Chapter

Vitamin D and Autism Spectrum Disorder

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Abstract

1,25(OH)2D is the hormonally active form of vitamin D known for its pleiotropic immunomodulatory effects. Via altering gene transcription, 1,25(OH)D exerts immunosuppressive effects and stimulates immune regulation. Recently, the interest in vitamin D in association with autism spectrum disorder (ASD) has been triggered. The prevalence of ASD has increased excessively over the last few decades, emphasizing the need for a better understanding of the etiology of the disorder as well as to find better treatments. Vitamin D levels in ASD patients are observed to be lower compared to healthy individuals and maternal vitamin D deficiency has been associated with an increased risk of ASD. Moreover, vitamin D supplementation improves ASD symptoms. These recent clinical findings strongly suggest that vitamin D is a factor in ASD onset and progression. Yet, possible mechanisms behind this association remain unknown. This review summarizes immunomodulatory properties of vitamin D and peripheral immune dysregulation in ASD, after which possible mechanisms via which vitamin D could rebalance the immune system in ASD are discussed. Although promising clinical results have been found, further research is necessary to draw conclusions about the effect and mechanisms behind the effect of vitamin D on ASD development.

Keywords: autism spectrum disorder, vitamin D, vitamin D receptor, vitamin D responsive element, immune system

1. Introduction

For many decades vitamin D has been known for its immunomodulatory effects. When metabolized into the active hormone calcitriol, it can bind to vitamin D receptors (VDRs). These VDRs are expressed by most cells in the human body, allowing vitamin D to have a broad range of functions. Upon binding of vitamin D to a VDR, gene transcription is altered. All types of immune cells in the human body express the vitamin D receptor, enabling vitamin D to alter immune responses [1]. In general, vitamin D has immunosuppressive properties and can therefore be beneficial in diseases characterized by inflammation and autoimmunity such as multiple sclerosis and inflammatory bowel disease [2]. Vitamin D deficiency is an increasing global problem with an estimated 30% of the population suffering from vitamin D deficiency and 60% being vitamin D insufficient [3]. Inadequate levels of vitamin D can have many adverse effects throughout the body due to the abundant expression of VDRs. Furthermore, maternal vitamin D deficiency has been suggested to affect development of the offspring. To date, the World Health

Organization does not recommend vitamin D supplementation to pregnant women. This illustrates the lack of awareness on the importance of vitamin D in health.

A disorder that recently received increased attention is autism spectrum disorder (ASD). ASD is a heterogeneous neurodevelopmental disorder, collectively describing autistic disorder, Asperger's syndrome and Pervasive Developmental Disorders Not Otherwise Specified (PDD-NOS). It is characterized by behavioral deficits, impaired communicative functioning and restricted and repetitive patterns of behavior [4]. ASD onset usually occurs in the first few years of life and proceeds into childhood and adulthood [5]. Genetics are of importance in the disorder – several studies show monozygotic twins share 60–90% of ASD symptoms [5, 6]. Additionally, ASD is four times more prevalent in boys, which is suggested to be due to the protective effects of estrogens in women [5, 7].

Despite the role of genetics, the prevalence of ASD has increased tremendously over the past few decades [8]. In the Netherlands, the prevalence of ASD increased from 90.000 to 190.000 cases between 2001 and 2009 [9]. More recently, the prevalence of ASD in the US was estimated at one in every 59 children aged eight years in 2014, which increased to one in every 54 children in 2016 [10]. Across all ages, the prevalence of ASD is estimated to be 1% of the worldwide population [11, 12]. Partially, this increase can be explained by improved diagnostics and increased awareness. However, the sudden and rapid increase also suggests the role of environmental factors in ASD onset. Research indicates that genetic predisposition predominates, requiring additional environmental triggers to develop ASD. Multiple environmental factors have been suggested, including antibiotic use, maternal infections during pregnancy and sun exposure. The strong increase in prevalence highlights the importance of understanding the role of environmental factors in the etiology of ASD [6].

