Hypovitaminosis D as Predisposing Factor for Atrophic Type A Gastritis: a Case–Control Study and Review of the Literature on the Interaction of Vitamin D with the Immune System

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Abstract 1,25-Dihydroxyvitamin D displays immunoregulatory and anti-inflammatory properties, and the cells involved in innate and adaptive immune response express the vitamin D receptor and can both produce and respond to this hormone. This article aims at describing the complex immune regulatory role of vitamin D and depicting whether a correlation exists between atrophic type A gastritis and hypovitaminosis. We studied 62 autoimmune gastritis (AIG) patients and compared them to 54 lymphocytic gastritis patients, 21 *Helicobacter pylori* gastritis patients and 212 healthy subjects. We also statistically analyzed vitamin D concentration in 36,384 outpatients referred to our clinical laboratories. 25-Hydroxyvitamin D levels, the measurable metabolite used to determine vitamin D status in plasma, were measured by a chemiluminescent method. Average level of 25-OHD in AIG subjects was 9.8±5.6 ng/mL (95% confidence interval (CI) 8.4–11.2), 11.1±8.4 (CI 7.5–14.7) in *H. pylori* gastritis patients, 22.2±13.5 (CI 18.6–25.8) in nonspecific lymphocytic gastritis patients, 21.3±12.2 (CI 19.7–22.9) in healthy subjects, and 21.8±13.1 (CI 21.7–21.9) in the 36,384 outpatients. Vitamin D levels in AIG patients were significantly lower than in patients with nonspecific gastritis or in the general population, supporting the hypothesis that hypovitaminosis D might be a risk factor for the development of autoimmune diseases. The low vitamin D concentration in *H. pylori* gastritis patients might act as predisposing factor for a more severe Th1-type aggression to the stomach epithelium.

Keywords Autoimmune diseases · Autoimmune gastritis · Tolerogenic dendritic cells · Transcriptional regulation · Vitamin D deficiency · Vitamin D receptor

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Introduction

1,25-Dihydroxyvitamin D ($1,25(OH)_2D$) or *calcitriol* is a steroid hormone derived from vitamin D, which plays an important role in maintaining an adequate level of serum calcium and phosphorus [1]. The two major precursors of vitamin D are vitamin D3 (*cholecalciferol*) and vitamin D2 (*ergocalciferol*). Both vitamin D precursors, resulting from exposure to the sunshine (D3) and the diet (D2), are converted to 25-hydroxyvitamin D (25(OH)D) (*calcidiol*) when they enter the liver [2].

More than 95% of 25(OH)D measurable in serum is typically $25(OH)D_3$, while $25(OH)D_2$ reaches measurable concentrations only in patients taking vitamin D supple-

mentation [3]. *Calcidiol*, an inactive form of the hormone synthesized by 25-hydroxylase CYP2R1, is carried in the blood as a complex with a vitamin D binding protein (DBP), transformed into $1,25(OH)_2D$ mainly in the kidneys by means of megalin-mediated endocytosis into the cells of the proximal nephrons, and again hydroxylated by the enzyme 1α-hydroxylase CYP27B1 [3, 4]. The renal production of $1,25(OH)_2D$ that is closely regulated by the plasma levels of parathyroid hormone (PTH), calcium, and phosphorus controls systemic endocrine actions such as the intestinal transport of calcium, the regulation of the bone metabolism, the increase in renal calcium reabsorption, the regulation of blood pressure, and the secretion of insulin [1].

Other types of cells are provided with enzymes apt to produce the active hormone on site: Extrarenal $1,25(OH)_2D$ appears to act firstly as an autocrine/paracrine factor with specific cellular functions, such as the inhibition of proliferation, the promotion of differentiation, and the regulation of the immune response [1, 5]. 1α-Hydroxylase is present in at least ten tissues other than the proximal nephron (Table 1), but its regulation in extrarenal sites is very different and aligned with autocrine/paracrine functions carried out in situ by $1,25(OH)_2D$ [6–15].

The synthesis and catabolism of *calcitriol* are controlled by local factors, cytokines, and growth factors which optimize the hormone levels for specific cellular actions by means of yet unknown mechanisms. This hormone performs its action by binding to the vitamin D receptor (VDR), part of the superfamily of steroid hormone receptors, acting as a transcription factor for a target gene [4]. The presence of VDR in tissues not taking part in mineral homeostasis led to the discovery of a myriad of other functions controlled by the D hormone (Table 2). The ability of *calcitriol* to inhibit growth and promote the differentiation of some cellular types suggests that the D endocrine system can have a very important role in the

prevention of tumors, in regulating the immune system, and in modulating other endocrine systems; these actions were recently demonstrated by observational studies which associated vitamin D deficiency with cancer, autoimmune diseases, hypertension, and diabetes and which detected that this hormone can act in some tissues also with autocrine and/or paracrine mechanisms [16, 17].

