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PEDIATRIC CLINIC

"The effect of breastfeeding and vitamin D status on the susceptibility of infants to infections"

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Midwife MSc

PhD THESIS

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«Η έγκριση της διδακτορικής διατριβής από το Τμήμα Ιατρικής του Πανεπιστημίου Ιωαννίνων δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέα Ν. 5343/32, άρθρο 202, παράγραφος 2 (νομική κατοχύρωση του Ιατρικού Τμήματος)».

Ημερομηνία αίτησης της κ. Ζήση Δήμητρας: 6-12-2016

Ημερομηνία ορισμού Τριμελούς Συμβουλευτικής Επιτροπής: 794ᵅ/31-1-2017

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Μέλη:
Χάλλα Άννα, Καθηγήτρια Παιδιατρικής με έμφαση στην Εργαστηριακή Διερεύνηση Νοσημάτων Μεταβολισμού των Οστών και άλλων Νοσημάτων παιδικής ηλικίας Πανεπιστημίου Ιωαννίνων
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Ημερομηνία ορισμού θέματος: 13-02-2017 (γλώσσα συγγραφής: αγγλική)
«Συσχέτιση του μητρικού θηλασμού και των επιπέδων βιταμίνης D με την προδιάθεση για λοιμώξεις στη βρεφική ηλικία»

«The effect of breastfeeding and vitamin D status on the susceptibility of infants to infections»

ΟΡΙΣΜΟΣ ΕΠΤΑΜΕΛΟΥΣ ΕΞΕΤΑΣΤΙΚΗΣ ΕΠΙΤΡΟΠΗΣ 960ᵅ/21-4-2021

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2. Χάλλα Άννα, τ. Καθηγήτρια Παιδιατρικής με έμφαση στην εργαστηριακή διερεύνηση νοσημάτων μεταβολισμού των οστών και άλλων νοσημάτων παιδικής ηλικίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων
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5. Ντζάνη Ευαγγελία, Αναπληρώτρια Καθηγήτρια Υγιεινής με ιδιαίτερη έμφαση στην Επιδημιολογία του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων
6. Γκαρτζονίκα Κωνσταντίνα, Αναπληρώτρια Καθηγήτρια Μικροβιολογίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων
7. Τσαμπούρη Σοφία, Αναπληρώτρια Καθηγήτρια Παιδιατρικής με έμφαση στην Παιδοαλλεργιολογία του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων

Έγκριση Διδακτορικής Διατριβής με βαθμό **«ΑΡΙΣΤΑ»** στις **25-05-2021**

Ιωάννινα 22-6-2021
ΠΡΟΕΔΡΟΣ ΤΟΥ ΤΜΗΜΑΤΟΣ ΙΑΤΡΙΚΗΣ
Άννα Μπατιστάτου
Καθηγήτρια Παθολογικής Ανατομίας

Η Γραμματέας του Τμήματος

ΜΑΡΙΑ ΚΑΠΙΤΟΠΟΥΛΟΥ

Αν σου πει κάποιος “δεν μπορείς”
πες του…. “κοίτα να μαθαίνεις”
Άγνωστος

ΠΡΟΛΟΓΟΣ

Στις μέρες μας η βιταμίνη D κερδίζει όλο και περισσότερο το παγκόσμιο ενδιαφέρον, αποκαλύπτοντας τον καταλυτικό της ρόλο - όχι μόνο στο ανθρώπινο μυοσκελετικό σύστημα αλλά και πέραν αυτού. Η βιταμίνη D, εκτός από την επίδραση στον οστικό μεταβολισμό, έχει και έξω-σκελετικές δράσεις ειδικά στο ανοσοποιητικό σύστημα. Η ανεπάρκεια της έχει ενοχοποιηθεί για την προδιάθεση εμφάνισης λοιμώξεων σε βρέφη και μικρά παιδιά, όμως, οι μέχρι του παρόντος μελέτες παρουσιάζουν αμφιλεγόμενα αποτελέσματα.

