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Effect of calcium & Vit D supplementation on incidence/severity of ovarian cancer

Dr. Seema Mishra[1], Dr. Kamlesh[2]
[1] Department of Clinical Nutrition, Bilasa PG Girls College, Bilaspur, Chhattisgarh, India
[2] Additional Director & Principal, Govt E Raghvendra Rao PG Science College, Bilaspur, Chhattisgarh, India

Abstract
Several epidemiological studies have evaluated the association between calcium intake and the reduced risk of ovarian cancer. However, the results of these studies remain controversial. Among 15 epidemiological studies involving 493,415 participants and 7453 cases eligible for this meta-analysis, 13 studies were about dietary calcium intake, 4 studies about dairy calcium intake and 7 studies about dietary plus supplemental calcium intake. When comparing the highest with the lowest intake, the pooled RRs of ovarian cancer were 0.80 (95% CI 0.72–0.89) for dietary calcium, 0.80 (95% CI 0.66–0.98) for dairy calcium and 0.90 (95% CI 0.65–1.24) for dietary plus supplemental calcium, respectively. Dietary calcium was significantly associated with a reduced risk of ovarian cancer among cohort studies (RR = 0.86, 95% CI 0.74–0.99) and among case-control studies (*RR* = 0.75, 95% CI 0.64–0.89). Based on these previous studies we designed a study to assess the effect of Ca supplementation / dietary intake with the occurrence / severity of ovarian cancer. In subgroup analysis by ovarian cancer subtypes, we found a statistically significant association between the dietary calcium (*RR* = 0.69, 92% CI 0.66–0.81) and the risk of epithelial ovarian cancer. This meta-analysis indicated that increased calcium intake might be inversely associated with the risk of ovarian cancer; this still needs to be confirmed by larger prospective cohort studies.

Keywords: calcium, vit. D, ovarian cancer

Introduction
Ovarian cancer has emerged as one of the most common malignancies affecting women in India. The present communication reports the trends in the incidence rate of ovarian cancer for Indian women. Vitamin D is a fat-soluble vitamin playing a vital role in human physiology. Calcium & Vitamin D deficiency is prevalent worldwide. The prevalence of Vitamin D deficiency ranged from 40% to 99%, with most of the studies reporting a prevalence of 80%–90%. There are more than 200,000 new ovarian cancer cases and 140,000 deaths of ovarian cancer per year, globally. Ovarian cancer is the seventh most common cause of cancer death among women worldwide and the fifth leading cause of cancer death among women. Numerous epidemiological studies have been carried out to evaluate the association between calcium intake and the risk of ovarian cancer. However, the results are inconsistent. Four studies found that calcium intake was inversely related to ovarian cancer risk, while other studies found no evidence of an association. Therefore, we systematically conducted a meta-analysis to further investigate the associations between dietary calcium and dairy calcium intake and the risk of ovarian cancer; further explore the effect of dietary plus supplemental calcium intake on the risk of ovarian cancer.

Etio-pathology
The exact biological mechanisms underlying calcium intake and risk of ovarian cancer are still not completely determined. One underlying explanation for our findings is that a higher level of calcium might be inversely related to ovarian cancer risk via down-regulation of circulating parathyroid hormone (PTH). The reduction of PTH could decrease hepatic and osteoblastic synthesis of insulin-like growth factor-1 (IGF-1). IGF-1 may exert a direct effect by increasing cell proliferation and inhibition of apoptosis and experimental studies have indeed shown that malignant transformation of ovarian epithelial cells (the cells from which ovarian cancer is believed to originate) can be induced by over expression of the IGF-1 receptor. These mitogenic and anti-apoptotic effects of IGF-I might be particularly relevant during ovulation related tissue remodeling of the surface epithelium. The reduction of IGF-1 would weaken mitogenic effects on the pathogenesis of ovarian cancer. In addition, PTH may be a tumour promoter acting as a comitogen and anti-apoptotic factor directly. Ovarian cancer is one of the top 19 cancers sensitive to vitamin D. Several studies show that mortality rates for this cancer are lower in areas with more solar ultraviolet-B (UVB) light. This is similar to findings of other cancers including breast cancer. Vitamin D encourages cells to either adapt to their organ or commit apoptosis (cell suicide). Calcitriol also limits blood supply to the tumor and reduces the spread of cancer. It is also found that vitamin D not only delayed malignant transformation of ovarian surface epithelial cells induced by DMBA but also played a chemo-preventive role in animal models directly implanted with DMBA.