The catabolism of 25(OH)D and $1,25(OH)_2D$ in calcitroic acid, biologically inactive and soluble in water, is performed by the enzyme 24-hydroxylase (CYP24A1), almost ubiquitously expressed. $1,25(OH)_2D$ itself is a very strong inducer of the expression of 24-hydroxylase, by means of the presence of two sequences of vitamin D responding elements (VDRE) in the promoter of this gene, inducing its own catabolism. This negative feedback is an internal security to avoid excessive levels of $1,25(OH)_2D$ [4, 18, 19].

While the best known action of vitamin D is on bone metabolism, it is clear today that this hormone displays other functions, mainly the regulation of immune response, with potential therapeutic effects on autoimmune diseases, and an anti-proliferative action, useful in treatment of psoriasis and neoplasms and in prevention of cardiovascular diseases [20].

The Vitamin D Hormonal System

The D hormonal system is modulated by intrinsic mechanisms which regulate its activity and effectiveness for the bone metabolism and many other tissue functions in which it is

Table 1 Sites of extrarenal $1,25(OH)_2D$ production in humans

Tissue	Reference
Colon	[6]
Dendritic cells	[7]
Endothelial cells	[8]
Human brain	[9]
Breast	[10]
Pancreatic islets	[11]
Parathyroid glands	[12]
Placenta, decidua	[13]
Prostate	[14]
Skin, keratinocytes	[15]

Table 2 Tissue distribution of vitamin D receptor

Adipose tissue	Activated T cells
Adrenal	Heart muscle
Bone	Aortic endothelial cells
Bone marrow	Osteoblasts
Brain	Islet cells
Mammary gland	Parathyroid cells
Neoplastic cells	Parotid
Chondrocytes	Pituitary cells
Colon enterocytes	Placenta
Ovarian cells	Prostate cells
Epididymis	Retina
Hair follicle	Keratinocytes of skin
Intestinal cells	Stomach
Distal renal cells	Testis
Liver cells	Thyme
Lung cells	Thyroid
Transformed B cells	Uterus

involved. The first regulation takes place with the close control of the circulating concentration of $1,25(OH)_2D$, the production of which is modulated according to the body requirements for calcium and other hormones and is inhibited by the same active compounds. Other regulating factors are the transport in blood, the capture in renal tubules, the activity of renal and extrarenal 1α-hydroxylases, and the interaction with the specific receptor.

The circulating metabolites of vitamin D, particularly 25 (OH)D, bind with high affinity to the DBP, which has plasma levels 20 times higher than the metabolites. This has an impact on their pharmacokinetics since bound compounds have limited access to target cells and are less susceptible to the hepatic metabolism; consequently, their average circulating life is increased [21]. DBP therefore acts as carrier as well as a modulator of vitamin D metabolism with respect to its blood concentration, optimizing the cellular catabolism and/or regulating its biological activity, related to the concentration of free hormones, protecting the tissues from toxic levels of vitamin D [22]. The concentration of DBP is decreased in the presence of hepatic diseases, nephrotic syndromes, and malnutrition, conditions where the risk of vitamin D intoxication is high; on the other hand, it increases during pregnancy and during estrogen therapy [5].

25(OH)D bound to DBP is actively transported inside the brush border cells of the proximal renal tubule, containing 1α-hydroxylase, by means of a megalin-mediated endocytosis process and not by means of diffusion through their basolateral surface [23]. Megalin is part of a group of complex proteins (protein associated to the cubilin receptor) located on the surface of the cells of the proximal renal tubule, capable of reabsorbing the calcidiol–DBP complex filtered by the kidney. DBP is broken up in the lysosomes (legumin) [24], and 25(OH)D is directed to 1α-hydroxylase by IDBP-3, or, depending on the concentration of active hormone, it returns in the blood, where it binds again with DBP [25, 26]. Megalin levels are increased by $1,25(OH)_2D$, through a positive control mechanism for the production of active hormones [27]. The regulation of renal 1α-hydroxylase ensures the limited regular concentration range of $1,25(OH)_2D$. The activity of 1α-hydroxylase CYP27B1 is strongly regulated by the axis calcium–phosphorus–parathyroid hormone. Diet-deriving calcium regulates the enzyme directly by means of serum variations or through the action on proximal tubule cells and indirectly by altering the PTH levels: Hypocalcemia in fact increases the levels of circulating hormones capable of stimulating 1α-hydroxylase [25]. Phosphate dietetic restrictions also increase the activity of renal enzymes, independently from the calcium and PTH concentration, probably by means of the action of phosphatonins (fibroblast growth factor 23, frizzled-related protein 4, phosphoglycoproteins