Στην παρούσα μελέτη εκτιμήθηκαν τα επίπεδα βιταμίνης D σε νοσηλευόμενα βρέφη και μικρά παιδιά κάτω των δύο ετών με λοίμωξη αναπνευστικού, γαστρεντερικού και ουροποιητικού συστήματος και διερευνήθηκε η ενδεχόμενη συσχέτιση. Επιπλέον, αξιολογήθηκε ο ρόλος του αποκλειστικού μητρικού θηλασμού, της εποχής του χρόνου αλλά και της χορήγησης συμπληρώματος βιταμίνης D με την εμφάνιση των λοιμώξεων αυτών.

Η εκτενής βιβλιογραφική αναζήτηση πάνω στο αντικείμενο της μελέτης οδήγησε σε μία συστηματική ανασκόπηση που δημοσιεύτηκε στο περιοδικό Hormones - International Journal of Endocrinology and Metabolism (impact factor 2.048): *Zisi D, Challa A, Makis A. The association between vitamin D status and infectious diseases of the respiratory system in infancy and childhood. Hormones. 2019 Dec;18(4):353-363. doi: 10.1007/s42000-019-00155-z. Epub 2019 Nov 25.*

Τα αποτελέσματα της μελέτης έχουν ανακοινωθεί στα παρακάτω συνέδρια ως προφορικές ανακοινώσεις:

- 28η Διημερίδα Συχνά και Σπάνια Νοσήματα στην Παιδική Ηλικία: Μια Συνεχής Πρόκληση για τον Παιδίατρο 2018, Ιωάννινα
- Στο 14ο Πανελλήνιο Συνέδριο Μαιευτικής 2018, Αθήνα
- Σε τοπικές Ημερίδες Υγείας των Ιωαννίνων 2018
- Στο ετήσιο Συνέδριο Συνεχιζόμενη Ενημέρωση του Παιδιάτρου: από το νεογνό στον έφηβο 2020, Ιωάννινα.

Τα αποτελέσματα της μελέτης έχουν παρουσιασθεί επίσης ως αναρτημένη ανακοίνωση:

- Στο 31ο Πανελλήνιο Συνέδριο της Ελληνικής Εταιρίας Κοινωνικής Παιδιατρικής και Προαγωγής της Υγείας 2019, στη Λευκάδα όπου έλαβε και το 3ο ΒΡΑΒΕΙΟ ΑΝΑΡΤΗΜΕΝΗΣ ΑΝΑΚΟΙΝΩΣΗΣ.

Ακόμα, τα αποτελέσματα της μελέτης έχουν υποβληθεί για δημοσίευση στο περιοδικό Archives de Pédiatrie.

Θα ήθελα να εκφράσω ένα μεγάλο ευχαριστώ στον επιβλέποντα κ. Αλέξανδρο Μάκη Αναπληρωτή Καθηγητή Παιδιατρικής / Παιδιατρικής Αιματολογίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων, που με πίστεψε και μου ανέθεσε το συγκεκριμένο θέμα. Του εκφράζω την ευγνωμοσύνη μου για την πολύτιμη επιστημονική καθοδήγησή του, την υπομονή του, τις εύστοχες συμβουλές και την υποστήριξή του σε όλη τη διάρκεια μέχρι την ολοκλήρωση αυτής της διατριβής.

Επίσης, θα ήθελα να ευχαριστήσω θερμά την Καθηγήτρια κα Άννα Χάλλα, μέλος της τριμελούς επιτροπής και υπεύθυνη του Εργαστηρίου που πραγματοποιήθηκαν οι εργαστηριακές αναλύσεις. Ευχαριστώ την κ. Χάλλα για την αμέριστη συμπαράσταση και καθοδήγηση ως την ολοκλήρωσης της συγγραφής της εν λόγω μελέτης με τις πολύτιμες συμβουλές, τις εύστοχες παρατηρήσεις στο κείμενο και τον πολύτιμο χρόνο που αφιέρωνε όταν είχα ανάγκη υποστήριξης.