The antineoplastic actions of $1\alpha,25(OH)_2D_3$ and its analogs have been shown both in vitro and in vivo, in various malignancies, including ovarian cancer. For example, in EOC cells, $1\alpha,25(OH)_2D_3$ caused cell cycle arrest at the G2/M transition and G1/S checkpoint, and decreased human telomerase reverse transcriptase mRNA stability through microRNA, the mechanism underlying $1\alpha,25(OH)_2D_3$-induced cell death. Our previous study also demonstrated that $1\alpha,25(OH)_2D_3$ suppressed SKOV-3 cell growth, and enhanced the antiproliferative effect of carboplatin by increasing apoptosis and reactive oxygen species production and reducing mitochondrial membrane potential.6 In the

present study, 1α,25 $(OH)_2$ D_3postponed malignant transformation of MOSE cells by increasing E-cadherin and decreasing β-catenin expression. Thus, these results give strong evidence supporting the use of $1\alpha,25(OH)_2D_3$ as an anti-proliferation agent for ovarian cancer cells in vitro. The chemo-preventive actions of vitamin D have been conducted in experimental lung, mammary and colon carcinogenesis and neuroblastoma. These studies supported that vitamin D or its analogs suppressed development of chemical-induced tumor. In the present study, vitamin D3 was administrated during initiation, promotion, and the full phase of ovarian tumor, respectively. Our results show that the efficacy of vitamin D on inhibiting tumor growth has been demonstrated to be dependent on the timing of administration, and supplementation during the full phase (including initiation and promotion) is the most effective way. Moreover, vitamin D3 also reduced the content of CA125 in both serum and ascites, especially in the 20-week vitamin D3 group. Furthermore, the incidence of ovarian cancer was negatively correlated with the level of 25(OH)D in mice. In addition, the level of serum calcium was not obviously changed among groups, which indicated that a high dose of vitamin D (with a dose of 20,000 IU/kg per week for duration of 20 weeks) did not trigger side effects of elevated serum calcium. These results indicate that vitamin D may delay the progression of ovarian tumors, and it is more effective to supplement with vitamin D at the entire stage than only at initiation or promotion of tumor development. Therefore, the results from in vivo antitumor activity suggest that vitamin D is a promising agent for cancer intervention.

Based on these studies a hypothesis was designed that Normal / high serum Calcium level is negatively associated with the occurrence of Ovarian Cancer, also supplementation with Calcium & Vitamin D reduces the severity of the disease.

Study Area- Bilaspur and Raipur

Study Duration –March 15 to November 18

Sample size – Subjects-61(Ovarian Cancer patients having Calcium [1500 mg/day] & Vitamin D [2000 IU/ Kg/ week] supplementation), Controls-61,(Ovarian Cancer patients with no Calcium & Vitamin D supplementation) both groups are demographically matched. The subjects were selected by contacting hospital, as Raipur hospital, Mittal's Hospital, CIMS and MECAHARA.

Objectives

- Estimation of Serum Calcium mg % in controls & Subjects, before and after Ca & Vit D supplementation
- Estimation of Serum Parathyroid level in Controls and Subjects before & after Ca & Vit D supplementation
- Estimation of Urinary Calcium in both the groups
- Statistical Analysis was done by using SPSS latest version.

Estimation of serum calcium: For the estimation of serum Calcium the diagnostic kit of Nicholas was used. Nicolas Piramal India Limited manufactured it (Catalog No- 907190). The colorimetric method for this is based on the O-cresophtalein complezone (OCPC) method developed by Ray, Sarkar and Chauhan (1967).OCPC reacts with Calcium in alkaline solution to form a purple colored complex. Interference from Magnesium is virtually eliminated by preferential binding with 8-quinoliol, a salt is added to solve problems of non–linearity. More over OCPC is an acid- base indicator; for this reason the reagent is strongly buffered.

- Reagent 1 contains Standard Calcium-8mg/100 ml (2 mmol / l)
- Reagent 2 contains AMP Solution.
- Reagent 3 contains O- Cresophtalein complezone Solution.