of the extracellular matrix), capable to act as mediators of phosphate regulation on the enzyme activity and/or on the gene controlling it [4, 28]. Calcitonin, GH, and IGF1 are also considered positive regulators of 1α-hydroxylase. Calcitriol limits its circulating levels by means of a negative feedback mechanism, through the inhibition of PTH-mediated induction of the activities of the promoter gene [29]. The product of the Klotho gene, induced by $1,25(OH)_2D$, is also considered a negative regulator of 1α-hydroxylase [30].

The regulation of 1α-hydroxylase located in extrarenal sites (Table 1) is very different compared to the renal enzyme and is in line with autocrine/paracrine functions performed by the locally produced $1,25(OH)_2D$, although the gene controlling the expression and activity of 1α-hydroxylase in extrarenal tissues is identical to that regulating it in the kidney (chromosomal locus 12q13.1–q13.3) [4]. The synthesis and catabolism are controlled by local factors, cytokines, and growth factors which optimize the levels of *calcitriol* for specific cellular actions by means of not yet fully understood mechanisms. In particular, the expression of 1α-hydroxylase in macrophages is significantly stimulated by immune signals mediated by interferon-γ (IFN-γ), lipopolysaccharides, and viral infections and in dendritic cells is associated with the p38 MAPK and NF-κB-dependent maturation of the same cells [31].

The local production of $1,25(OH)_2D$ depends on the levels of circulating precursors (condition that potentially explains the association between vitamin D deficiency and autoimmune diseases/neoplasms), is not subjected to a negative feedback mediated by $1,25(OH)_2D$ as it occurs for the renal enzyme, and does not increase the concentration of circulating active hormone.

Recent data demonstrate that T cells also express the enzymes responsible for the production of $1,25(OH)_2D$ [32]. The binding of $1,25(OH)_2D$ with a nuclear receptor, with a plasma membrane receptor or both in target organs, generates an appropriate biological response capable of regulating genetic transcription (genomic action) or of rapidly activating a variety of transduction signals on or near the plasma membrane (rapid nongenomic action) [33].

Calcitriol is one of the most important gene regulators, and their action is dependent to the binding of the high-affinity receptor VDR. VDR belongs to the superfamily of nuclear receptors for steroid hormones, which modulate the genetic expression of cells [34], and is present in many tissues (Table 2). The receptor is constituted by a single polypeptide of 427 amino acids, structured in three functional domains: the C terminus (ligand binding domain, LBD), which binds the hormone at the level of its A ring; the DNA binding domain, organized in two zinc finger DNA binding motifs, responsible for the high-affinity interactions with specific DNA sequences in the promoter

regions of the target gene, called VDRE; and the N terminus for transcription processes [4].

VDR regulates the transcription of the DNA in mRNA, performed by the RNA polymerase II, which migrates in the cytoplasm, transducing the information to the ribosomes for the protein synthesis [35]; it controls four main and subsequent phases: VDR binds $1,25(OH)_2D$ in the C terminus domain (LBD); this reaction causes conformational changes in the receptor which facilitate its heterodimerization with the retinoic acid X receptor (RXR) in LBD and the translocation to the nucleus; the newly formed complex $1,25(OH)_2D$–VDR/RXR binds, by means of the DNA binding domain, to specific sequences of VDRE and causes conformational changes of the genomic DNA [36, 37]; VDR recruits co-activator and/or co-repressor nuclear proteins [38, 39] that help it to modulate genetic transcription [4, 40, 41].