Τις θερμές ευχαριστίες μου για τη συμβολή τους στη διεκπεραίωση της διατριβής απευθύνω επίσης:

- Στον κ. Αντώνιο Βλάχο, Αναπληρωτή Καθηγητή Παιδοκαρδιολογίας, μέλος της τριμελούς επιτροπής, για την υποστήριξη και καθοδήγηση

- Στην κα Αικατερίνη Σιώμου, Αναπληρώτρια Καθηγήτρια, Διευθύντρια της Παιδιατρικής Κλινικής και στον κ. Νικόλαο Χαλιάσο, Ομότιμο Καθηγητή Παιδιατρικής, πρώην Διευθυντή της Παιδιατρικής Κλινικής για την άδειά τους να πραγματοποιηθεί η συλλογή δειγμάτων από τα παιδιά της Κλινικής.

- Στους ειδικευόμενους ιατρούς της Παιδιατρικής Κλινικής για την πολύτιμη βοήθειά τους στη διαδικασία συλλογής των δειγμάτων και το σύνολο του ιατρικού και νοσηλευτικού προσωπικού της κλινικής του νοσοκομείου για την άριστη συνεργασία που υπήρξε.

- Στο προσωπικό του Εργαστηρίου του Τομέα Υγείας Παιδιού κ. Βασίλειο Χολέβα και την κα Αφροδίτη Παπαγιάννη για την βοήθειά, τις συμβουλές και την εξαιρετική συνεργασία που είχαμε κατά τη διάρκεια παραμονής μου στο εργαστήριο αλλά και για την ανάλυση των δειγμάτων.

- Στον κ. Γεώργιο Δημακόπουλο για την ανάλυση των δεδομένων και τις επεξηγήσεις.

- Σε όλους τους γονείς που σε μία δύσκολη, γεμάτο φόβο ίσως, στιγμή της ζωής τους έχοντας το παιδί τους νοσηλευόμενο στην κλινική, με εμπιστεύτηκαν και δέχτηκαν να συζητήσουν, να απαντήσουν στις ερωτήσεις μου και να συμμετέχουν στη έρευνα.

Τέλος στην οικογένειά μου, στον σύζυγό μου Δημήτρη και τις κόρες μας, θα ήθελα να εκφράσω την ευγνωμοσύνη και αγάπη μου για την υποστήριξη και την υπομονή τους.

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ABREVIATTIONS

#	
$1,25(OH)_2D$	1,25 dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
7-DHC	7-dehydrocholesterol
A	
AAP	American Academy of Pediatrics
B	
BF	Breastfeeding
BM	Breast milk
C	
CMIA	Chemiluminescence microparticle immunoassay
CYP2R1	25-hydroxylase
CYP27B1	1α,25-hydroxylase
D	
DBP	Vitamin D binding protein
DRI	Dietary Reference Intake
DRV	Dietary Reference Values
E	
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
EFSA	European Food Safety Authority
F	
FM	Formula milk
H	
HES	Hellenic Endocrinology Society
HMOs	Human milk oligosaccharides
HSD	Honestly significant difference

I

IOM	Institute of Medicine
IU	International Units

M

MAs	Meta-analysis

P

PTH	Parathyroid hormone

R

RCT's	Randomized Control Trials
RSV	Respiratory Syncytial Virus

S

SACN	Scientific Advisory Committee on Nutrition
(SD)	Standard deviations

U

US	United States
UVB	Ultraviolet B radiation

V

VitD	Vitamin D
VDD	Vitamin D deficiency
VDT	Vitamin D Toxicity
VitA	Vitamin A
VDR	Vitamin D Receptor

W

WHO	World Health Organization

CHAPTER 1. INTRODUCTION

Infancy is a time of increased infectious disease susceptibility and severity. Immaturity of the infant's immune system predisposes to the development of viral, bacterial, fungal and parasitic infections. Infections can be generalized or localized to various systems such as the respiratory, gastrointestinal, urinary, central nervous systems and skin (1).