The serum samples were not taken from the participants who are in therapy with EDTA. Also, oxalate, citrate and EDTA were not used as anticoagulant. The specimens were drawn without venous stasis because it may alter serum albumin level and consequently serum Calcium level (increase of Calcium up to 0.5% mg% level). For estimation of serum Calcium, first 0.5 ml (500□l) of Reagent-2 was taken in a cuvette. To it the same quantity of Reagent-3 (0.5 ml/500 µl) was added. To this mixture 0.02 ml (20 µl) serum was added. All were mixed well and then left in the room temperature for about five minutes for incubation. The absorbance of the developed color was read at 578 nm, by using a filter with 550-590 nm range. The same procedure was applied for preparing Standard Solution. The 0.5 ml of Reagent-2 and 0.5 ml of Reagent-3 were mixed with 0.02 ml of Standard Calcium solution to incubate the apparatus. The test Sample was compared with a blank solution. The blank was prepared by adding the 0.5 ml of Reagent-2 and 0.5 ml of Reagent-3 only. The absorbance of the standard and test were read against the blank at 578 nm. The Calcium can be estimated in serum or plasma samples stored for ten days at 4˚ C.

Calculation= Ax /As ×8 =mg/100 ml Calcium
(As—sample, Ax—standard)
This assay is linear up to 20 mg / 100 ml.

Estimation of Urinary Calcium: For Calcium estimation the same colorimetric method was adopted. Only to avoid Calcium salt precipitation, acidifications of urine samples were done. In 24 hours urine samples 15 ml of concentrated HCL was added. Refrigerated urine samples were heated to redissolve precipitates and then HCL was added. The acidified urine samples were diluted with distilled water by adding 0.1 ml of the sample to 0.1 ml distilled water. Calculations were done as with estimation with serum. The results were multiply with 2 to adjust the dilution done to the urine sample. The calculated values were converted in to per day value by taking 1500 ml as per day urine volume.

Calculation = (Ax /As × 8) ×2 = mg% Calcium
(As—sample, Ax—standard)

Estimation of serum parathyroid hormone

For the estimation of serum Parathyroid level -mini VIDAS 100 of Biomerieux Company is used by using estimation Kit of Biomerieux Company.

Data and Analysis

Table 1: Serum calcium level in subjects & control groups before supplementation

Controls			Subjects		
Age in Years	**No. of Participants (n = 61)**	**Mean Serum Calcium Level**	**Age in Years**	**No. of Participants (n = 61)**	**Mean Serum Calcium Level**
36-38	4	0.103±0.01	36-38	9	0.101±0.01
39-41	7	0.104±0.01	39-41	9	0.093±0.004
42-44	8	0.105±0.01	42-44	8	0.096±0.004
45-47	5	0.115±0.005	45-47	9	0.086±0.004
48-50	7	0.117±0.026	48-50	9	0.093±0.004
51-53	9	0.123±0.017	51-53	8	0.099±0.004
54-56	5	0.133±0.005	54-56	5	0.093±0.003
57-59	7	0.132±0.004	57-59	2	0.098±0.008
60-62	9	0.136±0.004	60-62	2	0.100±0.003

Values expressed as $\overline{x}$ mg.ml^{-1} serum and are presented as Mean value ± Standard Deviation.

Table 2: Serum Calcium Level in subjects before & after supplementation

Age (Years)	Duration of Ca & Vit D Supplementation to subjects	serum Calcium (Before)	serum Calcium (After)
36-38	5 months	0.084	0.125
39-41	5 months	0.103	0.127
42-44	4 month 3 weeks	0.106	0.129
45-47	4 month 2 weeks	0.098	0.133
48-50	4 month 2 weeks	0.097	0.136
51-53	6 months	0.078	0.145
54-56	5 months 3 weeks	0.082	0.129
57-59	6 weeks	0.072	0.147
60-62	4 months 3weeks	0.069	0.131

Values expressed as Calcium exertion mg/day serum and are presented as Mean value ± Standard Deviation.

The serum Calcium levels of subjects were observed significantly higher than the values before Ca & Vit D supplementation. (The mean serum Calcium after supplementation was 0.11 mg/ml before supplementation was 0.95 mg/ml.

