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## Original Article

# Hypocalcitemia in Handigodu Disease: a spondylo epi (meta) physeal dysplasia

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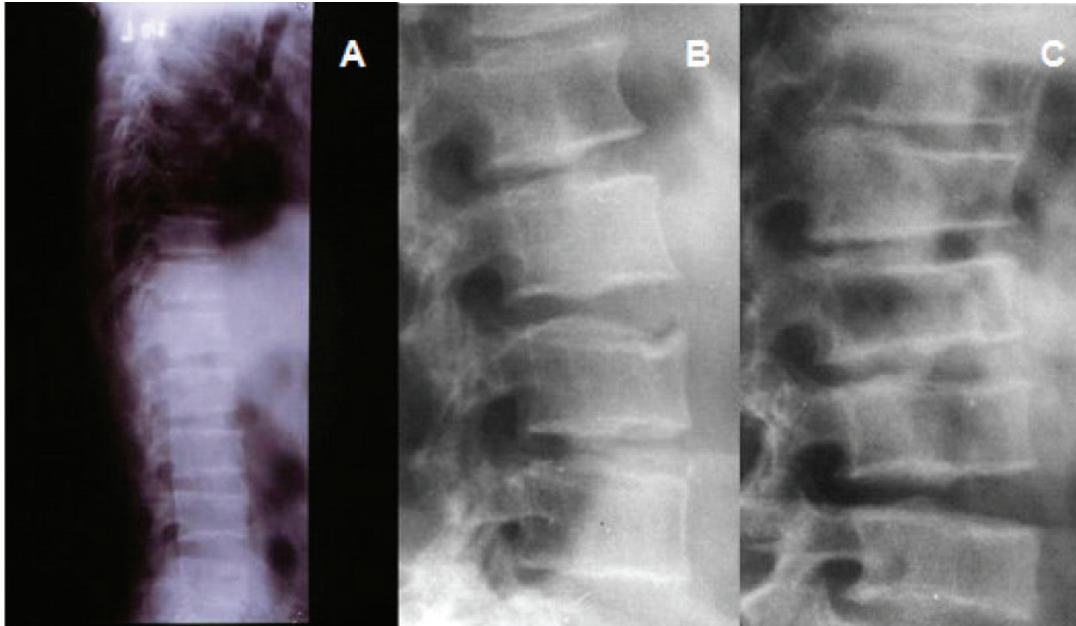
**Abstract:** Handigodu Disease (HD) is a disorder of the osteoarticular system which is highly prevalent in several villages of two districts viz, Shimoga and Chikmagalur of the state of Karnataka, southern India. The scientific name of the disease is Spondylo-epi-(meta) physeal Dysplasia, Autosomal Dominant variety, Handigodu syndrome. The same has been listed in the International Classification of Skeletal Dysplasias. The calcium homeostasis study was lack in HD. The serum calcium, phosphorus, parathyroid hormone and calcitonin levels after overnight fast state, and 24 hour urinary excretion of calcium and phosphorus were quantified. The decreased level of calcitonin associated with decreased serum total calcium and urinary calcium in HD were observed. The levels of parathyroid hormone, serum phosphorus and urinary phosphorus remain unchanged among HD affected. The Vitamin D3 levels also noticed unchanged in HD affected. Since calcitonin has antiresorption effect on bone, the observed low calcitonin in HD may imply reosrption of bone leading to deformity and causes hypocalcaemia and hypocalciuria. The hypocalcitemia without change in iPTH associated with hypocalcaemia may be a mutation in Vit D receptor (VDR) or may be an epiphenomenon.

**Keywords:** Calcitonin, hypocalciuria, parathyroid hormone

## Introduction

Handigodu Disease (HD) is a disorder of the osteoarticular system prevalent in several villages of two districts viz, Shimoga and Chickmagalur in the state of Karnataka, southern India. The disease was first identified in a patient from Handigodu village in 1975, hence its name [1]. The data obtained during subsequent multidisciplinary study by Indian Council of Medical Research (ICMR) showed that HD is a Spondylo epi (meta) physeal dysplasia, inherited as an autosomal dominant trait [2]. The clinical presentation of patients with HD has been identified into three sub types such as Arthritic type (Type I), Dysplastic type (Type II) and Dwarf type (Type III). All three sub types segregate in the same families implies often seen in the same family. The X-ray pictures showing the marked abnormalities are depicted in the **Figure 1** and **Figure 2**.