Infectious diseases in childhood are a leading cause of morbidity and mortality worldwide. According to World Health Organization (WHO) estimates in 2019, 5.2 million children under the age of 5 years died, mostly from preventable and treatable causes and with most of them living in low-and middle-income countries. Newborns (under 28 days of age) accounted for the 2.4 million deaths. Leading causes of death in children under 5 years are preterm birth complications, birth asphyxia/trauma, congenital anomalies, pneumonia, diarrhea and malaria. Nearly half of these deaths are in newborns (2)

Initially, the infant’s immune system is immature, and its maturation progresses gradually after birth reaching its full development long after the first years of life, and with the contribution of the child's environmental expose to pathogens. Breastfeeding (BF) is of primary importance for the protection of the infant from infectious diseases since the breast milk (BM) contains many anti-infective factors such as immunoglobulins, lysozyme, lactoperoxidase, complement components, immune cells and lipids. Other contributing factors to infants' immune system development include vaccinations, proper hygiene and adequate nutrition after ending breastfeeding (3).

In recent years, the beneficial effect of vitamin D (VitD) on the maturation and strengthening of the defensive system of infants has been highlighted (4, 5). VitD in addition to its role in bone metabolism, has also beneficial immune regulating functions (6, 7).

The adequacy of VitD during the prenatal and postnatal period has been associated with protection against infections of the respiratory, digestive, urinary and other systems during infancy (8-12). Hence, VitD deficiency has been suggested to be associated with susceptibility to infections (13).

In Greece, despite the plenty of sunshine, low levels of VitD have been found both in newborns and during infancy (14, 15). These findings in combination with the low rates of BF in Greece may contribute to the incidence of infections, their severity and the length of hospitalization of infants.

The purpose of this thesis, therefore, was to investigate the levels of VitD in young children aged less than two years hospitalized due to common infectious diseases and to find any correlations with several parameters such as the topography of infection (respiratory, gastrointestinal or urinary system), season of the year, supplementation of Vit D, effect of BF, mode of delivery, gestational age and birth weight.

CHAPTER 2. BREASTFEEDING

Breast milk is the natural food for infants. Due to the documented short-and long-term medical and neurodevelopmental advantages of breastfeeding (BF), infant nutrition should be considered a public health issue and not only a lifestyle choice.

2.1. Physiology of breastmilk (BM) production

Human milk production and secretion is a complex physiological process resulting from both the previous development of the mammary gland and tight regulations by systemic hormones and local factors (16). Lactogenesis is the process of developing the ability to secrete milk and it has 2 stages: lactogenesis I and lactogenesis II.

- Lactogenesis I, also known as secretory initiation, it occurs mid-pregnancy. High levels of progesterone supplied by the placenta and inhibit further differentiation. In this stage, small amounts of milk can be secreted by week 16 of gestation. During late pregnancy, many women may express colostrum.

- Lactogenesis II, also known as secretory activation, starts with copious milk production after delivery. This stage is stimulated by the rapid decline in progesterone that follows delivery of the placenta, and the elevated levels of prolactin, cortisol and insulin. Most women experience swelling of the breast along with copious milk production at days 2 or 3 postpartum. During this time, the ability of the mammary epithelial cells to synthesize milk rapidly develops, and milk volumes were reported to increase from 100 to 500ml by four days postpartum, and 650ml by eight days postpartum, and it is hormonally driven by the endocrine system (17, 18).

Milk production is dependent on a “supply demand” process. Milk removal and nipple stimulation are the primary control mechanisms for maintaining supply, as this procedure triggers prolactin release from the anterior pituitary gland and oxytocin from the posterior pituitary gland.