The association between vitamin D and ASD was suggested in 2008 when it was observed that the increase in ASD prevalence coincides with the medical advice to avoid sun exposure [7]. Since then, clinical trials have been performed, trying to prove the association between vitamin D and ASD. UV-B is the most important source of vitamin D in humans, illustrating the requirement for sunlight. Research shows ASD prevalence is higher in countries at higher latitudes, coinciding with reduced UV-B intensity. Moreover, ASD patients consistently exhibit lower vitamin D levels than healthy individuals and studies have shown maternal vitamin D deficiency increases the risk of ASD. These findings encouraged scientists to study the effect of vitamin D supplementation on improving ASD symptoms, and thus far promising results have been found [7, 13]. Yet, the mechanisms behind the possible association between vitamin D and ASD remain unknown. Neuroinflammation, oxidative stress, autoimmunity and immune dysregulation are all observed in individuals with ASD [14]. Of these phenomena, immune dysregulation is the least well-described in literature. ASD patients suffer from chronic systemic inflammation, which is illustrated by a disbalance in cytokine expression and the presence of comorbidities such as gastrointestinal problems in a large fraction of ASD patients [15]. Increased immune activation is observed in ASD patients and is associated with more severe symptoms [16]. Taking into consideration the immunosuppressive properties of vitamin D, this suggests that perhaps vitamin D could play a role in rebalancing the dysregulated immune system in ASD patients and thereby reduce systemic inflammation. However, to date there is no recommendation for vitamin D supplementation in ASD patients.

Therefore, in this review the role of vitamin D in immune dysregulation in ASD patients is examined. First, immunomodulatory properties of vitamin D in general and peripheral immune dysregulation in ASD are described, with a focus on CD4+ T cell activity. Next, possible mechanisms behind this effect of vitamin D on

immune dysregulation in ASD are discussed. This review is summarizing current knowledge on vitamin D and ASD and to examine possible mechanisms via which vitamin D might slow ASD development.

2. The immunomodulatory properties of vitamin D

2.1 Vitamin D: production and metabolism

Vitamin D is a steroid hormone with varying functions in the human body. Vitamin D precursors are extracted both from food and through the exposure to sunlight. Around 10% of the total amount of vitamin D in the body is provided by dietary sources and supplements [17]. There are two forms of vitamin D precursors: D2 (ergocalciferol) and D3 (cholecalciferol). Some plant products are rich in vitamin D2, whereas vitamin D3 is present in animal products, including fish and egg yolk [18]. Sunlight exposure accounts for about 90% of vitamin D and is thus the most important source of this vitamin [17]. When the human skin is exposed to UV-B, 7-dehydrocholesterol is converted into pre-vitamin D [19]. This process depends on factors such as UV-B intensity, skin color and coverage of the skin.

After the production of vitamin D3 in the body, it is first metabolized into the precursor 25-hydroxyvitamin D (25(OH)D). This reaction is performed in the liver by hydroxylases, of which CYP2R1 has the highest affinity for pre-vitamin D [20]. Vitamin D binding protein functions as a transporter of 25(OH)D to the kidney. Consequently, 1,25-dihydroxyvitamin D (1,25(OH)2D) is formed in the kidney by the enzyme CYP27B1. The activity of this enzyme is essential to produce bioactive vitamin D. 1,25(OH)2D is the hormonally active form of vitamin D [21]. In this review 1,25 (OH)2D reflects bioactive vitamin D. Besides renal CYP27B1, other cells in the human body can also express this enzyme. In this way, vitamin D can be directly synthesized not solely in the kidney but also in other tissues [20]. 1,25(OH)2D can be absorbed and then bind to the intracellular vitamin D receptor (VDR). Due to the lack of 1,25(OH)2D in its free form in the blood, vitamin D levels are based on 25(OH)D. This precursor is bound to vitamin D binding protein (DBP) in the circulation, allowing measurements to determine vitamin D levels [22, 23].

1,25(OH)2D can influence its own serum levels and binding to VDR. When serum 1,25(OH)2D levels are high, this enhances VDR expression. Moreover, 1,25(OH)2D has a negative feedback on CYP27B1, the enzyme involved in 1,25(OH)2D synthesis. Besides self-regulation, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) are important regulators of vitamin D metabolism. To sustain normal systemic vitamin D levels, CYP24A1 is stimulated by 1,25(OH)2D and degrades vitamin D. The CYP24A1 enzyme is present in all vitamin D target cells, resulting in the ability to regulate intracellular vitamin D levels [20].

