Medical Hypotheses 123 (2019) 67–71

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Is vitamin D deficiency behind the scenes for high incidence of Giant cell tumor amongst the Indian population? Unraveling the vitamin D – RANKL association

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ARTICLE INFO

Keywords:
Giant cell tumor
Vitamin D
RANKL pathway
Endemic hypovitaminosis D
Indian population

ABSTRACT

Introduction: The major neoplastic and proliferative component of GCTB is the stromal tumor cells; that they have shown no evidence of bone destruction, instead the massive tissue destruction appears to be a result of tumor induced osteoclastogenesis.

The discovery of receptor activator of nuclear factor kB (RANK) and RANK binding ligand (RANKL) uncovered the bone homeostasis and molecular mechanism by which multiple compounds (including vitamin D) regulated osteoclast differentiation; a function mediated by osteoblastic cells and osteoclast-precursor cells.

Hypothesis: In a country burdened by vitamin D deficiency, causal relation between hypovitaminosis D and GCTB was hypothesized based on the vitamin D mediated RANKL expression and osteoclastogenesis, as India is also a population with higher incidence of GCTB as compared to Western populations described in the literature.

The possibility of vitamin D regulated osteoclastogenesis in GCTB is postulated on the evidence from molecular research linking it to the RANK/RANKL/OPG pathway.

The aim of this study was to analyse the prevalence of Vitamin D deficiency in patients with primary GCTB and to elucidate any difference in serum Vitamin 25(OD)D_3 levels amongst the matched control population data.

Materials and results: 130 patients of primary GCTBs were matched to 310 controls from the general health check population and serum levels of 25(OH)D_3 were analyzed. Statistical analysis performed on the non-parametric data and Mann Whitney U Test used to derive inference with significance set at $p < 0.05$.

56 females and 76 males with median Vitamin D level in the GCTB group was 15.9 ng/ml (Mean 19.41; Range 1.03 to 92) as compared to the control population with median level of 22.2 ng/ml (Mean 25.1; Range 2.6 to 87.9). The results were significant (p value < 0.05) as compared to the control population in all decades except the third decade (p value 0.0548).

Discussion: The differential expression of RANKL and OPG in response to levels of vitamin D has been established. The stromal cells of osteolytic GCTB express high levels of RANKL, which is a key signal regulator in development of this disease and bone destruction typical of GCTBs. This has resulted in research targeting this pathway for therapeutic approach in GCTBs.

As vitamin D supplementation is simple and safe, increased awareness to assess and if necessary correct vitamin D status of patients is warranted, however the question as to whether patients with low vitamin D levels are more prone to develop GCTB and thus would profit from vitamin D supplementation remains unanswered.

To conclude, it is essential to assess vitamin D levels in patients with GCTB as deficiency is pronounced. Future research on this hypothesis might lead to an association between Vitamin D deficiency and the onset/natural history of GCTB that may in the future help us cure or prevent GCTBs.

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https://doi.org/10.1016/j.mehy.2018.12.010
Received 28 October 2018; Accepted 20 December 2018

Introduction

Giant cell tumor of bone (GCTB) is an osteolytic primary bone neoplasm occurring in young adults and characterized by the presence of numerous osteoclasts. The name GCTB or osteoclastoma falsely implies neoplasm of proliferating osteoclast or osteoclast precursors [1]. The major neoplastic and proliferative component of GCTB is the stromal tumor cells [1–3]; that they have shown no evidence of bone destruction, instead the massive tissue destruction appears to be a result of tumor induced osteoclastogenesis [1,4].

The discovery of receptor activator of nuclear factor kB (RANK) and RANK binding ligand (RANKL) uncovered the bone homeostasis and molecular mechanism by which multiple compounds (including vitamin D) regulated osteoclast differentiation; a function mediated by osteoblastic cells and osteoclast-precursor cells [2,5–9] (Fig. 1). RANKL which promotes bone resorption by inducing osteoclast maturation [10] as well as Osteoprotegrin (OPG) which blocks RANKL by acting as a decoy receptor for the ligand [11]: are both produced by the osteoblasts [12]. The key to bone formation:resorption cascade is the RANKL:OPG ratio which is regulated by osteoblast genome [13].