Prolactin, initially, required for the morphological development and differentiation of the mammary gland. Moreover, plays a crucial role in stimulating milk protein and lactose synthesis (19). Emptying of the breast by the infant's suckling is thought to be the most important factor. Prolactin concentration increases rapidly with suckling of the nipple which stimulates nerve endings located there.

Oxytocin is produced more quickly than prolactin. It makes the milk that is already in the breast flow. The tactile stimulation of the nipple-areolar complex by suckling leads to afferent signals to the hypothalamus that trigger release of oxytocin. Oxytocin also has psychological effects. It induces a state of calm, reducing stress and enhance feelings of affection which is an important factor in bonding between mother and child.

2.2. Breastmilk composition

The composition of breast milk is believed to be specifically modified by each mother to precisely reflect the requirements of her own infant.

The nutritional components of human milk derive from three sources: Some of the nutrients originate by synthesis in the lactocyte, some are dietary in origin, and some originate from maternal stores (20). Moreover, its composition varies by stage of lactation and between term and preterm infants.

Human milk changes in composition from colostrum to transitional milk and finally to mature milk over the course of lactation. Colostrum occurs in small quantities during the first days of parturition, and it is different from mature milk in terms of color, consistency and composition. Although the nutrients in colostrum and mature milk remain similar, levels of the nutrients vary throughout lactation. Human milk is composed of the correct amount of nutrients and bioactive compounds to provide complete nutrition for the developing infant as well as beneficial bacteria which protect vulnerable immune systems against disease.

2.2.1. Nutritional components (macronutrients and micronutrients)

The most important nutrients in human milk are lipids, carbohydrates, proteins, vitamins, minerals and water.

Maternal diet may have a significant influence on the production and/or composition of several nutrients such as VitD, VitA, water-soluble vitamins and the composition of fatty acids. That is why Institute of Medicine (IOM) and other health committees recommend supplementation of BF infants or their mothers with VitD and other nutrients.

Fats/lipids

BM contains about 3.5g of fat per 100ml of milk, which provides about one half of the energy content of the human milk and its composition in fatty acids defines its nutritional and physio-chemical properties. The fat is secreted in small droplets, and the amount increases during feeding. As a result, the milk secreted at the end of BF is richer in fat.

Human milk fat is an important energy source and one of the most important components of BM. Furthermore, human milk fatty acids are essential for the development of the central nervous system (21), organs and tissues.

In addition, the enzyme lipase helps break down lipid acids, making human milk more digestible than other milks. It is well absorbed and does not leave much residue, which is why the feces of exclusively BF babies have a special color, texture and smell.

Carbohydrates

A huge variety of different and complex carbohydrates are present in milk with lactose being the main carbohydrate. BM contains about 7g lactose per 100ml (22) which is the largest percentage of the milk of other mammals.

Another type of carbohydrate is human milk oligosaccharides (HMOs). One liter of mature human milk contains 10–15g HMO and is 100- to 1000 -fold higher than the concentration of oligo-saccharides in cow milk, and concentrations are even higher in colostrum. HMOs act as receptors that block the attachment of disease causing pathogens, which may help to prevent or reduce intestinal infectious diseases, and potentially also respiratory and urinary tract infections (23).

Proteins

BM contains over 400 different proteins which perform a variety of functions; providing nutrition, possessing antimicrobial and immunomodulatory activities, as well as stimulating the absorption of nutrient (24). Milk proteins are the fourth most abundant component in human milk, consisting of both caseins and whey proteins with a total concentration range of 10–20mg/ml (25). Caseins and whey proteins are very important because they play many roles in the newborn's health and development. The three main types of caseins present in human milk are β-, k-, and α-casein. Caseins have been considered to be of predominantly nutritional

value because they are completely digested by the infant's gastrointestinal tract (26). The whey proteins present in significant quantities in the whey fraction are α-lactalbumin, lactoferrin, immunoglobulins [secretory IgA (SIgA), IgM, and IgG], osteopontin, albumin and lysozyme. In addition to providing essential amino acids and energy, these proteins have exhibited a wide range of biological actions, including antimicrobial activity, prebiotic activity, and assisting the digestion of proteins, all of which promote healthy development of the newborn (26).