2.2 Vitamin D: mode of action

By binding to VDR, which has a DNA-binding domain, 1,25(OH)2D can exert effects on the body through gene transcription. VDRs are located intracellularly in a wide range of cells. Due to this, vitamin D can exert effects on many different biological processes in the body [21, 24]. The regulation of genes by VDR is cell specific. After the binding of vitamin D to VDR, VDR interacts with the retinoic X receptor (RXR). The VDR/RXR heterodimer binds to vitamin D responsive elements (VDRE) in the promoter region of vitamin D responsive genes, influencing gene transcription [21, 25]. These VDREs are upstream of many genes and thereby exert an effect on different functions of the body. The most well-known activity

of vitamin D in the body is its role in calcium homeostasis. By stimulating calcium absorption, vitamin D enhances bone density. However, vitamin D also plays a role in many other biological processes, including the control of cancer cell proliferation, skin function, cardiovascular disease and regulation of the immune system [20]. It has vasculo-protective roles, especially in blood vessels that are sensitive to inflammation [26]. Moreover, vitamin D is important in neurocognitive development through its stimulation of nerve growth factor production [27].

2.3 Vitamin D: modulating immune responses

Vitamin D has also been described to affect both innate and adaptive immunity and is therefore considered to be immunomodulatory, including control of effector functions, increasing barrier function and stimulating regulatory T cells [28]. 1,25(OH)2D binds to a VDR which is located intracellularly. Consequently, the VDR/RXR complex translocates to the nucleus and binds to a VDRE, thereby altering gene transcription [29]. Studies show that the required 1,25(OH)2D levels are likely to be higher than the average serum vitamin D levels to facilitate immunomodulation. To maintain bone health, 1,25(OH)2D serum levels should be around 20 ng/mL or higher [30]. In contrast, 1,25(OH)2D levels should approximately be 40–80 ng/mL to reach sufficient amounts necessary for immunomodulation. These high 1,25(OH)2D levels can be achieved by the autocrine and paracrine functions of immune cells regarding vitamin D [31]. As stated before, vitamin D exerts its effects through VDRs. These receptors are expressed in all immune cells, although in ranging amounts [31]. By binding of 1,25(OH)2D to VDRs, vitamin D can activate or suppress gene transcription. Additionally, 1,25(OH)2D can exert rapid non-genomic responses. In contrast to genomic responses which require hours to days to become apparent, these rapid responses take 1 to 45 minutes [32]. Unfortunately, the exact mechanism of how this works has yet to be discovered.

Interestingly, immune cells can also affect 1,25(OH)2D levels. Most immune cells, including macrophages and dendritic cells, express CYP27B1 and CYP24A1, the enzymes needed for active vitamin D synthesis and degradation respectively. This allows immune cells to directly control 1,25(OH)2D levels in their direct local microenvironment, exerting autocrine and paracrine effects [33, 34]. This contrasts with systemic 1,25(OH)2D levels, which are regulated by CYP27B1, PTH and FGF23. Previous studies show that the negative feedback loop present in renal CYP24A1 and 1,25(OH)2D does not apply to immune cell hydroxylases. Due to this, CYP24A1 is not activated by high levels of 1,25(OH)2D, resulting in increased vitamin D levels [35].

3. Peripheral immune dysregulation in ASD

ASD is characterized not only by behavioral deficits, but also by comorbidities, including gastrointestinal problems. In addition, there is an involvement of the immune system based on the increased inflammation, autoimmunity and oxidative stress in ASD patients compared to healthy individuals [36]. Additionally, the prevalence of allergies and infections among ASD patients is higher compared to healthy individuals [37, 38]. A recent study states that approximately 60% of all ASD patients suffers from immune dysregulation [39, 40].

3.1 Antigen presenting cells

Studies indicate that innate immune activation with activated antigen presenting cells and associated cytokine production is observed in ASD patients [41]. Increased

numbers of monocytes with increased amounts of cytokines, with a shift towards pro-inflammatory cytokines are found in ASD patients compared to healthy individuals. IL-1β is one of these cytokines and is associated with more severe ASD symptoms. Upon TLR signaling, monocytes in ASD patients show increased activation and pro-inflammatory cytokine production [42, 43]. Macrophage or microglial activity associated with increased production of macrophage migration inhibitory factor (MIF) neuroinflammation in the brain is also altered in ASD patients [44]. MIF is a mediator of innate immunity by enhancing pro-inflammatory cytokine release and higher MIF levels result in less suppression of macrophage activity. Moreover, MIF levels are positively correlated with increased macrophage activity and thus ASD severity [45, 46]. Individuals with ASD show an increased number of dendritic cells, which is associated with more severe ASD symptoms [47]. These different findings thus illustrate increased innate immune activation in ASD patients.

