RESEARCH COMMUNICATION

Impact of Total and Ionized Serum Calcium on Prostate Cancer Risk in North Indian Men

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Abstract

Introduction: Calcium has anti-proliferative and pro-differentiation effects on cells *in vitro* and can inhibit the development of various cancers. While there is some epidemiologic evidence for an inverse relation between dietary calcium intake and prostate cancer risk, only few have focused on serum calcium levels in this respect. Materials and Methods: We assayed total serum calcium and ionized serum calcium in a pilot study of 40 prostate cancer patients and compared with 40 healthy controls. Results: Our observations provided evidence for an association between prostate cancer risk and total and ionized serum calcium levels(p=0.020 and p≤0.001 respectively). The mean difference of total serum calcium was also significant in patients with serum PSA >20ng/ml (p=0.017). Conclusion: This is an important and interesting finding which requires further exploration into mechanism involved in calcium channel and prostate cancer risk in a larger cohort of different ethnic population.

Keywords: Serum calcium - ionized calcium - prostate cancer - PSA - North India

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Introduction

Prostate cancer affects men worldwide, being most common in Europe and United States as compared to the other Asian countries. Many possible risk factors have been identified; both endogenous factors such as ethnicity and genes, and exogenous factors such as diet, environment etc. (Gronberg, 2003). There are a number of studies which focus on high intake of dietary calcium, such as dairy products, that may increase the risk of prostate cancer (Giovannucci et al., 2006, Kesse et al., 2006 and Tseng et al., 2005). Calcium levels in serum are sturdily controlled over a wide range of dietary calcium and generally are not correlated with dietary calcium levels. Approximately half of the total serum calcium is in the ionized or physiologically active state. Another 40% is bound to serum proteins, principally albumin, and the remaining 10% is bound to anions such as lactate and phosphate (Skinner et al., 2009). The fact that 1, 25-dihydroxyvitamin D (1, 25-D) might protect against prostate cancer (Schwartz et al., 1990) suggests another possible mechanism: that at sufficiently high amounts, dietary calcium suppresses production of 1, 25-D, thereby increasing risk of prostate cancer (Giovannucci et al., 1998). The hypothesized reason for this association is based on the relationship between calcium and vitamin D.

So therefore if the calcium levels are controlled in a PCa patient then it can be assumed that the disease could be less aggressive. Although numerous studies have investigated prostate cancer with respect to calcium intake from the diet, the subject of calcium in serum has received scarce attention, perhaps because calcium levels in serum are believed to be under strict homeostatic control. Increase in extra-cellular serum calcium causes a decrease in apoptosis and an increase in proliferation and migration of metastatic prostate cancer cells (Schwartz et al., 2009). Thus, high levels of total calcium in serum might also give rise to malignant cancers.

Based on this objective we aimed to analyse the association of total and ionized serum calcium with prostate cancer risk in North Indian men. Therefore in this pilot study we assessed the relationship between total and ionized serum calcium along with serum creatinine levels for prostate cancer risk in North Indian population.

Materials and Methods

Study subjects

Forty healthy individuals and 40 prostate cancer patients were recruited for the assay. Of the 40 patients attending the Urology Department at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow (U.P.) between August 2009 and April 2010 satisfied the eligibility criteria. For PCa patients, the base line diagnostic work-up included digital rectal examination (DRE), prostate specific antigen level (PSA) and prostate biopsy. The pathological grades were categorized

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according to the Gleason grading system (Gleason and Mellinger, 1974) and classified into three groups: Low Grade (< 7), Intermediate grade (7) and High grade (> 7). Every effort was made to enroll control subjects that matched each case patient in age, and ethnicity. Therefore, simultaneously 40 unrelated healthy, age matched controls having no history of cancer or any other chronic disease were recruited for the study. The controls were selected from the individuals from health awareness camp held at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow (U.P.) and were free of any chronic or urological disease or any type of cancer. The controls were free of any voiding symptoms (American Urological Association); their prostate-specific antigen (PSA) levels were within the normal limit (< 4 ng/ml). Blood samples (3ml) were collected in plain vials after the interview from each of eligible subjects. Serum was isolated by centrifugation at 5000rpm for 5 minutes, and stored in -20ºC until further analysis. The total PSA levels were determined in controls and PCa patients using CanAg PSA ELISA kits (Fugirebio, Sweden). The study protocol was approved by Ethical Committee of the Institute. Informed consent was obtained from each subject at time of recruitment.