Vitamins and minerals

BM normally contains sufficient vitamins and minerals for an infant, unless the mother herself is deficient. The only exception is vitamin K and D. Newborns have low levels of vitamin K, and are at risk of developing hemorrhagic disease. Therefore, parenteral vitamin K supplementation is recommended by the American Academy of Pediatrics, immediately after birth (27). Likewise, it is known that BM has low VitD and as it is dependent mainly on the mother's exposure to sun. Infants who are exclusively BF receive below the minimum recommended intake of VitD, and much lower than the recommended dietary intake and are at risk for VitD deficiency. Infants need exposure to sunlight to generate endogenous VitD, and furthermore a supplementation is required (22).

2.2.2. Bioactive components and immune factors

In addition to providing correct nutrients required for energy, human milk provides many bioactive components and immune factors such as antibodies, immunoglobulins, lactoferrin, lysozyme, antimicrobial peptides, growth factors, white blood cells, microRNAs, and HMOs which play a vital role in boosting the developing infant immune system and providing defense against pathogens (28).

2.3. Comparison to other milks/ Formula versus BF

Formula milk (FM) is very different from BM in both quantity and quality of the various nutrients, and cannot be equivalent or have the same anti-infective properties as BM. It is an effective substitute and is formulated to mimic the nutritional composition of BM for normal infant growth and development. It is usually made from industrially-modified cow milk or soy products. For infants who are unable to tolerate cow milk or soy-based formulas another option is Hypoallergenic Formulas and Amino Acid Formulas.

BF infants have IgA antibodies, that destroy bacteria and protects the mucosal surface of the gut, from the first day of life. A study by Jatsyk et al. showed that IgA is found in BF infants' faeces on the second day of life, compared to 30% of the formula-fed infants (formula does not contain IgA), whose faeces only contains IgA at one-month post-partum (29). Additionally, studies showed that there are differences BF and formula-fed infants both with respect to short-term and long-term outcomes. Fewer infections (gastrointestinal and respiratory infections) reduced risk for chronic diseases such as obesity, type 1 and type 2 diabetes, lower blood pressure and LDL cholesterol (30-33). Moreover, the longer duration of BF may be associated with an increase in cognitive development (34).

A recent review comparing feeding with FM versus BM in preterm or low birth weight infants, concluded that feeding preterm infants with artificial formula is associated with faster rates of growth, but with a near-doubling the risk of developing necrotizing enterocolitis (35).

Lastly, BM is costless, it is always fresh, in the right temperature, and always available for the infant while FM is expensive and needs to get warm. Also, skin-to-skin contact can enhance the emotional connection between mother and infant.

2.4. Benefits of breastfeeding

BF offers many advantages for infants that cannot be replaced by any other form of feeding. The benefits of BF begin from the first moments after birth - as it helps build up the infant's defense system and protect against infectious diseases - lasting for many years after BF ends. It has immunological properties and works like a baby's first vaccine, protecting infants from potentially deadly diseases and giving them all the nourishment, they need to survive. It is well known that BF has also emotional and psychological benefits for both mothers and infants.

BF infants are believed to experience fewer and shorter infections and this protective effect is enhanced with greater duration and exclusivity of BF (30, 36, 37). BM seems to reduce the frequency and severity of some various infections in infants such as bacterial meningitis (38, 39), diarrhea, (30, 31, 40-43), respiratory infections (30, 31, 44), necrotic enterocolitis (45) and urinary tract infections (46, 47), as well as reduces the risk of chronic diseases, such as obesity, and type 1 and type 2 diabetes (32, 33).