Monocyte and macrophage activity are increased in ASD patients, both due to increased cell numbers and increased pro-inflammatory cytokine production. Contradictory, vitamin D suppresses pro-inflammatory cytokine release by M1 macrophages, while antimicrobial activities and differentiation into M2 macrophages are stimulated. Like a balance between Th1 and Th2 cells, a balance between M1 and M2 macrophages is required for immune homeostasis. An increase in both types of macrophages could thus be beneficial, if a balance is maintained [48, 49]. Altogether, vitamin D balances macrophage function and is thereby likely to positively affect macrophage function in ASD patients. The number of dendritic cells is also increased in individuals with ASD, resulting in increased T cell activation and, indirectly, development of more severe symptoms [47]. In contrast, vitamin D can induce a tolerogenic state in dendritic cells. The expression of surface molecules required for antigen presentation and T cell activation is inhibited and a shift from pro-inflammatory to anti-inflammatory cytokine secretion arises. Via these pathways, vitamin D could affect dendritic cells in ASD patients in such a way that it facilitates immunosuppression. Besides altered cytokine profiles that illustrate changes in CD4+ T cell differentiation, this shift in subsets is also shown by absolute cell numbers. Increased Th1 and Th17 populations are observed in ASD patients, combined with a decreased Treg population. Moreover, Tregs exhibit a reduced expression of Foxp3, CD25 and CTLA-4, which are all required for regulation of immune responses. Opposingly, vitamin D positively influences Th2 and Treg populations and hereby shifts immune responses to a more anti-inflammatory state [50, 51].

3.2 Pro-inflammatory cytokines

Altered cytokine expression has been observed in ASD patients with increased levels of pro-inflammatory cytokines IFN-y, IL-6, TNF-alpha, IL-8, IL-12, IL-17, IL-1ß, GM-CSF and MCP-1. IL-2 and IL-23. A meta-analysis described the strongest elevations were seen for IFN-y and thus Th1 cells stimulating inflammation and inhibiting Th2 proliferation in ASD patients compared to healthy individuals [52]. Besides, IL-6 is increased which as an important B cell activator enhances antibody production. In addition, IL-6 induces innate immune responses via the production of acute phase proteins [53]. Besides, IL-6 is important in signaling pathways in the central nervous system (CNS) by impairing synaptic plasticity and mediating behavioral deficits seen in ASD patients [54, 55]. IL-1ß has been shown to play a role in depression and anxiety through the hypothalamus-pituitary–adrenal (HPA) axis. Moreover, the role of IL-1ß has been suggested in training of the immune system. Upon excessive IL-1ß production, the immune system is characterized by less tolerance induction and increased prevalence of chronic inflammation [56]. However, a study reported no change in IL-1ß levels in ASD patients [57].

A study described significantly increased TNF-alpha, IL-6 and IL-17 levels and a decrease in IL-2 by peripheral blood samples of thirty ASD individuals compared to healthy controls [58]. Another study did not find significant alterations in IL-2 levels of ASD patients compared to healthy individuals [59]. TNF-alpha and IL-12 expression are consistently proven to be elevated in ASD patients [57, 60]. IL-17 expression is shown either to be increased [61–63] or similar in ASD patients compared to healthy individuals [64, 65]. Besides IL-17, IL-21 and IL-22 are two other important Th17 cytokines. These are both shown to be increasingly expressed in ASD patients [66]. Contradicting findings exist on the expression of IL-23, a cytokine important in Th17 differentiation [64, 65, 67]. GM-CSF is shown to be elevated in ASD patients. This cytokine is important in the activation of Th17 cells and hereby plays a role in autoimmunity [68]. Contradictory, GM-CSF is also suggested to have beneficial effects on ASD symptoms. For example, GM-CSF can cross the blood brain barrier and can act as neuronal growth factor [68]. GM-CSF was associated with improved development and behavior in ASD patients. Several chemokines, including IL-8 and MCP-1, are also elevated in ASD patients. These chemokines have the capacity to attract T cells to tissue inflammation sites [69].