The type I was characterized by late presentation of disease (45-50 years of age), characterized by pain in hip joints with difficulty in walking; and on examination these individuals had near normal height and body proportions (without significant truncal shortening), inability to sit cross-legged, fixed flexion deformity of hips and compensatory lumbar lordosis. Radiologically these individuals showed characteristic changes of osteoarthritis at the hip joints bilaterally, without any abnormality of spine or involvement of other bones. The type II of Handigodu disease was characterized by short stature, particularly truncal shortening. These patients were relatively younger (25-35 years of age) and the main complaint was inability to walk for longer distance. The X-rays of these patients showed dysplastic femoral heads bilaterally, and varying degrees of platyspondyly. The type III Handigodu syndrome was marked by



**Figure 1.** X-Ray radiograph of hip joints from Handigodu Disease (HD) (A-E): The radiograph osteoarthritis in hip joints of Type I subgroup are shown in A-C whereas dysplasia of hip joints from Type II and Type III subgroups are presented in D and E respectively.

dwarfism. They also had varying types of associated skeletal anomalies, particularly at the knees and hands. In these patients there was marked epiphyseal dysplasia at hips, knees, hands and wrists besides significant platyspondyly [2, 3]. The similarities between the Handigodu Disease and Mseleni joint disease reported from South Africa [3].

Unpublished findings of alterations in calcium homeostasis were observed by the group of Dr. Teotia and colleagues (as part of the ICMR multidisciplinary study). Since there was a deficit of study on mineral metabolism of bone with respect to hormones in HD. The calcium homeostasis was studied by quantifying serum calcium, phosphorus, parathyroid hormone and calcitonin in overnight fast state of serum. The calcium and phosphorus excretions for 24 hrs urine volume were also quantified to avoid interference of diurnal rhythms and food habits.

### Materials and methods

#### Subjects

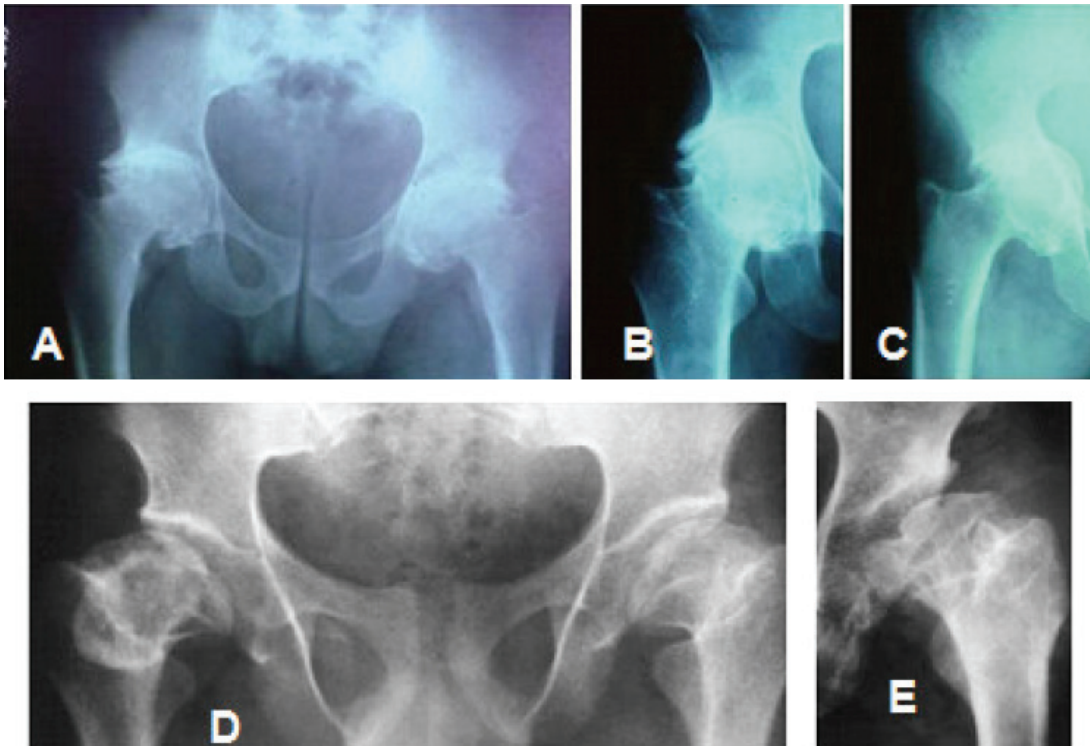
The HD affected patients clinically and x-ray radiological confirmed (Type I; n=19, Type II; n=