The most important co-activator proteins are part of the family of steroid hormone receptor co-activators (SRC-1, SRC-2, and SRC-3) and the CBP/p300 protein. They recruit enzymes which remodel chromatin [42, 43] and DNA transcription mediator complexes of the transcription gene [44, 45]. The main co-repressors are NcoR-1, NcoR-2, and Hairless which compact chromatin and inhibit gene transcription [44, 46]. The VDR co-modulator, NCoA-62/Ski, can act both as promoter or inhibitor of the transcription activity, depending on NcoR and CBP/p300 levels [47, 48]. *Calcitriol* itself can modulate the transduction activity by means of the selective induction of TIF2 (p160), co-activator, and/or SMRT, co-repressor of the NcoR family; the final balance between co-activators and co-repressors determines the control of genetic transcription

in the presence of physiological or pathological stimuli [49].

The genomic actions of vitamin D are divided in classic and nonclassic (Table 3). With regard to the classic actions, the endocrine system of vitamin D acts as a fundamental actor in the interactions between kidney, bone, parathyroid, and intestine in order to maintain extracellular calcium levels within the normal levels, a vital process for the physiology of normal cells and the integrity of the skeleton. Among the nonclassic genomic actions, the recent discovery of VDR in the cells of the immune system and the fact that many of these cells have an enzyme collection capable of producing the hormone indicated that it carries out important immunoregulatory properties [4, 32].

The Vitamin D Hormonal System and the Immune Response

$1,25(OH)_2D$ modulates the immune system, causing direct regulatory effects on the functions of B and T lymphocytes and influencing the phenotype and function of antigen presenting cells (APCs) and dendritic cells (DCs), promoting tolerogenic properties that facilitate the induction of T regulators (Tregs) instead of T effectors [50]. This regulation is mediated by the action of vitamin D on nuclear transcription factors such as NF-AT and NF-κB or by the direct interaction with VDRE in the promoter regions of the genes of cytokines. The vitamin is produced by the macrophages, DCs, and T and B cells and is therefore capable of physiologically contributing, by means of the VDR expressed in their nucleus, to the autocrine and

Table 3 Genomic actions of vitamin D

Classical genomic actions
Key player to maintain extracellular calcium levels within the normal limits
Increase in the intestinal absorption of calcium and phosphate from the diet
Regulator of development and maintenance of mineralization of bone
Potent modulator of parathyroid function
Reabsorption of calcium in the proximal tubule through the simultaneous suppression of 1 alpha-hydroxylase and stimulation of 24-hydroxylase
Non-classical genomic actions
Cell growth inhibition
Apoptosis regulation
Control of cell differentiation
Modulation of the immune response
Prevention of neoplastic transformation
Control of the renin–angiotensin system
Control of insulin secretion
Control of muscle function
Control of the nervous system

paracrine regulation both of innate and adaptive immunity [51, 52]. The strict control of the hormone production by the cells of the innate and adaptive immune system confirms the significance of the D endocrine system in modulating the immune response in healthy or sick subjects.

Strengthening of the Innate Immune System Response The innate immune response involves the activation of Toll-like receptors (TLR) in the polymorphonuclear granulocytes, monocytes, macrophages, as well as in the epithelial cells of epidermis, gums, intestine, vagina, bladder, and lungs. TLRs are transmembrane receptors with the task of recognizing pathogens, which react to specific membrane patterns expressed by etiological agents and unleash the innate immune response of the host [53]. *Calcitriol* represents the key link between the activation of TLRs and the antibacterial response of the innate immune system, especially in the case of tuberculosis, where it is capable of causing the production of an antimicrobial peptide, cathelicidin, coded by a primary gene which responds and is strongly regulated by vitamin D [54]. This suggests that a sufficient quantity of active hormone contributes to decreasing the susceptibility to this infection. Other studies demonstrated that vitamin D allows keratinocytes to defend the wound repairing process from infections and confirmed that the hormone has a key role in the innate immune response against bacterial attacks [32, 55]. Furthermore, the autocrine production of $1,25(OH)_2D$ by DCs is capable of programming the homing of T cells associated to the skin, including Tregs capable of fighting the proinflammatory effects caused in the tissue. The hormone also regulates the expression of the receptor for epidermotropic chemokines CCR10 in activated T cells, coded by a primary gene responding to vitamin D. This allows the CCR10^{+} T cells to migrate to the skin in response to epidermic chemokine CCL27 produced by keratinocytes, where they can perform their tissue action [56]. Hence, the innate immune system is largely distributed and operates not only in the cells of the lymphopoietic system but also within the epithelium of those tissues that face external factors, where it is a fundamental protective barrier.