The GCTB neoplastic cells are dysfunctional mesenchymal stromal-like cells that are believed to be precursors of osteoblastic cells due their potential to produce osteoid [1]. Expression of RANKL was shown in cultured mesenchymal stromal-like spindle shaped mononuclear neoplastic cells of GCTB [4,14,15] and suggested its role to induce formation of tumor like giant cells of GCTB [4,16] (Fig. 2).

Hypothesis

In a country burdened by vitamin D deficiency [17–20], causal relation between hypovitaminosis D and GCTB was hypothesized based on the vitamin D mediated RANKL expression and osteoclastogenesis, as India is also a population with higher incidence of GCTB as compared to Western populations described in the literature [21,22].

The possibility of vitamin D regulated osteoclastogenesis in GCTB is postulated on the evidence from molecular research that direct action of hypovitaminosis D and upregulation of RANKL expression on osteoblasts resulting in osteoclastogenesis has been studied in vivo [5,23]. Also pharmacological concentrations of active vitamin D compounds have shown direct suppression of osteoclast precursor cells [5,24,25].

Evaluation of hypothesis

The aim of this study was to analyse the prevalence of Vitamin D deficiency in patients with primary GCTB and to elucidate any difference in serum Vitamin 25(OD)D_3 levels amongst the matched control population data; to ascertain the critical role of Vitamin D depletion that may be involved in producing the large osteoclast population typical of GCTBs.

Empirical data

130 patients of primary GCTBs presenting to our center were subjected to venous blood samples for serum 25(OH)D_3 vitamin levels prior to initiation of treatment. Age and sex matched 310 controls were identified from the general health check population that underwent the investigation without any obvious clinical presentation of Vitamin D deficiency and data entered into a spreadsheet. The study was conducted in accordance to the guidelines of the Institutional Review Board and Ethics Committee. Guidelines of the endocrine society defining vitamin D deficiency at 25(OH)D_3 level of < 20 ng/ml was used [26]. Statistical analysis performed on the non-parametric data and Mann Whitney U Test used to derive inference with significance set at $p < 0.05$.

Results

56 females and 76 males with median age 31 years (Range 14–67 years) presented to our center with primary GCTB. Median Vitamin D level in the GCTB group was 15.9 ng/ml (Mean 19.41; Range 1.03–92) as compared to the control population with median level of 22.2 ng/ml (Mean 25.1; Range 2.6–87.9) (Table 1). The mean vitamin

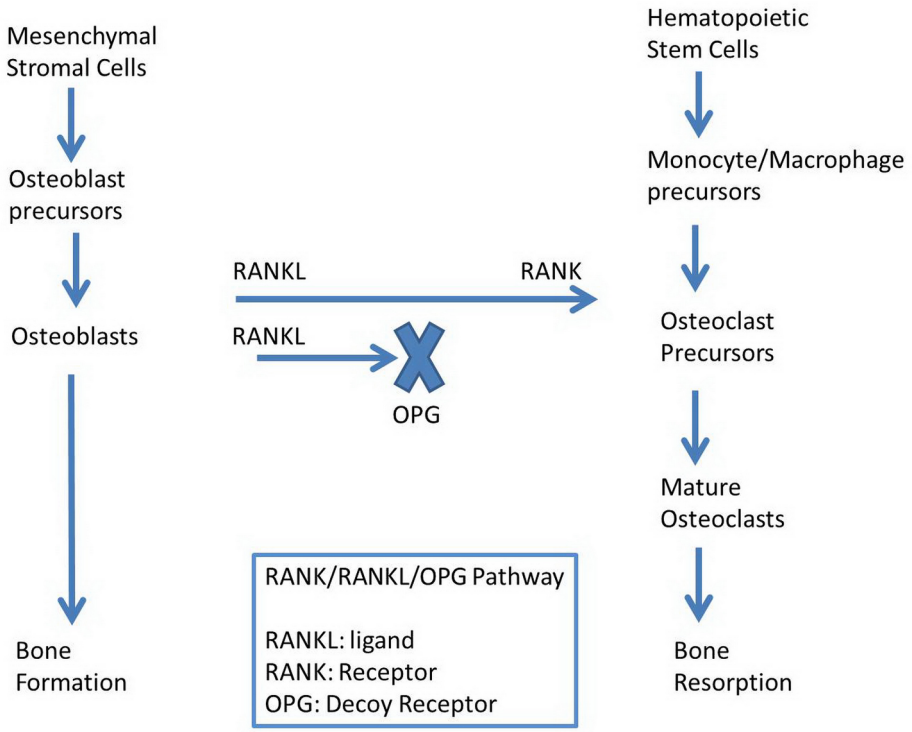

Fig. 1. Bone Homeostasis interlinking osteoblast-osteoclasts via the RANK/RANKL/OPG pathway.

