association with CS treatment [29, 30, 31, 32] using a prevalent-user design (meaning that individuals already using the treatment were included as “users”) which might introduce different types of biases such as the duration of treatment use before the study initiation. To the best of our knowledge, there is a notable absence of studies that have methodically assessed the impact of CS on cancer risk employing a more robust “new user” design. This newer approach involves the examination of a cohort of patients who commence a specific drug and initiates follow-up assessments post-treatment initiation, mirroring the methodology commonly found in randomized controlled studies [33].

Therefore, we undertook the current study to evaluate the effect of CS on cancer risk using the new-user design in the publicly available longitudinal data from the database Osteoarthritis Initiative (OAI) [34].

**Aim of the study**

The aim of this study was to evaluate the association between CS use and incidence of self-reported cancer during 6-year follow-up in the publicly available OAI cohort participants.

**Materials and Methods**

The current study included analysis of 6-year longitudinal data obtained from the OAI progression (n = 1390) and incidence (n = 3284) subcohorts that are publicly available at https://nda.nih.gov/oai/ [35].

The inclusion criteria for OAI were the following: age between 45 and 79 years for both subcohorts, symptomatic tibiofemoral knee OA for the progression subcohort, and the presence of established or putative risk factors for incident knee OA for the incidence subcohort. The OAI participants were recruited and enrolled between February 2004 and May 2006 at four recruitment centers in the United
States. This study received ethical approval from each recruitment center. All participants provided written informed consent.

To obtain information about the use of CS, a medication inventory method was used. This method is based on that the participants bring in all of the medications they are taking at the time of visit, and the brand name, generic name or active ingredients are recorded and matched to an entry in an online medication dictionary [36].

To reduce the risk of bias, only participants who did not take CS at baseline were included in the analysis (a “new-user” design) [37]. Incident cancer was defined as an occurrence of self-reported cancer, other than skin cancer, leukemia or lymphoma. The information on self-reported cancer was collected from the Charlson Comorbidity Index. CS users with cancer were defined using the following criteria 1. a person who used CS for at least 6 months 2. chondroitin use was at least 1 year before the incident self-reported cancer. For CS users without a cancer, the duration of CS use had to be at least 6 months. All other participants were classified as non-users. To examine the cancer risk for CS users compared with non-users, the incidence rate ratios (IRRs) were calculated, both unadjusted and adjusted for age (2-year age groups), race (white or non-white), and smoking status (current smoker/non-smoker) using the Mantel-Haenszel test. A 95% CI was applied to determine statistical significance. All statistical analyses were performed using R software (version 3.5.1).

**Results**

The flow diagram of analysis is shown in Fig. 1. A total of 3167 participants neither having cancer nor taking CS at baseline were included in the analysis. 570 (18%) new users of CS and 2597 (82%) CS non-users were compared. In the CS user group, 294 (51.6%) participants received CS for more than one year, 291 (33.5%) for more than 2 years, 123 (21.6%) for more than 3 years, and 60 (10.5%) for more than 4 years. None of the participants received CS for more than 5 years.

CS users and non-users were of the same age, had similar male/female ratio, and had similar body mass index (BMI). In the non-user group, there were more African-American participants and there were more current smokers.

160 (6.2%) and 37 (6.5%) cases of incident self-reported cancer in the non-users and user groups were identified. There was no association between the use of CS and incident self-reported cancer in the non-adjusted analyses. The adjustments for age, race and smoking status did not change the results (Table 2). There were missing data about the race for 2 cases in CS users and 2 subjects in non-users.

**Discussion**

In the current study, no association between the use of CS and incident cancer was found. The results concur with those reported by Brasky et al. (2012) who have analyzed 35,239 male participants, at the age between 50-76 years, who were members of the ViTamins And Lifestyle (VITAL) cohort, residents of western Washington State. The patients have completed an extensive baseline questionnaire and provided information about the frequency (days/week) and duration (years) of supplement uses. Overall 1,602 incident invasive prostate cancers were registered. There was no link between CS use and prostate cancer risk [29].