Regulation of the Adaptive Immune System Response $1,25$ $(OH)_2D$ exercises an inhibitory action onto the adaptive immune system: It suppresses the proliferation and production of immunoglobulins and delays the differentiation of precursors B in plasma cells [57]; it inhibits the proliferation of Th1, capable of producing IFN-γ and interleukin 2 (IL-2), and activating macrophages [58, 59]; it also acts on the DCs/APCs facilitating the production of IL-4, IL-5, and IL-10 and moving the T differentiation in favor of phenotype Th2 [32].

The action of the hormone also increases the quantity of Tregs CD4^{+}/CD25^{+} which produce IL10, by means of which they block the development of Th1, and inhibits the secretion of IL-17 by the T effectors [60]. These actions regulate subsequent presentations of antigens to the lymphocytes by the APC system and therefore the recruitment (facilitated by IFN-γ) and proliferation (facilitated by IL-2) of T effectors.

Induction of Tolerance Properties in Dendritic Cells DCs are a highly specialized system of APC, critical for the initiation of the T cells CD4^{+} response. They are circulating in different maturation stages as well as being present in lymphoid and nonlymphoid organs, where they act as sentinels. After contact with the antigen, the DCs migrate toward T-dependent areas of afferent lymph nodes, where they can transform the native T cells in Th1 [61].

According to the expression of CD11c, two subpopulations of DCs are recognized: (a) M-DCs belonging to the myeloid line, characterized by a monocyte morphology, myeloid-like markers (CD13, CD33, β2-integrin, CD11c, immunoglobulin-like transcript (ILT)1 activation receptor) and low levels of CD123 receptor for IL-3 and (b) P-DCs belonging to the lymphoid line, characterized by plasma cell morphology, without myeloid markers, expressing high levels of CD4 and CD123.

M-DCs produce high levels of IL-12, while P-DCs produce high levels of IFN-α, with distinct effects on the activation and differentiation of T cells. Therefore, DCs can be immunogenic but can also induce tolerance both in the thymus and in the periphery and can modulate the development of T cells; in particular, immature DCs seem capable of inducing tolerance and proliferation of Tregs [61].

The VDR agonists facilitate the proliferation of DCs with tolerance properties [62], stopping the differentiation and maturation of DCs, decreasing the expression of CD40, CD80, and CD86 co-stimulatory molecules, significantly decreasing the production of IL-12, increasing the production of IL-10 [63], and finally inducing the expression of ILT3, an inhibitory molecule associated with tolerance induction [64].

Experimental studies demonstrated that DCs with tolerance properties induced by VDR agonists are capable of inducing differentiation of CD4^{+} CD25^{+} Tregs capable of stopping the development of autoimmune diabetes (T1DM) [62]. We can derive that the active synthesis of 1,25(OH)D within DCs not only inhibits the differentiation from monocyte precursors in immature DCs but also blocks their ability to undergo a final differentiation in response to maturation stimuli [65]. Recent studies highlighted that during inflammatory processes calcitriol negatively regulates, with a paracrine mechanism and by means of the ligand-induced recruitment of histone deacetylase, the

expression of the nuclear factor κB, fundamental both for the differentiation and maturation of DCs and to trigger the inflammatory response. This explains the inhibitory effect of the active hormone on the maturation of DCs and on their production of proinflammatory mediators [66, 67]. *Calcitriol* and the VDR agonists perform their action exclusively on the M-DCs, regulating their production of cytokines and chemokines and modulating their ability to inhibit the development of T helpers and to increase the Treg function [61, 68]; on the contrary, P-DCs are not subjected to regulation by the active hormone in inducing tolerance [32].

Modulation of the Function of Effector Lymphocytes The VDR agonists regulate the activation and differentiation of T cells by modulating the DC functions [32]. Furthermore, they can also have direct effects on B and T cells, inhibiting (a) selectively the development of Th1 and Th17 [58, 69] and (b) directly type 1 cytokines such as IL-2 and IFN-γ [70].

It was also demonstrated that $1,25(OH)_2D$ increases the number of Th2 through a direct effect on native CD4s [71]. Therefore, both Th1 and Th2 are regulated by the action of VDR agonists, both with respect to their activation and differentiation [72]. *Calcitriol* has powerful and direct effects also on the B cell response, causing induction of apoptosis, inhibition of proliferation, generation of B memory cells, plasma cell differentiation, and immunoglobulin production [57].