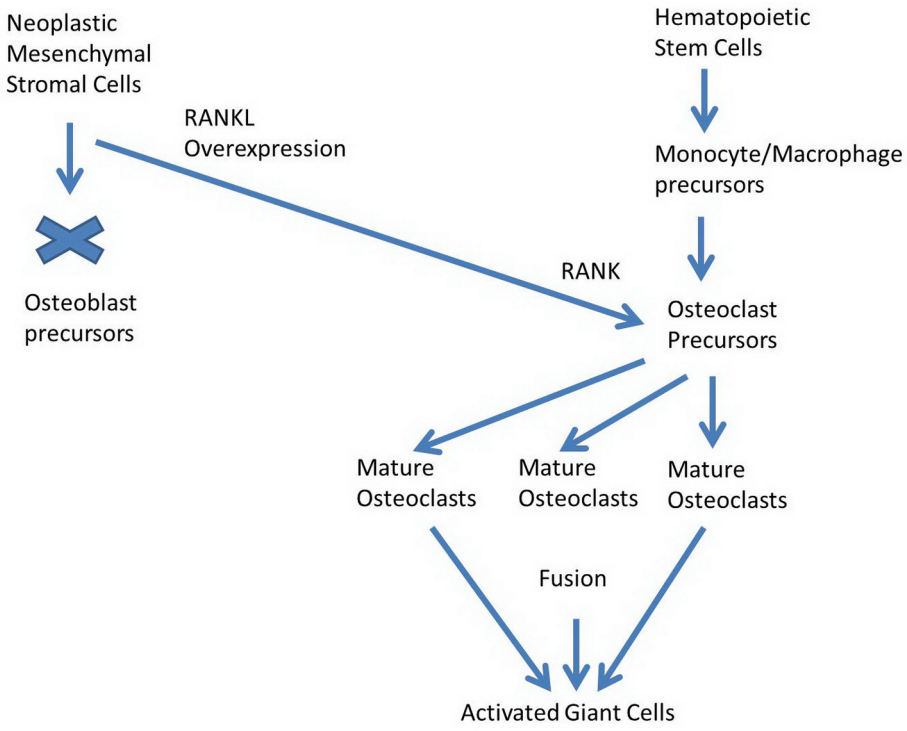

Fig. 2. The neoplastic paradigm of physiological osteoblast-osteoclast structure in Giant Cell Tumor of Bone.

D levels across the various age groups in the control population were consistent with results observed in literature for Indian and South Asian populations. Although there was no significant difference in Vitamin D levels between males and females in both the groups (p value 0.116 for GCTBs and p value 0.575 for control group); the results were significant (p value < 0.05) as compared to the control population in all decades except the third decade (p value 0.0548).

Discussion

The etiology of GCTB is still unknown, significant reduced vitamin D levels observed in the GCTB population as compared to the control population supports the hypothesis that vitamin D – RANKL pathway and resultant osteoclastogenesis due to vitamin D deficiency may play a role in course and natural history of GCTB. Endocrine changes resulting from vitamin D deficiency lead to an increase in bone resorption and alterations within the bone microenvironment that further results in release of growth factors potentially promoting the formation and growth of bone tumors [27,28].

The neoplastic component of GCTB is mainly dysfunctional mesenchymal stromal cells that produce chemokines and cytokines to recruit osteoclast precursors of monocyte/macrophage lineage from blood and promote osteoclastogenesis [1]. The neoplastic stromal cells of GCTB share traits such as cytokine and gene expression to osteoblasts [14,29,30]; however the cytokine environment coupled with tumorigenic gene expression causes neoplastic GCTB cells to fail to differentiate into osteoblasts. This suggests a neoplastic paradigm of physiological osteoblast-osteoclast structure involving GCTB stromal cells and monocytes [1,30] (Fig. 2).

The receptor activator of nuclear factor kB ligand (RANKL) and osteoprotegerin (OPG); both produced by osteoblasts are major components of the RANK-RANKL-OPG pathway, a key signaling pathway in bone remodeling by differentiation and activation of osteoclast precursors. RANKL expression of osteoblasts results in osteoclast differentiation via the RANK receptor on osteoclast precursors; whereas OPG production by osteoblasts blocks RANKL action by acting as a decoy receptor for the ligand (Fig. 1). This emphasizes the role of osteoblast and osteoclast cell lineages in osteoclastogenesis [8,31].The role of serum $25(OH)D_3$ levels in determination of bone volume has well been established [32]. It has been recently established that osteoblasts are

Table 1
Results.