In addition, it is reported to reduce overall infant mortality by 20% (48) even in rural settings like Ethiopia, where infant mortality rate is still high and hygienic standards are poor (49).

BF is also recommended for preterm infants as it significantly reduces complications associated with prematurity such as necrotizing enterocolitis, late-onset sepsis and promotes brain development (50).

Due to its vital importance, the WHO recommends exclusive BF for the first 6 months of life, during which the infant receives only milk, while after that solid foods are introduced. BF can be continued for at least 2 years or longer as desired by mother and child, as long as solid foods are the main source of feeding (51).

However, despite the fact that the scientific community has accepted that BF infants are less prone to infections, the rates of BF in Greece are small, in contrast to the administration of modified cow's milk (52, 53). The most recent study in Greece shows that the WHO recommendation for exclusively BF during the first 6 months of life has not been adopted by mothers neither has been promoted adequately by health-care professionals. Unfortunately, introduction of human milk substitutes in the first months of life is predominant and early introduction of solids after the 4th month of age is considered the ‘norm’ (54).

CHAPTER 3. PHYSIOLOGY OF VITAMIN D

3.1. Vitamin D (VitD)

VitD was originally recognized as a vitamin needed for the metabolism of calcium and phosphate in human body and its role in bone health particularly during childhood. Rickets was the first disease recognized to be caused by the lack of this important compound and VitD administration was found to be the cure for it which affected infants, toddlers and older children during the 19^{th} and 20^{th} century (55). Over the past few years, many published studies, identified multiple health benefits of VitD beyond its role in the regulation of calcium and phosphorus homeostasis and bone metabolism not only in infants but for all age groups also.

3.2. Vitamin D Synthesis and Metabolism

VitD is a fat-soluble steroid with endocrine function. It is mainly synthesized in the skin after exposure to the ultraviolet B radiation (UVB) of the sun or absorbed from food sources and has two major forms: D_3 (cholecalciferol) and D_2 (ergocalciferol). VitD itself is biologically inactive and requires sequential enzymatic conversions in the liver and kidneys before it can become active.

Once VitD is produced in the skin or absorbed it is transported by its main carrier protein, vitamin D-binding protein into the liver (DBP). There the first hydroxylation catalyzed by the enzyme 25-hydroxylase (CYP2R1) takes place to produce and release into circulation 25(OH)D (56). This metabolite in the circulation is a marker of 25(OH)D status. Then depending on the needs 25(OH)D undergoes a second hydroxylation in the kidneys by the mitochondrial cytochrome P450 enzyme, 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) and is converted into $1,25(OH)_2D$ (9, 57). This metabolite is the active one and its production is tightly feedback-regulated. Its main role is in the regulation of calcium /phosphate and bone homeostasis through genomic activation of number of genes in its target tissues (intestine, bone, kidney, parathyroid gland) (Figure 1).