28 and Type III; n=8) have been selected for this study within age group of 20-60 years old with their consent. The sixty three (Nonaffected; n=63) healthy individuals with age group of 20-60 years old from the non related family members of HD affected were enrolled in this study as controls. The patients were enrolled from the field. A door to door survey had been done of affected communities in all the affected villages of the Sagar taluk of District Shimoga in the state of Karnataka. X-ray examination had been done on all the family members (except pregnant women), in affected families with their consent. Only cases confirmed by both physical examination and evaluation of X-rays were included in the study, from amongst those who were willing to be admitted in the hospital for collection of 24 hour urine sample and overnight fast serum. The X-ray pictures were elucidated in **Figure 1** and **Figure 2**. All the controls clinical and by x-ray diagnosis were normal.

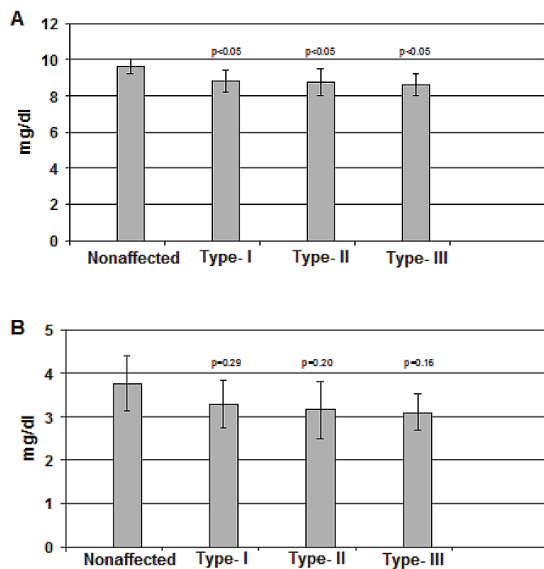
#### Ethics

Both the affected and control subjects were explained the purpose of the study and written informed consent was obtained prior to the collection of specimens. The study was approved

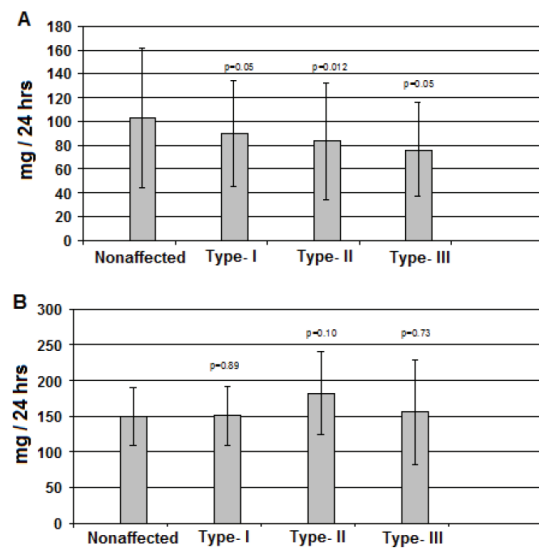
## Hypocalcitemia in Handigodu disease



**Figure 2.** X-Ray radiograph of Lumbar spine from Handigodu Disease (HD) (A-C): Lateral view Lumbar spine showing varying degrees of platyspondyly in Type I (A), Type II (B) and Type III (C) subgroups.