3.3 Anti-inflammatory cytokines

Several studies observe alterations in anti-inflammatory cytokines in ASD patients, like reductions in TGF-ß expression [70, 71]. TGF-ß being involved in immune regulation and is associated with severity of ASD symptoms; the lower the TGF-ß status, the more severe ASD symptoms are [72]. The levels of IL-10 in ASD patients remain debatable. Some studies observed increased IL-10 levels [69], while others found similar IL-10 levels [73] or even lower IL-10 levels [71, 74] in ASD patients compared to healthy individuals. IL-10 modulates inflammatory response and thus the observed increased inflammation in ASD patients could be expected to be increased. Lack of this compensatory activity of IL-10 suggests immune dysregulation. Lastly, IL-35 is also connected to regulatory T cells and was found to be reduced in ASD patients [75]. Meta-analysis findings suggest that the changes in IL-4, IL-5 and IL-13 levels in ASD patients are insignificant [70]. On the other hand, multiple studies observe increased concentrations of IL-4, IL-5 and IL-13 in ASD patients [76]. In general, a decreased level of anti-inflammatory cytokines is found in ASD patients. This can result in chronic inflammation in ASD patients [6].

In summary, pro-inflammatory cytokines and chemokines are all increasingly expressed in ASD patients while anti-inflammatory cytokines are downregulated. Nevertheless, other studies showed an increased expression of anti-inflammatory cytokines combined with a decreased expression of pro-inflammatory cytokines upon vitamin D treatment. Upon exposure to vitamin D, immune cells secrete increased amounts of the anti-inflammatory cytokines IL-10 and TGF-ß. At the same time vitamin D suppresses the production of pro-inflammatory cytokines and chemokines. This cytokine expression profile indicates that vitamin D might have protective effects against ASD development.

3.4 CD4+ T cell populations

In general, an increase in inflammatory Th1 and Th17 cells can be observed in individuals with ASD and were directly correlated with severity of symptoms [71, 77]. In contrast, increased Th2 responses are associated with improved behavior in children with ASD [72]. This suggests also beneficial effects of a Th2-skewed immune system in ASD patients. While mostly an increased Th1/Th2 ratio is observed in ASD patients compared to healthy individuals [78], others describe

increased Th2 relative to Th1 [72]. This contrast illustrates immune dysregulation in ASD patients [70]. In addition, a decreased Foxp3 expression positive Treg population is observed [] as well as decreased CTLA-4 expression [72]. Also, CD25 expression in activated CD4+ and CD8+ T cells is decreased [66, 71], while others showed a decreased ratio [79].

The observed dysregulation of the immune system in individuals with ASD is important not only for developing symptoms, but also affects the severity of the symptoms. In general, it can be concluded that ASD patients have increased Th1- and Th17-mediated immune responses and decreased Th2 and Tregs cytokines. Due to the increased immune activation, chronic inflammation can occur and worsen ASD symptoms. Children with genetic heritability have a higher chance of developing ASD, and the role of a disbalanced immune system in ASD development should be acknowledged.

3.5 Inflammation in ASD

In addition to peripheral immune dysregulation in ASD, other immunological dysfunctions in ASD are also of importance. The role of neuroinflammation, autoimmunity and oxidative stress have been investigated more widely in ASD patients and the importance of these processes should be noted. The difference between systemic inflammation and neuroinflammation is reflected by the fact that some cytokines are differentially expressed in the brain versus systemically. Increased TGF-ß levels are measured in the cerebellum of ASD patients, in contrast to decreased levels in the cerebrospinal fluid or the periphery [80]. Upon cell death, cells often secrete TGF-ß to reduce local inflammation. Neurons that showed degeneration were high in TGF-ß, suggesting the increased TGF-ß levels found in the brain of ASD patients are targeted at controlling neuroinflammation. Increased microglial activation, combined with increased pro-inflammatory cytokines and i-NOS activation results in neuroinflammation [41]. This is observed in a large fraction of all ASD cases and could lead to impaired connectivity in the CNS, resulting in the pathophysiology observed in ASD patients. Moreover, oxidative stress is increased in ASD patients, which is among others shown by increased i-NOS activation and the presence of reactive oxygen species. Oxidative stress can affect both immune cells and neurons, thereby causing neuroinflammation and neuron degeneration [36]. Vitamin D has been shown to increase glutamine, an antioxidant capable of counteracting the negative activities of free oxygen radicals, and to decrease nitric oxide. Via these ways, vitamin D could reduce oxidative stress in ASD patients [13].

