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## Implications of Raised Folate and Lowered Vitamin B<sub>12</sub> in Osteosarcoma

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### ABSTRACT

Osteosarcoma is the most common primary tumor of bone, occur mainly during childhood and adolescence. No reports are available in literature where serum folate and vitamin B<sub>12</sub> have been assessed in osteosarcoma patients. Hence, the present study was planned to analyze status of folate and vitamin B<sub>12</sub> in thirty osteosarcoma patients and compare it with thirty controls (subjects with musculoskeletal pain). Serum calcium, alkaline phosphatase levels were higher and phosphorus levels were comparable in osteosarcoma patients as compared to controls. Serum folate levels were significantly raised in osteosarcoma patients as compared to controls (p<0.001). Serum vitamin B<sub>12</sub> levels were significantly lowered in osteosarcoma patients as compared to controls (p<0.001). Folate-mediated one-carbon metabolism (FOCM) is unequivocally linked to multiple health outcomes, including birth defects, several types of cancer and possibly cardiovascular disease and cognitive function. Tumors are known to up regulate FR $\alpha$  modulating the folate uptake in serum. Vitamin B<sub>12</sub> diminishes osteoblastic activity and lowered serum vitamin B<sub>12</sub> levels are possibly due to increased metabolic demand of the tumor. The present study suggests that these parameters can serve as useful markers for diagnosis and follow up of disease.

**Key words:** Folate, vitamin B<sub>12</sub>, osteosarcoma, osteoblastic activity, serum, tumor

### INTRODUCTION

Osteosarcoma is the most common primary tumor of bone, occur mainly during childhood and adolescence. Osteosarcoma develops at the sites of greatest bone growth (Rosier, 1999). Several markers for diagnosis and prognosis have been proposed in osteosarcoma namely, VEGF, bone alkaline phosphatase, osteocalcin, survivin, Erb B<sub>2</sub> (Palmieri *et al.*, 2009).

Alterations in one carbon metabolism related nutrients (folate, vitamin B<sub>12</sub>) in body leads to insufficient methyl group for DNA synthesis, methylation and repair, thus promoting carcinogenesis (Stover, 2009; Bailey and Gregory, 1999; Eto and Krumdieck, 1986). Several epidemiological studies have reported protective role of folate in preventing development of colorectal, breast and lung cancers (Johansson *et al.*, 2010; Zhang *et al.*, 1999; Heimburger *et al.*, 1988). Some studies have reported that diet deficient in vitamin B<sub>12</sub> enhance tumor growth (Bailey and Gregory, 1999; Eto and Krumdieck, 1986; Zhang *et al.*, 1999; Heimburger *et al.*, 1988).

There are conflicting results in several studies in relation to folate or vitamin B<sub>12</sub> status and neoplasia. Epidemiological studies have demonstrated association between folic acid supplementation and increased risk of colorectal cancer (Mason *et al.*, 2007). Also, high vitamin B<sub>12</sub>

intake has been reported to increase risk of prostate, esophageal and gastric cancer (Hultdin *et al.*, 2005; Mayne *et al.*, 2001).

However, no reports are available in literature where serum folate and vitamin B<sub>12</sub> have been assessed in osteosarcoma patients. Though, deficiencies in vitamin B, along with the consequent elevated homocysteine level have been reported to be associated with bone loss, decreased bone strength and increased risk of fracture (Ahmadiéh and Arabi, 2011).

Hence, the present study was planned to analyze status of folate and vitamin B<sub>12</sub> in osteosarcoma patients.

## **MATERIALS AND METHODS**

Thirty histopathologically confirmed cases of osteosarcoma (localized, without metastasis) were selected for the study and 30 age-matched subjects with musculoskeletal pain served as control. The study was carried out between July 2010 to June 2011.

Five milliliter blood was collected aseptically and serum was separated by centrifugation. Serum folic acid and vitamin B<sub>12</sub> were analyzed by competitive immunoassay using direct chemiluminescent assay (Brewster, 1989; Chen *et al.*, 1987).

Folate in the patient sample competes with acridinium ester labelled folate in the Lite Reagent for a limited amount of biotin-labelled folate binding protein. Biotin-labelled folate binding protein binds to avidin that is covalently coupled to paramagnetic particles in the Solid Phase. The sample is pre-treated to release the folate from endogenous binding proteins in the sample. The system automatically performs these steps.