The B cells, like the macrophages, DCs and T cells, express VDR and can synthesize the active hormone, which can perform autocrine/paracrine regulatory actions. Treatment with VDR agonists inhibits the production of IL-17 by T cells, a proinflammatory cytokine produced by pathogenic T cells in several organ-specific autoimmune diseases affecting the brain, heart, synovium, and intestine [60, 73]. The production of IL-17 is sustained by IL-23, a member of the IL-12 family, formed by p19 and p40 chains, the latter of which is strongly inhibited by VDR agonists [69].

Increase in Treg Functions Tolerance-inducing DCs, produced after a short treatment with $1,25(OH)_2D$, lead to the development of $CD4^+CD25^+$ Tregs capable of mediating the tolerance to transplants and stopping the development of T1DM [74]. VDR agonists not only facilitate the induction of Tregs and increase their inhibiting activity but can also facilitate their recruitment in inflammatory sites [75]. Circulating M-DCs also produce high levels of chemokines CCL17 and CCL22, increased by binding with the CD40 ligand [61], capable of selectively recruiting $CD4^+CD25^+$ $CCR4^+Foxp3^+$ Tregs. In particular, the high production of CCL22 by immature M-DCs leads to a preferential attraction of such Tregs [76]. The production of this chemokine is significantly increased by $1,25(OH)_2D$ in

blood M-DCs, indicating that VDR agonists can facilitate the recruitment of Tregs by DC subgroups [61].

Hypovitaminosis D and Autoimmune Gastritis

There is no full agreement on the optimal serum levels of $25(OH)D_3$, the measurable metabolite to assess the level of vitamin D in man, but deficiency is defined when hormone levels are lower than 20 ng/mL [20, 77]. Studies carried out in the past decade indicated a high prevalence of deficiency and/or insufficiency of vitamin D in the general population, and this low concentration is correlated with an increased incidence of autoimmune diseases, bone pathologies, and cancer [20]. Epidemiological studies clearly showed that a sufficient concentration of hormone (>30 ng/mL) decreases the risk of multiple sclerosis, T1DM, chronic intestinal inflammatory diseases, rheumatoid arthritis, osteoarthritis, and systemic lupus erythematosus, confirming that the level of vitamin D has an effect on the prevalence of autoimmune diseases.

As no experiences are available on vitamin D levels in patients with autoimmune gastritis (AIG), we studied the concentration of the hormone in a group of AIG patients, diagnosed by histology, in order to verify if hypovitaminosis D can be a predisposing factor for this pathology as well. AIG is an organ-specific disease, found in 2% of the general population, characterized by the presence of circulating autoantibodies against the enzyme H^+/K^+-adenosintriphospha-tase (H^+/K^+-ATPase), the protonic pump of the parietal cells (PCA), and against the intrinsic factor (IFA), another product of the parietal cells [78]; it is often an asymptomatic disease that may cause atrophy of the gastric mucosa and in 10% of cases develops into an adenocarcinoma or a carcinoid tumor.

The chronic autoimmune attack against H^+/K^+-ATPase takes place on the fundus and body of the stomach and does not affect the antrum; it is characterized histologically by a mononucleate inflammatory infiltrate in the mucosa, consti-tuted by T CD4 and CD8 lymphocytes, macrophages, and B cells, which causes the loss of parietal cells and zymogenic cells and the subsequent decrease of gastric acid secretion, hypergastrinemia, and, finally, anemia due to iron deficien-cy. In the latter stages of the disease, pernicious anemia (AP) occurs, caused by a deficiency of vitamin B12 that is no longer absorbed by the intestine due to the presence in the serum and/or gastric juices of the patients of IFA; in this phase, the biopsy shows a more advanced degree of atrophy and metaplasia of the gastric body [78, 79].

The aim of our study was (a) to determine vitamin D concentration in patients affected by autoimmune gastritis, (b) to compare it with concentration in patients affected by nonautoimmune gastric pathologies, and (c) to compare it with the concentration in an Italian reference population.

This allowed to verify whether the average concentration of vitamin D in these patients was lower than that in healthy subjects of the same age and sex, as well as in patients affected by nonautoimmune gastritis.

Materials and Methods

We determined vitamin D concentration in 62 patients with AIG (aged 37–80) and compared it with the concentration found in 54 PCA-positive patients with nonspecific lymphocytic gastritis [20–80], 21 patients with *H. pylori* gastritis [20–80], and with a control group formed by 36,384 subjects (29,401 women, 6,983 men aged 1–80); the latter were chosen among outpatients of the northeast of Italy (Veneto and Friuli). They had access in the last 2 years to laboratories at Cittadella, Vicenza, Udine, and Latisana and underwent a vitamin D test. All dosages of $25(OH)D_3$ were determined by a quantitative chemiluminescent competitive direct method (Liaison, Diasorin, Saluggia, Italy) using a specific anti-vitamin D antibody linked to a solid phase and a vitamin D tracer bound to isoluminol. In order to classify the vitamin D status of our population, we used Holick's decision-making levels, which have been determined by putting them in relationship with calcium and PTH concentration [80].