Lastly, improving ASD symptoms is touched upon most in this review by discussing immune dysregulation in ASD, prevention of ASD is another topic that requires attention. While vitamin D is presumed to play a role in immune dysregulation, and thereby systemic inflammation in ASD patients, this is thought to be limited to the progression of ASD symptoms. ASD is a neurodevelopmental disorder, indicating the importance of the CNS in the etiology and pathophysiology of ASD. Considering the onset of ASD, neuroinflammation, rather than systemic inflammation, should be focused on. Vitamin D is proven to play an important role in neuronal development, which is also illustrated by the abundance of VDRs in the CNS [81]. Maternal vitamin D deficiency and risk of ASD have been commonly shown to be associated. When maternal vitamin D deficiency occurs, insufficient vitamin D impairs neurodevelopment in the infant [82] This illustrates the importance of adequate vitamin D levels during gestation. A recent study tested the efficacy of vitamin D supplementation in pregnant mothers of children with autism on reducing the risk of autism in the newborn sibling [83]. After maternal vitamin D

supplementation and supplementation during the first three years of the newborn's lives, the risk of autism was shown to be reduced from 20% to 5%. This illustrates the importance of adequate maternal vitamin D status and the influence on ASD risk. Vitamin D supplementation is likely to be effective at reducing inflammation in ASD patients and improve symptoms of ASD. However, to prevent ASD it is more relevant to look at maternal vitamin D supplementation and the role of vitamin D in neurodevelopment. Therefore, further research should be performed to examine the possible mechanisms of vitamin D during gestation and the association with ASD development in the infant.

4. Vitamin D and ASD: clinical results

The possibility of an association between vitamin D and ASD was found when studies concluded that ASD prevalence is increased in high-latitude countries and with more cloud coverage, resulting in reduced UV-B intensity. Many studies observe the connection between low sun exposure and risk of ASD [84]. UV-B exposure is required for the conversion of 7-dehydrocholesterol into previtamin D underscoring the link between UV exposure, vitamin D generation and ASD development [85]. However, it was shown that vitamin D insufficiency in ASD patients is independent of sun exposure, ruling out the environmental factor causing vitamin D deficiency later in life. Moreover, ASD prevalence is suggested to be higher in dark skin-colored people compared to light skin-colored people [86]. It is suggested that increased skin pigmentation lowers the production of previtamin D, due to UV-B radiation that is absorbed by melanin and thereby less available for vitamin D synthesis [11]. For example, a study showed only 4.1% of the dark skin-colored pregnant women had sufficient vitamin D levels, compared to 37.3% in light skin-colored pregnant women [87]. However, other studies state skin pigmentation does not influence vitamin D synthesis and that a different lifestyle, i.e. less exposure to sunlight, could explain lower vitamin D levels in dark skin-colored people [88, 89]. Thus, common vitamin D deficiency in dark skin-colored people might explain the higher ASD prevalence among this group, however results are contradictory regarding the cause of lower vitamin D status.

4.1 Maternal vitamin D deficiency

ASD prevalence is increased in children of whom the mother was vitamin D deficient during gestation [87] and thus it is suggested that maternal vitamin D deficiency increases the risk of ASD in the infant [84, 90].

The possible role of maternal vitamin D deficiency is also illustrated by the influence of season of birth on ASD risk. Maternal vitamin D levels are often lowest in winter and spring months [91], which could be explained by differences in sun exposure and UV-B intensity [85, 91]. However, studies observe conflicting results regarding seasons most positively associated with ASD risk, questioning whether birth season is indeed a cofactor influencing the risk of ASD. Multiple studies observe highest ASD prevalence in children born in March [92]. These children have a higher risk of maternal vitamin D deficiency in the second half of gestation, since maternal 25(OH)D levels are lowest in winter and spring months. On the other hand, studies observing highest ASD prevalence among children born in May, July or August also exist [93–95]. Autumn months coincided with highest ASD prevalence, while birth in spring months reduced the risk of ASD [96]. Studies show that the first six months of gestation are most important for neurocognitive development in the infant, a process which is influenced by vitamin D [97, 98]. Therefore,

it is suggested that maternal vitamin D deficiency increases the risk of ASD most when occurring in the first six months of gestation. As vitamin D levels are lowest in winter and spring, this would result in highest ASD prevalence among children who are born in summer. However, contradicting results on the association between birth season and ASD risk hinder a definite conclusion.