Biochemical Tests

Serum total calcium assay was done by Arsenazo III method on the fully automated clinical chemistry autoanalyser, Imula. Mac Ranolux Diagnostics, U.K. using internal quality control sera, calibrators from Ranolux Diagnostics, U.K. Serum ionized calcium was calculated by the formula developed by Pottgen and Davies (Pottgen and Davies, 1976) using serum total calcium, total protein and albumin.

Statistical Analysis

In order to estimate the significant difference between pre-diagnostic mean calcium levels of healthy controls and PCa patients, we used Student's t-test and the result is expressed in terms of Mean±S.D. and p value <0.05 was considered statistically significant. Post-Hoc Comparisons were made to see exactly which pairs of groups are significantly different. Distribution of age and substance use was examined using Chi-square statistics and logistic regression analysis was used to evaluate the association in controls and patients calcium level as well as with clinical parameters. All statistical analyses were performed with the SPSS ver. 13.0 (SPSS Inc, Chicago, USA) statistical software package.

Results

Demographic details

The mean age (years±SD) for PCa patients and controls were (62±8.7) and (68±8.5) respectively, which was not significantly different. A considerable significant difference was observed in total PSA between patients (35±43.6) and controls (0.41±0.42) (p=0.036) . Bone Scan was done only for 36 cases, with 21 (58.3%) positive metastasis, while 15 (41.7%) demonstrated negative metastasis. The Gleason grade data was available for only 27 PCa patients.

Table 1. Frequency Distribution of Total and Ionized Calcium in Controls and PCa patients

Parameter	Controls	Patients	OR (95%CI)	p[c] value
Total serum calcium				
Normal	37 (92.5)	27(67.5)	1[a]	
Hypocalcaemic	2 (5.0)	4 (10.0)	4.31 (0.607-30.7)	0.144
Hypercalcaemic	1 (2.5)	9 (22.5)	18.9 (2.103-170)	0.027
Ionized serum calcium				
Normal	20 (50)	0 (0.0)		
Hypocalcaemic	4 (10)	0 (0.0)	NC	NC
Hypercalcaemic	16 (40)	40 (100)	NC	NC

[c]Bonferroni corrected; NC, not calculated; [a]adjusted for age

Biochemical analysis

Mean total calcium was 10.1mg/dL, and mean ionized calcium was 7mg/dL and a remarkable increase was seen in ionized serum calcium levels in case of PCa patients which was found to be significantly associated with prostate cancer risk (p value, <0.001) as none of the 40 PCa patients had normal ionized serum calcium levels. A correlation was also observed between total serum calcium levels and PCa risk (p, 0.020) .

Association between controls and PCa patients and with clinical parameters

Total serum calcium was associated with a much higher risk in PCa patients as compared to controls (Table 1), 22.5% of the PCa patients were found to be hypercalcaemic in comparison to controls (2.5%). In case of ionized serum calcium all the 40 PCa patients were hypercalcaemic ($\chi 2$ p value 0.003 compared to controls), so therefore further multivariate analysis was not done. Post-Hoc analysis between serum PSA and serum calcium levels revealed that mean difference of total serum calcium in group of patients with PSA >20ng/ml was significantly different from the other 2 groups with a PSA level between 5-10ng/ml and 11-20ng/ml. Whereas, no significant difference was observed between serum PSA levels and ionized serum calcium levels in PCa patients.

Total and ionized serum calcium associated risks with clinical parameters, such as Gleason score and Bone metastasis were also evaluated. For Gleason group grading the patients were categorized in three groups (Low grade <7; Intermediate grade =7 and High grade >7). Overall no significant association was observed statistically with any of the grading stages and with bone metastasis status in PCa.