![Fig. 1. Analysis of CS use and self-reported cancer](image-url)
Association between the use of CS and incidence of colorectal cancer using VITAL cohort was studied by Kantor et al. (2013) who have analyzed 75,137 western Washington residents aged 50–76 who completed the VITAL questionnaire between 2000-2002 regarding the association between glucosamine and chondroitin use and colorectal cancer risk. Glucosamine and chondroitin use during the 10-year period prior to baseline was assessed. People who have taken the supplements more than three years, 4 or more days weekly had a non-statistically significant 45% lower risk for colorectal cancer than non-users and this inverse association was present for overweight/obese patients, but not in those with normal body weight or under-weight. The use of glucosamine alone was not significantly associated with colorectal cancer risk [38]. In over 77,000 participants (VITAL cohort), Satia et al. (2009) have found that any use of both glucosamine and chondroitin was also associated with lower risk for colorectal cancer. Glucosamine, chondroitin, and methylsulfonylmethane were adjusted for each of the other two supplements for both lung and colorectal cancers. The participants were followed for an average period of 5 years [39].

Brasky et al. (2010) who have analyzed 35,016 postmenopausal women (mean follow-up time 6 years) in the VITAL cohort and have found no association between breast cancer and glucosamine and chondroitin intake both in cases of recent use or 10-year average use [30]. In the VITAL cohort studies, an association between glucosamine and CS use and self-reported urothelial cell carcinoma of the bladder [40] and hematologic malignancies has also not been observed [41].

Analysis in the above-mentioned studies had a prevalent user design and thus could have been subjected to bias leading to incorrect results [42]. It should be emphasized that in the current study, a new-user design was used, which simulates a randomized controlled study and allows to prevent sev-

Table 1. Characteristics of CS non-users and users

<table>
<thead>
<tr>
<th></th>
<th>Non-users</th>
<th>Users</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2597</td>
<td>570</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>61.01 (9.42)</td>
<td>60.61 (8.90)</td>
<td>0.351</td>
</tr>
<tr>
<td>Male gender</td>
<td>1528 (58.8)</td>
<td>348 (61.1)</td>
<td>0.354</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.74 (4.88)</td>
<td>28.82 (4.92)</td>
<td>0.725</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Other non-white</td>
<td>54 (2.1)</td>
<td>8 (1.4)</td>
<td></td>
</tr>
<tr>
<td>White or Caucasian</td>
<td>1921 (74.0)</td>
<td>468 (82.1)</td>
<td></td>
</tr>
<tr>
<td>Black or African American</td>
<td>604 (23.3)</td>
<td>84 (14.7)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>16 (0.6)</td>
<td>8 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>Never</td>
<td>1371 (52.8)</td>
<td>311 (54.6)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>209 (8.0)</td>
<td>22 (3.9)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>996 (38.4)</td>
<td>234 (41.1)</td>
<td></td>
</tr>
<tr>
<td>Current, but never regular</td>
<td>6 (0.2)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Data presented as mean (SD) or n (%). BMI Body Mass Index

Table 2. Incidence Rate Ratio of Cancer in Chondroitin Sulfate Users vs Nonusers

<table>
<thead>
<tr>
<th></th>
<th>IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>1.00 (0.98, 1.03)</td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>1.10 (0.78, 1.56)</td>
</tr>
<tr>
<td>Adjusted for race</td>
<td>1.02 (0.72, 1.45)</td>
</tr>
<tr>
<td>Adjusted for smoking status</td>
<td>1.03 (0.72, 1.46)</td>
</tr>
</tbody>
</table>

IRR Incidence Rate Ratio, CI confidence interval
eral types of biases inherent in studies with prevalent user design [33].

The results of the current study may be due to lack of effect of CS on cancer risk or due to the limitations of the study. The main limitation of our study is that we used a self-reported cancer as a main outcome. The usage of self-reported cancer as an outcome in this analysis may reduce the validity of the results. Overall, the self-reported cancer lacks sensitivity but for some tumor locations like breast and thyroid cancers self-reported diagnoses are highly valid [43]. The strength of the study is that a large population was analyzed with a well-defined cohort and we report for the first time results from a study with a new-user design to address a question of association between CS use and cancer.

**CONCLUSION**

The results of the current study did not show an association between CS use and self-reported cancers. Considering the complex function of CS in the development and progression of malignant tumors, there is a need for further epidemiological studies evaluating CS effects on specific types of cancers and in different genetic backgrounds.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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