Vitamin B<sub>12</sub> from the patient sample competes with vitamin B<sub>12</sub> labeled with acridinium ester in the lite reagent, for a limited amount of purified intrinsic factor, which is covalently coupled to paramagnetic particles in the solid phase. The assay uses releasing agent (sodium hydroxide) and DTT to release the vitamin B<sub>12</sub> from the endogenous binding proteins in the sample and cobinamide to prevent rebinding after the solid phase is added to the sample. The system automatically performs these steps.

**Statistical analysis:** SPSS ver.18 was applied for various statistical analysis and student's t-test and regression analysis was carried out. Level of significance was 0.05.

## **RESULTS AND DISCUSSION**

Table 1 analysed various parameters in both the groups. Serum calcium levels were higher in osteosarcoma patients as compared to controls ( $p < 0.001$ ). Serum phosphorus levels were comparable among both the groups. Serum alkaline phosphatase were raised in osteosarcoma as compared to controls ( $p < 0.05$ ).

In the present study, serum folate levels were significantly increased in osteosarcoma patients as compared to controls ( $p < 0.001$ , Table 2). To the best of our knowledge, no study is available where folate levels have been reported in osteosarcoma. Though low levels of folate have been reported in Paget's disease (Polyzos *et al.*, 2010). Rapidly dividing cells have an increased requirement for folate to maintain DNA synthesis and findings of raised serum folate levels in the present study supports the wide-spread use of antifolate in chemotherapy.

There are conflicting reports in relation to folate and cancer development. Some studies support the concept that diminished folate status predisposes to development of several cancers such as colon, breast, lung, pancreas and cervix (Glynn *et al.*, 1996; Almadori *et al.*, 2002;

Table 1: Various parameters in both osteosarcoma and control groups (Mean±SD)

Parameters	Osteosarcoma	Controls
Serum calcium (mg dL <sup>-1</sup> )	10.01±0.58**	9.46±0.70
Serum phosphorous (mg dL <sup>-1</sup> )	4.41±0.55 <sup>ns</sup>	4.58±0.51
Serum alkaline phosphatase (U L <sup>-1</sup> )	331.73±173.69***	120.23±31.78

ns: Not significant, \*\*p<0.01, \*\*\*p<0.01, \*\*\*p<0.01, significant when compared to control

Table 2: Serum folic acid and vitamin B<sub>12</sub> levels in both osteosarcoma and control groups (Mean±SD)

Group	Serum folic acid (ng mL <sup>-1</sup> )	Serum vitamin B <sub>12</sub> (pg mL <sup>-1</sup> )
Osteosarcoma	14.30±0.55*	235.80±121.71*
Control	5.30±0.86	522.50±206.80

\*p<0.001, significant when compared to control

Table 3: Correlation between various parameters (r-values)

Parameter	Serum vitamin B <sub>12</sub>	
	Osteosarcoma	Control
Serum calcium (mg dL <sup>-1</sup> )	-0.464**	-0.410**
Serum alkaline phosphatase (U L <sup>-1</sup> )	-0.116 <sup>ns</sup>	0.201 <sup>ns</sup>
Serum folic acid (ng mL <sup>-1</sup> )	-0.405**	0.359 <sup>ns</sup>

ns: Not significant, \*\*p<0.01, significant when compared to control

Kato *et al.*, 1999). Increased risk of colorectal cancer has been reported with folate supplementation and high serum folate levels (Weinstein *et al.*, 2008), Reduced Folate Carrier (RFC), a folate carrier protein is expressed in almost all tissues and its primary physiological function is tissue redistribution of folate (Whetstine *et al.*, 2002). Decreased RFC expression and transport mediated defects of folate have been reported in osteosarcoma (Yang *et al.*, 2008).

Tumors are known to up regulate folate receptors and folate receptor (FR $\alpha$ ) has been reported to over expressed in osteosarcoma tissue samples (Yang *et al.*, 2007). It has been reported that FR $\alpha$  is found in normal tissues and in malignant cells it loses its polarity and become contactable to blood stream, thus modulating cell folate uptake (Yang *et al.*, 2007).

In the present study, serum vitamin B<sub>12</sub> levels were significantly decreased in osteosarcoma patients as compared to controls (Table 2, p<0.001). Status of vitamin B<sub>12</sub> in osteosarcoma is not known and no report of vitamin B<sub>12</sub> status in osteosarcoma is available in literature. However, high serum cobalamin has been reported in chronic myeloid leukemia, multiple myeloma (Ermens *et al.*, 1993). Several *in vitro* and clinical studies have indicated that cobalamin may have effect (direct or indirect) on osteoclastic activity (Whetstine *et al.*, 2002; Yang *et al.*, 2008; Yang *et al.*, 2007; Ermens *et al.*, 1993).