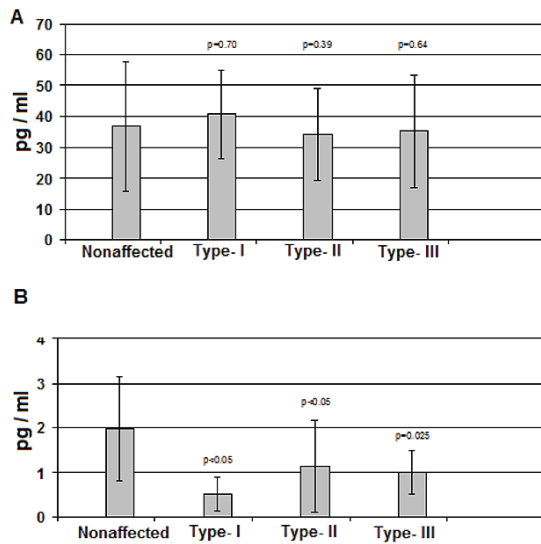


**Figure 3.** Calcium and Phosphorus level of sera in Handigodu Disease (HD) (A-B): Serum total Calcium (A) and Phosphorus (B) levels in HD subgroups Type I, Type II, and Type III compared with nonaffected family group. Note significantly decreased level of Calcium but unchanged Phosphorus in HD subgroups compared to nonaffected family group.



**Figure 4.** The excretory level of Calcium and Phosphorus in Handigodu Disease (HD) (A-B): The 24 hours urinary excretion of Calcium (A) and Phosphorus (B) levels in HD subgroups Type I, Type II, and Type III compared with nonaffected family group. There was significant decrease in level of Calcium but unchanged Phosphorus excretion in HD subgroups compared to nonaffected family group.

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**Figure 5.** The Parathyroid hormone and Calcitonin level of sera in Handigodu Disease (HD) (A-B): Serum Parathyroid hormone (A) and Calcitonin (B) levels in HD subgroups Type I, Type II, and Type III compared with nonaffected family group. There was significant decrease in level of Calcitonin and unchanged parathyroid hormone level in HD subgroups compared to nonaffected family group.

by institute's human ethics committee.

### Biomarkers

The subjects were asked to empty their bladder prior to the collection of 24 hrs urine and Hydrochloric acid (HCl) was used as preservative to avoid microbial deterioration. The serum samples were collected after an overnight fasting state. All of them (affected and control group) remained on their usual home diet during the 24 hours urine collection. The serum and urinary levels of calcium and phosphorus were determined by automated serum chemistry analyzer (Olympus). Calcium and phosphorus excretions were related to 24 hrs urine volume collected and creatinine levels excreted. The serum iPTH and Calcitonin levels were determined by Immulite 2000 reagents.

### Statistical analysis

The Data were analyzed by using SPSS programme of Windows 2000. Statistical significance was determined with one-way ANOVA followed by Bonferroni T-test and represented by 'p' value. The 'p' values were determined for

different HD groups in comparison with non affected group as controls. The probability values,  $\leq 0.05$  were considered as statistically significant. The p values were shown with respective data presented in Figures.

### Results

The calcium homeostasis was studied by quantifying calcium, phosphorus in urine and serum, and hormones from serum.

The serum total calcium (mg/dl) levels (Nonaffected:  $9.58 \pm 0.39$ , Type I:  $8.83 \pm 0.59$ , Type II:  $8.74 \pm 0.73$ , Type III:  $8.61 \pm 0.65$ ) were found to be significantly less in affected group (**Figure 3A**). Interestingly, serum phosphorus (mg/dl) levels (Nonaffected:  $3.77 \pm 0.64$ , Type I:  $3.29 \pm 0.54$ , Type II:  $3.15 \pm 0.66$ , Type III:  $3.10 \pm 0.43$ ) were found to be remain unchanged (**Figure 3B**). The excretory levels of calcium in 24 hrs urine volume (mg/24 hrs) (Nonaffected:  $103.16 \pm 58.65$ , Type I:  $89.51 \pm 44.43$ , Type II:  $83.65 \pm 49.13$ , Type III:  $76.42 \pm 39.81$ ) were significantly lowered in affected groups and urinary phosphorus levels (mg/24 hrs) (Nonaffected:  $149.43 \pm 39.77$ , Type I:  $150.90 \pm 41.15$ , Type II:  $182.59 \pm 58.56$ , Type III:  $155.14 \pm 73.43$ ) found to be remain unchanged (**Figure 4A** and **Figure 4B**).