Discussion

Current guidelines recommend an intake of 1200mg/day of calcium over 50 years of age in men. However, calcium from diet or supplemental sources has been linked to a higher risk of prostate cancer in an epidemiological study (Sonn et al., 2005). Calcium may support vitamin D-related anti-proliferative effects in prostate cancer. The evidence linking prostate cancer risk to the afore-mentioned dietary factor remains compelling but inconsistent. Comparison of these studies is hampered

by differences in sample size, duration of follow-up, and extensiveness of dietary evaluation.

In our study, all of the PCa patients demonstrated high ionized calcium concentration than the control group. Whereas the serum calcium concentration exhibited an increased trend in PCa patients irrespective of the tumor stage i.e. localized as well advanced stage patients all had elevated levels of ionized serum calcium as compared to that of healthy controls. Thus, it can be suggested that ionized serum calcium can turn out to be a novel and promising marker, but on the other hand total serum calcium was also found to be at risk for PCa as evident from our results. It was also associated with serum PSA levels. The fact that some of the controls also had elevated levels of total and ionized serum calcium may be linked to several other factors, like diet and hormones (Skinner et al., 2009).

Our findings are consistent with studies that observed an elevated risk of prostate cancer with greater calcium (Berndt et al., 2002 and Chan et al., 2000). During the last 20 years, the introduction of the prostate-specific antigen (PSA) screening has enhanced the early detection of prostate cancer with the consequence of a higher proportion of non-fatal cancers among the diagnosed (Kvale et al., 2007). Notably, several previous studies that included larger proportions of cases diagnosed after the widespread adoption of PSA for screening saw stronger associations with calcium for more advanced disease than for the early, preclinical disease often detected by PSA screening (Kristal et al., 2002 and Rodriguez et al. 2003). This was in conjunction with our findings as we also found that mean difference of total serum calcium was significant in patients with serum PSA >20ng/ml in comparison to those individuals with PSA<20ng/ml. We also observed that 70.3% of the PCa patients recruited in our study were in advanced stage (Gleason grade ≥7) of prostate cancer and showed an elevated risk for the ionized serum calcium levels.

But on the contrary it has been it is evident from literature that association between serum calcium and prostate cancer is more prominent and relevant in aggressive prostate cancer, a high proportion of PSA detected cases would dilute the association (Halthur et al., 2009). The possibility of the anomaly to evaluate the proportion of bound calcium in relation to biologically available calcium is generally overlooked when analyzing total calcium, as compared to only measuring free ionized calcium. If a man were to have, e.g., elevated levels of calcium binding plasma proteins, typically albumin, then total calcium level would be misguiding. Unfortunately, data on albumin levels are unavailable which precludes the possibility of calculating the amount of free calcium. However, total calcium has been considered as a good measure of calcium homeostasis

In summary, we found that prostate cancer risk was significantly elevated with higher total and ionized calcium levels. Our findings therefore suggest that high serum calcium levels might increases risk of prostate cancer, probably through its calcium content. A stronger association between calcium intake and symptomatic prostate cancers (rather than asymptomatic cancers detected through PSA screening) is also consistent with the effect on progression of prostate cancer (Lokeshwar et al., 1999).

Reasons for the elevated risk are unclear, although the reduced content and bioavailability of vitamin D may play a role. The limitation of our study is that we did not record any data regarding the calcium intake in study subjects. Given the implications of our findings with respect to increase calcium intake, the mechanisms by which calcium might increase prostate cancer risk should be clarified and confirmed to verify that calcium is indeed the critical risk factor. To the best of our knowledge this is first study evaluating the association between serum calcium and prostate cancer risk in Indian population. But this preliminary finding needs to be validated in population with large sample size and taking the relevant dietary and hormonal factors into consideration which are correlated with the calcium regulation as is evident from studies in other populations, so as to confirm the scenario of the present study.

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