Serum alkaline phosphatase was inversely correlated with vitamin B<sub>12</sub> in osteosarcoma and positively correlated with controls and was not statistically significant (Table 3). Effect of vitamin B<sub>12</sub> on proliferation and cellular alkaline phosphatase activity in human bone marrow stromal osteoprogenitor cells (hbMSC) and osteoblastic cells (UMR106 cells) has been reported (Kim *et al.*, 1996). They suggested that vitamin B<sub>12</sub> deficiency suppresses osteoblastic activity that may contribute to osteoporosis and fractures.

A significant negative correlation was observed between serum calcium and vitamin B<sub>12</sub> in both osteosarcoma and controls and values were higher in osteosarcoma (Table 3). Low vitamin B<sub>12</sub> levels

suppress osteoblastic activity and release calcium from bone (Kim *et al.*, 1996). Finding of inverse correlation between vitamin B<sub>12</sub> and serum calcium in the present study lend support to this statement.

In the present study a significant inverse correlation was observed between serum folate and vitamin B<sub>12</sub> levels (Table 3). It is not clearly documented in literature whether imbalance between folate and vitamin B<sub>12</sub> associated with any other adverse effects, low serum folate and vitamin B<sub>6</sub> concentrations but not low serum vitamin B<sub>12</sub> concentrations, have been reported to be associated with an altered morphology of human bone (Holstein *et al.*, 2009).

There is compelling evidence that habitual intake of adequate quantities of folate helps to reduce the risk of developing colorectal cancer in general population (Ratan *et al.*, 2008). In addition, there is little question that folate may act in a paradoxical, cancer promoting fashion under certain experimental conditions. Ample evidence now exists to indicate that increasing folic acid intake of women in the periconception period substantially diminishes the risk of pregnancies complicated by a neural tube defect (Ratan *et al.*, 2008). Evidence from animal studies suggests a possible association between high intakes of folic acid and promotion of cancer development and progression. There is also a time trend study from the USA and Canada that suggests colorectal cancer incidence increased at around the same time mandatory fortification with folic acid was introduced (Wald and Oakley, 2007).

Vitamin B<sub>12</sub> has been shown to stimulate osteoblast proliferation and alkaline phosphatase activity and vitamin B<sub>12</sub> deficiency has been associated with defective functional maturation of osteoblasts. Recent publications indicate a shift to more evidence of osteoclast stimulation by high homocysteine and low vitamin B<sub>12</sub> concentrations (Van Wijngaarden *et al.*, 2011). Kim *et al.* (1996) suggested that a suppressed activity of osteoblasts may contribute to osteoporosis and fractures in patients with vitamin B<sub>12</sub> deficiency. Herrmann *et al.* (2007) demonstrated a strong stimulatory effect of low concentrations of folate, vitamin B<sub>12</sub> and B<sub>6</sub> on osteoclasts activity, suggesting a mechanistic role of low B-vitamin concentrations for bone degradation.

Folate has a dual effect on cancer, protecting against cancer initiation but facilitating progression and growth of preneoplastic cells and subclinical cancers, which are common in the population. Thus, a high folic acid intake may be harmful for some people. At present, no information concerning the effect of maternal folic acid supplementation on bone cancer risk in the offspring exists in humans. Epigenetic and metabolic programming takes places during embryogenesis and hence the embryonic stage is highly susceptible to changes in the intrauterine environment, which may influence the risk of developing cancer in adulthood (Ly *et al.*, 2011).

Findings of raised folate and vitamin B<sub>12</sub> in young subjects with osteosarcoma in the present study raise the possibility of supplementation of folate and occurrence of osteosarcoma in these subjects. It may be added that in India, periconception maternal folic acid supplementation is in practice owing to high risk of neural tube defects. Further studies are required to explore dual effect of folate on cancer, especially bone cancer.

Tumors are known to up regulate FR $\alpha$  modulating the folate uptake in serum. Vitamin B<sub>12</sub> diminishes osteoblastic activity and lowered serum vitamin B<sub>12</sub> levels are possibly due to increased metabolic demand of the tumor. Thus, vitamin B<sub>12</sub> supplementation, along with newer antifolate derivative and drugs targeting FR $\alpha$  proteins may be of value in treatment of osteosarcoma in future.

## CONCLUSION

Serum folate levels were significantly raised and vitamin B<sub>12</sub> levels were significantly lowered in osteosarcoma patients as compared to controls. The present study suggests that these parameters can serve as useful markers for diagnosis and follow up of disease and raise the possibility of supplementation of folate, vitamin B<sub>12</sub> and occurrence of osteosarcoma in these subjects.

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