To calculate the average concentration of vitamin D in the control group, representing the population of the northeast of Italy, we used the statistical indirect method of Kairisto and Poola [81] and processed 36,384 vitamin D concentration values obtained in subjects without metabolic diseases, not under vitamin D therapy, living indoors as the majority of the population, and with PTH values within reference limits. These were the same criteria used by Holick and Chen in selecting the individuals for the control group in their study [82]. The first three criteria were assessed by questionnaire administered to the patients before blood drawing and the fourth by automatic cross-matching performed by the Laboratory Information System.

This indirect statistical method is based on the theory that most of the laboratory data regarding outpatients derive from screening and refer to individuals who do not have the disease or a correlated pathology. It assumes that the mode of the frequency distribution of all data is the same as that of the reference population if the outliers are eliminated. This objective model has a performance comparable with the International Federation of Clinical Chemistry standard when the asymmetry and kurtosis of frequency distribution are not very large. The data collected (Fig. 1) showed a scatter of values too high to calculate the reference range in the population; however, the statistical elaboration allowed us to calculate the average concentration in the subjects recruited in our territory. To verify whether the data obtained by means of the indirect statistical method of
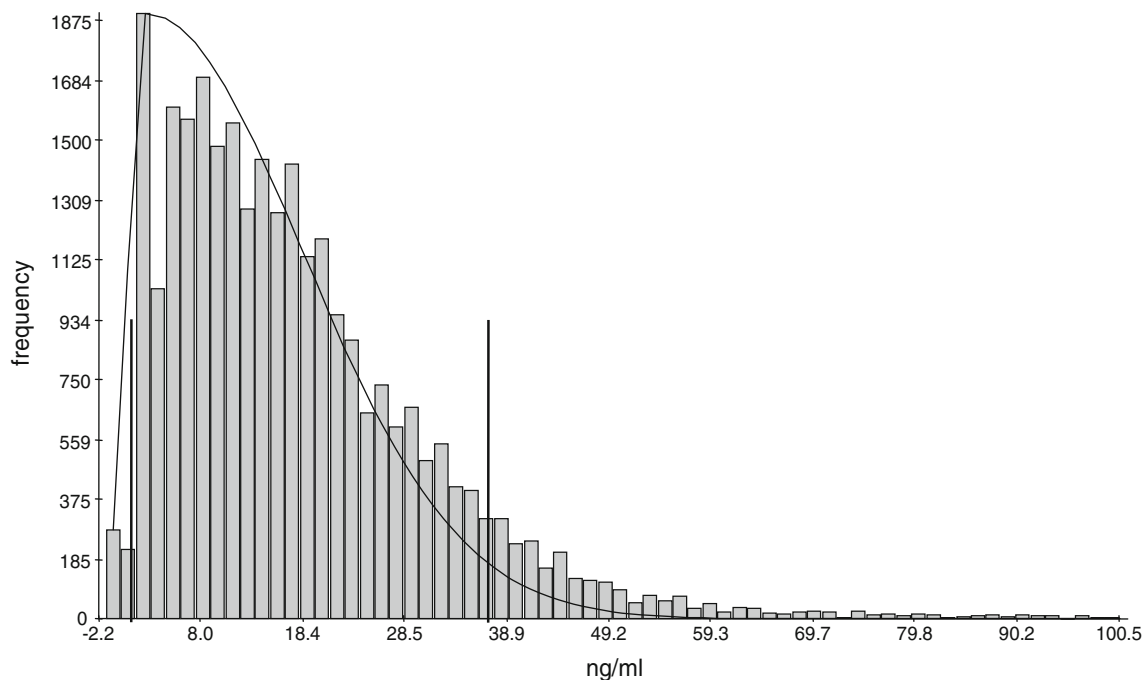

Fig. 1 Mode of the frequency distribution of vitamin D concentration in 36,384 subjects of the northeast Italian population analyzed by means of the indirect Kairisto and Poola's statistical method. Lower and upper reference limits were 9.4 and 43.7 ng/mL, respectively; mean vitamin D level was 21.7 ng/mL

Kairisto and Poola were representative of vitamin D levels in the general population, we studied also 212 healthy subjects (181 females, 31 males), 49 aged 1–10, and 163 aged 20–80, selected with the same Holick's criteria.