4.2 Vitamin D deficiency in ASD children

Studies show that children with ASD have lower vitamin D levels than healthy children. Since 2011, vitamin D insufficiency is classified as a 25(OH)D level between 20–30 ng/mL, whereas levels below 20 ng/mL are considered vitamin D deficient [3]. Individuals with ASD on average show 25(OH)D levels below 30 ng/mL [99–101]. In a recent study, 48% of the ASD cases was vitamin D insufficient and 40% vitamin D deficient, whereas none of the healthy children were deficient and only 20% was insufficient [101]. This study used different cut-off values, resulting in the fact that when using the standard cut-off of 20 ng/mL for vitamin D deficiency, the percentage of deficient children would even be higher than 40%. An average vitamin D level of 28.5 ng/mL was measured in ASD children, compared to 40.1 ng/mL in healthy children [102]. A significant negative correlation between vitamin D levels and severity of ASD symptoms was found, indicating low vitamin D levels can increase the severity of ASD [100]. When combining this finding with the previously mentioned association between season and vitamin D levels, this suggests the effect of season on ASD symptoms. Several case studies indeed observe that children with ASD experience less symptoms during summer compared to other seasons, which supports the plausible association between vitamin D and ASD [103].

In addition to the above-mentioned environmental factors that could cause vitamin D deficiency in ASD patients and are associated with progression of the disorder, genetics also play a role. In a study that compared vitamin D levels in ASD children and their healthy siblings, lower 25(OH)D levels were found in ASD children, suggesting genetics are upstream of vitamin D deficiency in ASD patients, rather than environmental factors [104]. Moreover, most studies on neonatal vitamin D levels and the association with ASD risk have found a negative correlation, illustrating that vitamin D deficiency presumably develops during gestation and is dependent on either or both genetics and maternal environmental factors [105, 106].

Furthermore, genetic polymorphisms are shown to be associated with impaired vitamin D metabolism and binding to VDR and can therefore predispose ASD. VDR gene polymorphisms were studied of which two were significantly associated with ASD [86]. Measured 25(OH)D serum levels did not significantly correlate with gene polymorphisms, suggesting vitamin D deficiency itself is not the cause of increased ASD risk, but rather genetic mutations. However, not all ASD patients suffer from these gene mutations and thus gene polymorphisms cannot explain all ASD cases [107]. To conclude, it is uncertain whether genetic or environmental factors alone predispose vitamin D deficiency in ASD patients. Nonetheless, clinical trials agree that reduced vitamin D levels are observed in ASD patients.

4.3 Vitamin D treatment in ASD patients

Due to the suggested association between vitamin D levels and ASD symptom severity, it is being investigated whether vitamin D supplementation could work as treatment to reduce ASD symptoms. The effect of vitamin D, n-3 fatty acids and the combination of the two were tested on ASD symptoms [108]. In the study,

children with ASD received a daily dose of 2000 IU vitamin D3 for twelve months. This study did not find a positive effect of only vitamin D supplementation on reducing ASD symptoms. However, treatment with n-3 fatty acids only or the combined treatment with vitamin D and n-3 fatty acids did improve social awareness scores in children with ASD. In contrast, a positive effect of vitamin D treatment was observed on ASD symptoms [109]. Autism Behavior Checklist (ABC) and Childhood Autism Rating Scale (CARS) scores were used, two methods commonly used for scoring ASD symptoms. Children with ASD received one monthly dose of 150,000 IU vitamin D intramuscularly and daily doses of 400 IU vitamin D orally. After three months, both total ABC and total CARS scored were decreased significantly. These reductions were prominent in ASD children under the age of three compared to ASD children above the age of three, suggesting vitamin D treatment possibly is more effective at a younger age. Similarly, vitamin D treatment can be effective at reducing ASD symptoms. Upon receiving daily doses of 300 IU vitamin D3 per kg bodyweight orally, 67 out of 83 children with ASD experienced improved symptoms [110]. The positive effect of vitamin D was most prominent in the group with 25(OH)D levels above 40 ng/mL at the end of the study, suggesting higher vitamin D levels correlate with increased improvement of behavior. Although research is limited, recent studies on the effect of vitamin D treatment in ASD children show promising results.

A review supported the need of a high vitamin D dose for its efficacy [31]. Whereas the recommended daily intake of vitamin D is 30 ng/mL, a minimum dose of 40–80 ng/mL is suggested for vitamin D to exert its immunomodulatory effects in general. In ASD, most improved ASD symptoms upon vitamin D administration above 40 ng/mL. Worldwide it is estimated that 30% of all children and adults are vitamin D deficient, and around 60% has insufficient vitamin D levels [110]. This high percentage of insufficiency cases illustrates the need for vitamin D supplementation and/or increased sun exposure when the effect of vitamin D on the immune system is wished upon. Presumably, this would not cause adverse effects as studies on the toxicity of vitamin D have found little disease outcomes, except possibly hypercalcemia [111]. However, findings on hypercalcemia are inconsistent and it is thus not known whether hypercalcemia will indeed occur in the case of vitamin D levels above 40–80 ng/mL and most likely at a dose of 150 ng/mL [3, 91].