Parathyroid hormone (iPTH) (pg/ml) levels in fasting state serum between control and affected groups (Nonaffected:  $36.76 \pm 20.82$ , Type I:  $40.7 \pm 14.37$ , Type II:  $34.04 \pm 14.91$ , Type III:  $35.15 \pm 18.08$ ) (**Figure 5A**) remain unchanged, whereas calcitonin values (pg/ml) were significantly lowered in HD affected subgroups (Nonaffected:  $1.97 \pm 1.16$ , Type I:  $0.522 \pm 0.372$ , Type II:  $1.125 \pm 1.034$ , Type III:  $0.996 \pm 0.488$ ) (**Figure 5B**).

The serum profile of other biochemical parameters such as total protein, albumin and Alb/Glb ratio, the markers for nutritional deficiency were measured and found without significant differences. Similarly, SGOT, SGPT, Urea, Creatinine and Alkaline Phosphatase values, the markers for liver function test and kidney function test were found to be remain unchanged in both the HD affected and unaffected members of HD families (data not shown).

### Discussion

Parathyroid hormone (PTH) synthesized as 115-

amino acid polypeptide called preproPTH in parathyroid cell by polyribosomes located within cell matrix of parathyroid gland. When the growing polypeptide chain ~20-30 amino acids long, the NH<sub>2</sub>- terminus of chain emerges first, and at this time two NH<sub>2</sub> terminal methionine residues cleaved by methionyl amino peptidase. As the nascent chain continues to grow the hydrophobic NH<sub>2</sub> terminal sequence of preproPTH emerges and associates with membrane of endoplasmic reticulum. The sequence of 23 amino acids from prepro PTH is removed by cleavage of gly-syl-lysyl bond near or in reticular membrane by enzymatic activity to proPTH (90 amino acids). The pro PTH arrives at Golgi apparatus and the trypsin like activity accomplishes conversion of proPTH to PTH (iPTH of 1-84 amino acids) by removal of hexapeptide from NH<sub>2</sub> terminal sequence. The transport of PTH at the site of release by plasma membrane occurs through secretory granules. PTH is the predominant form of hormone stored in parathyroid gland [4].

Calcium is the physiologically important regulator of parathyroid glandular activity for secretion of PTH. Other secretagogues have been identified for the regulation of PTH secretion, however their physiological role not understood completely. The secretion of PTH is inversely dependent on concentration of extracellular calcium. PTH increases the concentration calcium in blood and extracellular fluid (ECF) through its effects on bone, kidney, and gut. The negative feedback inhibition of parathyroid gland contributes to the regulation of ECF calcium concentrations. Adenylate cyclase and cAMP appears to play as an intermediate in the regulation of PTH secretion in the control of calcium, however the exact role is not known. It may be that cAMP in some manner involved in the phosphorylation of substrate phosphoprotein in parathyroid gland. This substrate is membrane protein involved in the fusion of secretory granule with plasma membrane resulting in discharge of hormone to extracellular space. The PTH released to extracellular space by mechanism exocytosis [4].

PTH is responsible for regulation of calcium levels in blood and ECF through principal actions on kidney, bone and intestine. The parathyroid hormone acts on kidney to enhance the reabsorption of calcium and diminish the reabsorption of phosphate. PTH stimulates the formation of 1, 25 (OH)<sub>2</sub>D (vitamin D<sub>2</sub>) through its action on renal 1- α- hydroxylase, which in turn has

direct biological effects on intestine. VitD<sub>2</sub> increases the absorption efficiency of dietary calcium through intestine (4, 5).

PTH has several actions at different sites along the nephron. PTH enhances the calcium reabsorption at cortical site within the distal tubular portion and blocks the reabsorption of sodium, calcium, phosphate, and bicarbonate in proximal tubule. The iPTH decreases tubular reabsorption of phosphate by accelerating degradation of the Type-II sodium dependent phosphate transporter in the proximal tubules [5] The enzymatic activities of glucose-6-phosphate dehydrogenase and reduced nicotinic adenine dinucleotide phosphate (NADPH) - diaphorase increases by exposure to PTH. The activity of latter enzyme may involve in the activation of Vitamin D metabolites by 1- α- hydroxylation. PTH action in the kidney occurs through the formation of cAMP as second messenger cause to changes in calcium transport. PTH stimulated adenylate cyclase activity is found at basolateral renal tubular cells adjacent to the renal capillaries. The intracellular receptor proteins for cAMP are found in brush borders of these cells at luminal side. The cAMP generated at basolateral region of cell migrates through cytoplasm to activate cAMP dependent kinases, in turn activate enzymes responsible for ion transport [4].