Results

Using the Kairisto and Poola indirect method, we were allowed to consider many samples. The statistical calculations on which this mathematical model is based hypothesize that the mode of frequency distribution of all data is the same as that of the reference population; each side of the frequency distribution is considered a half gaussian distribution, with the same mode and the same mode frequency but with a different standard deviation. By means of this method, we have processed data for 36,384 patients who were referred to our laboratories in the past 2 years for measurement of $25(OH)D_3$. By means of the statistical elaboration, we found that once the outliers were removed from the study population, the lower limit was 5.42 ng/mL and the upper limit was 43.8 ng/mL, with an average concentration of 21.8± 13.1 ng/mL. These data are a statistical–mathematical expression of the distribution of values in a control population, allowing us to calculate the average value of the hormone content in the study group; however, they do not allow us to establish a reference range since the values are extremely scattered and include deficient, insufficient, and optimal levels. As seen in Table 4, the average level of vitamin D found in 212 control subjects that is 21.3± 12.2 ng/mL (95% confidence interval (CI) 19.7–22.9) exactly corresponds with that found in the northeastern population obtained through the indirect statistical method that is 21.8±13.1 ng/mL (CI 21.7–21.9); it was slightly higher in children aged <10 years (22.9±11.3 ng/mL (CI 19.7–26.1)) than in the group aged 20–80 but still inadequate according to Holick's. Vitamin D average concentration in subjects with AIG was 9.8±5.6 ng/mL (CI 8.4–11.2), in nonspecific gastritis patients 22.2± 13.5 ng/mL (CI 18.6–25.8), and in *Helicobacter pylori* gastritis patients it was 11.3±8.4 ng/mL (CI 7.5–14.7).

Discussion and Conclusions

Data from the present study show that patients with AIG have significantly lower levels of vitamin D than the control group and the outpatient population and than patients affected by nonautoimmune gastric pathologies. This confirms the hypothesis, as seen in subjects affected by systemic lupus erythematosus and multiple sclerosis that a deficiency of vitamin D is a risk factor for autoimmune diseases in genetically predisposed patients. The low level of $25(OH)D_3$ in northeastern Italy detected from as early as childhood could explain the high frequency of osteoporosis in our population and the fact that the dietetic administration of vitamin D in the first months of life eliminated only the onset of rickets but not vitamin D insufficiency [18]. Evidence emerging from literature, as well as from our study, suggests that if we want to prevent autoimmune aggression in genetically predisposed subjects with vitamin D deficiency, it is very important to supply them with this hormone before the aggression has already developed into the autoimmune disease [66]. Supplying medications after that is completely useless. This should be taken into consideration for a therapeutic strategy with first degree relatives of patients affected by these autoimmune diseases.

The unexpected result was the finding of an important hormone deficiency in patients with *H. pylori* gastritis; by studying the pathogenic mechanism, it becomes clear that the host response to a chronic *H. pylori* infection is of the Th1 type, exactly the same of that in an autoimmune disease, and it participates in the induction of damage to the gastric epithelium, thus having an integral role in the pathogenesis of this gastritis. Vitamin D deficiency may facilitate Th aggression because fails its ability to differentiate Th0 into Treg and regulate the secretion of nuclear factor-kappa B and the early-response transcription-factor activator protein 1, intracellular messengers involved in the initiation of this chronic inflammatory process that are negatively regulated by vitamin D. This point needs to be addressed by specific studies, designed to determine how much a significant hypovitaminosis D in subjects with *H. pylori* gastritis influences the severity of gastric mucosal immune aggression.

Table 4 Vitamin D concentration, SD, and 95% CI in the different patient groups

Group	Age	No.	Mean (ng/mL)	SD (ng/mL)	C.I. (95%)
Current data on LIS	20–80	36,384	21.8	13.1	19.4–25.8
Healthy children	<10	49	22.9	11.3	19.7–26.1
Healthy adults	20–80	163	21.3	12.2	19.7–22.9
NSLG	20–80	54	22.2	13.5	18.6–25.8
AIG	37–80	62	9.8	5.6	8.4–11.2
H. pylori gastritis	20–80	21	11.3	8.4	7.5–14.0

AIG autoimmune gastritis, *NSLG* nonspecific lymphocytic gastritis, *LIS* laboratory information system

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