Sr No.	Age Group	GCTB		Non GCTB Controls		P value
		Median $25(OH)D_3$ levels	(No.)	Median $25(OH)D_3$ levels	(No.)	
1.	Second Decade	18	11	27.1	28	0.0348
2.	Third Decade	14.8	47	21.2	108	0.0548
3.	Fourth Decade	12.2	39	19.5	90	0.0146
4.	Fifth Decade	11	20	22.01	43	0.0366
5.	Sixth Decade	14.9	10	22.5	35	0.098
6.	Seventh Decade	17.43	3	32.8	7	0.023
	Total	15.9	130	22.2	310	< 0.001
	Males	Females	P value			
GCTBs	76	56	0.116			
Non GCTB	184	126	0.575			

GCTB: Giant Cell Tumour of Bone.

also a site of vitamin D metabolism [13,33,34]. The differential expression of RANKL and OPG in response to levels of vitamin D has been studied at the molecular level. Reduced RANKL expression (RANKL:OPG mRNA ratio) was shown by Anderson et al in cultured human osteoblasts in response to higher serum vitamin 25(OH)D3 levels [32] resulting in reduced osteoclast mediated bone resorption [5,9]. Pharmacological concentrations of active vitamin D compounds have shown direct suppression of osteoclast precursor cells [5,24,25] 0.25(OH)D_3 enhanced – RANKL mediated metabolism is an important intrinsic mechanism for optimizing osteoclast differentiation and coupling bone resorption to formation [35].

Dysregulation of RANKL pathway by mutation or epigenetic alterations may induce aberrant expression of components of this pathway leading to osteoclast mediated bone destruction and even bone metastasis or progression of existing skeletal tumors [36,37]. The stromal cells of osteolytic GCTB express high levels of RANKL, which is a key signal regulator in development of this disease [38] and bone destruction typical of GCTBs. This has resulted in research targeting this pathway for therapeutic approach in GCTBs [39–43].

Laboratory cultured mesenchymal stromal cells of GCTB have shown to induce differentiation of RANKL expression in presence of active 1,25$(OH)_2D_3$ and dexamethasone [4,15].Further research on characteristics of neoplastic cells of GCTB which are believed to be dysfunctional mesenchymal stromal precursors of osteoblastic cells could hold the key to the association of RANKL mediated osteoclastogenesis of GCTB and Vitamin D deficiency.

In a country burdened by vitamin D deficiency [17–20] as well as higher incidence of GCTB as compared to Western populations described in the literature [21,22], causal relation between hypovitaminosis D and GCTB was hypothesized based on the vitamin D mediated RANKL expression.

To the best of our knowledge, there is currently no report of vitamin D status in patients with GCTB. We found widespread and significant lower levels of vitamin D in patients with GCTB as compared to the endemic low levels in the control population. We are aware that this study has certain limitations that need to be taken into consideration. Hypovitaminosis D is frequent in the general population in India [18–20] and direct comparison between studies is often difficult and can be misleading. The results achieved here only propose but cannot prove a causal relationship and further studies are certainly needed to confirm the results.

As vitamin D supplementation is simple and safe, increased awareness to assess and if necessary correct vitamin D status of patients is warranted, however the question as to whether patients with low vitamin D levels are more prone to develop GCTB and thus would profit from vitamin D supplementation remains unanswered. Molecular research and the ability to induce formation of giant cell tumor from osteoblast precursor mesenchymal stromal cells in a vitamin D deficient environment may ascertain a direct relationship.

To conclude, it is essential to assess vitamin D levels in patients with GCTB as deficiency is pronounced and may warrant supplementation. Future research on this hypothesis might lead to an association between Vitamin D deficiency and the onset/natural history of GCTB that may in the future help us cure or prevent GCTBs.

Conflict of interest statement

Each author certifies that neither he, nor any member of his immediate family, has funding or commercial associations (consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

Each author certifies that his institution waived approval for the human protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

This work was performed at P. D. Hinduja Hospital and Medical Research Center, Mumbai, India

Acknowledgement

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2018.12.010.

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