All in all, many clinical trials have been performed on the association between vitamin D and ASD. Low vitamin D levels are observed more often in ASD children compared to healthy children. Moreover, research indicates the role of maternal vitamin D deficiency in ASD is plausible and recent studies have illustrated the effectiveness of vitamin D treatment on improving ASD symptoms. Thus, clinical trials show promising results on the association between vitamin D and ASD and the effectiveness of vitamin D treatment in ASD patients. Lastly, rather than in ASD children there is still little research performed on immune functioning and vitamin D levels in adults with ASD as lower 25(OH)D levels were observed in adults with autistic disorder compared to healthy individuals [112]. The lack of research in this target group complicates extrapolation of the discussed results to adults. It is therefore uncertain whether vitamin D supplementation could improve ASD symptoms in adults.

5. Association vitamin D and ASD

The association between vitamin D and ASD is proven through clinical research. Clinical trials show promising results on vitamin D supplementation and the improvement of ASD symptoms. Characteristics of ASD, including behavioral deficits and impaired communicative functioning, impair the lifestyle of both

patients and their close relatives – improvement of symptoms therefore would be highly beneficial [113]. Insights into the mechanisms would support a better understanding of the etiology of the disorder. Consequently, this can enhance the finding of better preventive measures and treatments. Besides the lack of research into the mechanisms behind the effect of vitamin D on ASD, further research is also required to better understand immunomodulatory properties of vitamin D in general, as well as immune dysfunction in individuals with ASD [31]. Similarly, there is a lack of research on several important cytokines in ASD patients, like IL-21, IL-22 and IL-35. Additionally, IL-10 expression in ASD patients remains debatable. Different studies have found either reduced, similar or increased IL-10 levels compared to healthy individuals. This suggests the high interpersonal variability between ASD patients and the heterogeneous etiology of the disorder, emphasizing the need for further research to determine possible subgroups on which tailored treatment design could be based [114]. In addition, the role of IL-2 in regulating immune responses in ASD remains elusive. IL-2 is a Th1 cytokine, important for T cell proliferation of effector T cells but also for regulatory T cells. Vitamin D is shown to reduce IL-2 levels. This implies a reduction in Th1 cytokines, but as IL-2 is required for TGF-ß-mediated induction of CTLA-4 and Foxp3 expression on Tregs this suggests that increased IL-2 expression would enhance immunosuppression [115–117].

Although research on the association between vitamin D and ASD is receiving increased attention, no causality has been proven. As discussed, it is unknown whether vitamin D deficiency is caused by genetic or environmental factors. The possibility of reduced endogenous vitamin D production in ASD patients raises the question whether vitamin D insufficiency is a cause or consequence of ASD. To date, it is uncertain whether vitamin D deficiency predisposes ASD onset or is developed because of ASD. Effectiveness of vitamin D supplementation is irrespective of the outcome of causality, as clinical trials have shown promising results on the positive effect of vitamin D on ASD symptoms. However, increasing sun exposure could be less effective in the case of impaired endogenous vitamin D production in ASD patients. Research on the mechanisms behind the role of vitamin D in ASD could support a better understanding of a possible causal relationship.

6. Conclusion

Vitamin D can have immunosuppressive effects on the immune system that could be of interest in ASD. By shifting immune responses away from Th1- and Th17-mediated towards Th2- and Treg-mediated, vitamin D promotes a tolerogenic state in the immune system. This could rebalance immune dysregulation in ASD, consequently reducing systemic inflammation among others. Clinical trials on the effect of vitamin D supplementation on improving ASD symptoms and reducing ASD risk are promising, highlighting the relevance of investigating vitamin D when studying ASD. This relevance is best illustrated by the finding that increased immune activity is positively correlated with severity of ASD symptoms, a process which could be counteracted by vitamin D. However, studies on the direct mechanisms of vitamin D on the immune system in ASD patients are absent. Therefore, further research is necessary to draw conclusions about a possible causal relationship. Moreover, further research into the mechanisms behind maternal vitamin deficiency and neuroinflammation are advised to investigate possible preventive actions of vitamin D in relation to ASD. Since vitamin D toxicity is rare, it is advised to increase vitamin D levels in pregnant women and ASD patients. However, insufficient research exists to state the effectiveness of vitamin D in regulating immune dysregulation in ASD patients with confidence.

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