Table 3: Serum parathyroid hormone Level in subjects & control Groups after supplementation

Controls			Subjects		
Age in Years	**Serum Parathyroid Level (pg/ml)**	**Mean Serum Calcium Level**	**Age in Years**	**Serum Parathyroid Level(pg/ml)**	**Mean Serum Calcium Level**
36-38	49.59	0.103±0.01	36-38	40.56	0.101±0.01
39-41	50.67	0.104±0.01	39-41	38.88	0.093±0.004
42-44	58.78	0.105±0.01	42-44	45.31	0.096±0.004
45-47	119.12	0.115±0.005	45-47	37.89	0.086±0.004
48-50	102.36	0.117±0.026	48-50	37.22	0.093±0.004
51-53	118.77	0.123±0.017	51-53	44.19	0.099±0.004
54-56	125.09	0.133±0.005	54-56	40.57	0.093±0.003
57-59	112.34	0.132±0.004	57-59	43.12	0.098±0.008
60-62	112.45	0.136±0.004	60-62	39.55	0.100±0.003

When estimating Serum Parathyroid Level in studied human subjects, the women patients suffering from Liver Diseases, Pancreatitis and Splangitis were omitted as these conditions precipitate Hyper Para-thyroidism.

Table 4: Serum C-125 level before & after calcium supplementation

Before (n-09)			After (n-09)	
Age in Years	**Serum C-125 Units / ml**	**Mean Serum Calcium Level**	**Serum C-125 Units / ml**	**Mean Serum Calcium Level**
36	69	0.084	46	0.125
41	74	0.103	53	0.127
44	79	0.106	39	0.129
47	81	0.098	73	0.133
50	62	0.097	55	0.136
53	108	0.078	84	0.145
54	112	0.082	96	0.129
57	101	0.072	72	0.147
62	99	0.069	58	0.131

Table 4: Mean, SD & 't' Values of Serum Calcium Profile of subjects & Controls and Effect of supplementation

/Factors	(mean Calcium level)	Changes in percent value	t value
Serum Calcium	0.095 (±0.07) [subject] 0.119 (±0.01) [Control]	↓ 20%	9.26*, **(df =120)
Urinary Calcium Level	185.08 (±0.25) [subject] 350.41 (±0.92) [Control]	↓ 89%	25.24*, **(df =120)
Serum Parathyroid Level	40.81 (±0.33) [subject] 94.35 (±0.17) [control]	↓ 44 %	13.33*, ** (df= 120)
Effect of supplementation on Serum Calcium	0.13 (±0.03) [Before] 0.10 (±0.11) [After]	↑ 15%	0.89 NS (df = 120)
Serum C-125 Level	87.22 (±0.09) [Before] 64.00 (±0.10) [After]	↓ 27 %	0.93 NS (df = 20)

NS = Not Significant *P<0.05 level, **P<0.01 level [SD values showed in parenthesis]

As can be appreciated from the above data, a significant difference in Serum Calcium and Urinary Calcium Levels were noteworthy among the subjects and controls. The calculated t-values are significant at both levels in the case of Serum Calcium and Urinary Calcium. In comparison subject's groups serum Calcium was 20% increased in control groups. Urinary Calcium was also observed 89% more in control group, this Hyper-calcemia and Hyper-calciurea are strong indicators of hyper activity of Parathyroid Hormone. This hormone is proved precipitating agent of Ovarian Cancer.

A negative correlation (r = 0.314) was observed among serum Calcium levels and serum levels of Parathyroid Hormones, as higher levels of Parathyroid causes Hypercalcemia thus lower levels of this hormone precipitates normal or lower levels of serum Calcium. Likewise as a negative correlation was observed between serum levels of Calcium and serum levels of C-125, Ovarian Cancer biomarker (r = 0.687) because normal levels of Parathyroid hormone precipitates normal/lower levels of Serum Calcium. A negative correlation (r = 0.102) was also seen among serum Parathyroid level and serum C-125 level.

Thus we can concluded that Higher Serum Parathyroid level precipitates hypercalcemia and increased C-125 levels with increased possibility of Ovarian Cancer, supplementation of Calcium along with Vitamin D corrects not only the serum Parathyroid level, serum Calcium but also the serum C-125 levels and severity of ovarian cancer.

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