In the skeleton, PTH stimulates to resorb the bone and subsequent release of calcium and phosphate into the circulation. PTH stimulates osteocytes, osteoclasts and their precursors. In hyperparathyroidism bone destruction and concomitant release of calcium, phosphate and other salts occurs. Osteoclasts can breakdown large volume of bone when stimulated at chronic levels; osteocytes resorb areas of surrounding calcified matrix stimulated by PTH. The action of PTH on bone may largely mediated by intracellular cAMP. The production of cAMP and elevation of intracellular calcium levels are first events in the cascade of hormonal action. In the subsequent steps cAMP dependent protein kinases have been activated. The increase in the intracellular calcium level causes increased RNA synthesis and the release of lysosomal and other enzymes associated with bone resorption [4, 6].

The calcitonin is a potential calcitropic hormone that has an inhibitory effect on osteoclastic activity in-vitro and can also stimulate urinary

loss of calcium and phosphate [7]. The physiological role of calcitonin in maintenance of bone mass is suggested by studies showing that conditions associated with osteoporosis such as age and female sex show lower serum calcitonin level than observed in young individuals and in males [8,9,10]. Furthermore, the calcitonin administration increases bone mineral density (BMD) in postmenopausal women. Its effectiveness in treatment of osteoporosis has been shown in a randomized trial [11, 12, 13]. Calcitonin inhibits extracellular Ca<sup>2+</sup> sensing, a potent antiresorptive signal, and by implication, calcitonin withdrawal should enhance Ca<sup>2+</sup> sensing and limit resorption [14]. Calcium Sensitive Receptor (CaSR) signaling is required for homeostasis of iPTH levels in serum [15, 16].

The lowered concentrations of calcium and phosphorus in extra-cellular fluid lead to defective mineralization of organic bone matrix. The Defective bone matrix mineralization of newly formed bone and growth plate cartilage leads to characteristic morphological and clinical signs of rickets, while at sites of bone remodeling, it causes osteomalacia [17, 18]. However, despite negative calcium and phosphorus balance clinical and radiological signs of rickets and osteomalacia have not been observed in HD. It is also paradoxical that in spite of low levels of serum and 24 hours excretion of calcium, the serum iPTH levels were normal in HD subgroups. On the basis of results observed, we can provide the following hypothesis in the HD perspective to calcium homeostasis. 1) During subsequent studies of ICMR on HD observed no changes in Vitamin D<sub>3</sub> level (30.4 ± 5.8 ng/ml), values were in laboratory reference ranges. Hence these results suggest suspicious over possible mutation of Vitamin D Receptor in HD. The uptake of calcium from intestine may disturb causing low level of calcium in serum, subsequently leads to low level excretion. 2) The low level of calcium without change in PTH causes diminished secretion of calcitonin. Hence observed hypocalcitemia may be an epiphenomenal. 3) Calcitonin has antiresorption effect on bone; the observed low calcitonin in HD may imply resorption of bone leading to deformity and causes hypocalciuria and hypocalcaemia. 4) The observed calcitonin withdrawal should enhance Ca<sup>2+</sup> sensing, limit resorption, and increased extracellular calcium. Surprisingly in HD the calcium level is low and has no effect on PTH homeostasis. This result lead to suspicious over possible mutation of Calcium Sensitive

Receptor (CaSR).

The peptide bound increased Pro / Hyp ratio suggests HD may involve reduced bone turnover or a possible defective hydroxylation of prolyl residues during posttranslational modification of collagen molecule biosynthesis [19, 20]. We have earlier reported deficiency of magnesium in HD may cause defect in bone matrix formation [21]. The varied involvement of these factors may result in different subtypes observed in HD. The scientific name of the disease is Spondylo-epi-(meta) physeal Dysplasia, Autosomal Dominant variety, Handigodu syndrome. The same has been listed in the International Classification of Skeletal Dysplasias. It is considered to be genetically determined. The disease is highly prevalent in a localized pocket, restricted to a particular geo-ethnic community. It would be interesting to carry out a follow up study to evaluate it as an early biomarker of the disease.

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