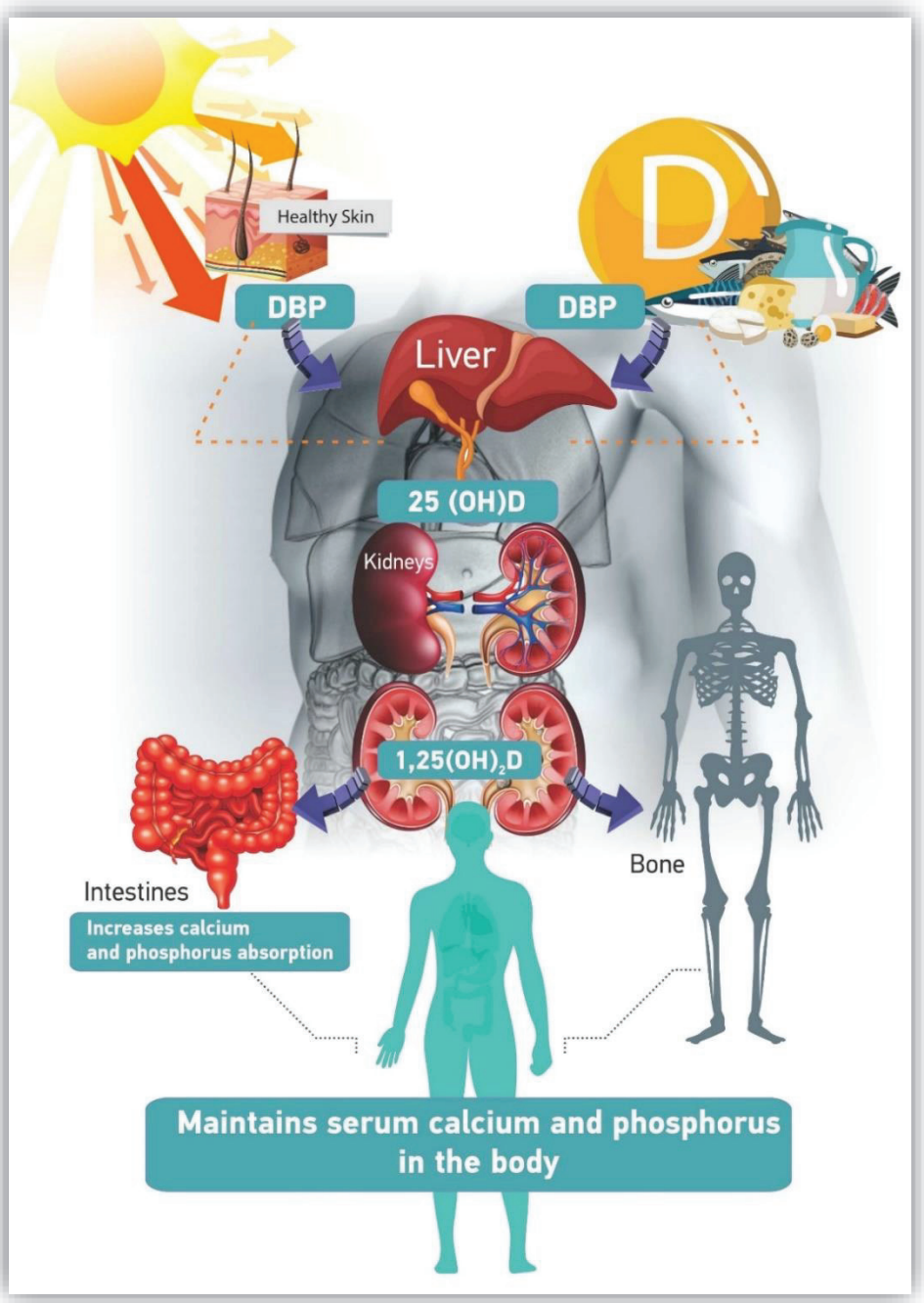

Figure 1. Schematic representation of the synthesis and metabolism of Vitamin D. A description of the production of the active form, $1,25(OH)_2D$, and the regulation of its amount is illustrated.

However, enzyme activity studies using a variety of tissues have revealed that synthesis of $1,25(OH)_2D$ occurs at several key peripheral sites, including the cells of the immune system (58, 59). In contrast with the kidney, which supports the systemic endocrine actions of $1,25(OH)_2D$, the extrarenal metabolite appears to act in an autocrine or paracrine fashion by modulating cell differentiation and/or function at a local level (60).

These actions are mediated by the binding of the $1,25(OH)_2D$ hormone with the nuclear receptor VDR leading to regulation of target gene transcription which in turn through a putative plasma membrane-associated receptor [VDR (mem)] initiates signal transduction pathways and generates rapid biological cellular responses. Regulation of transcriptional activity is cell specific like $1,25(OH)_2D$ inhibiting parathyroid hormone (PTH) secretion but promoting innate immunity, insulin secretion and inhibiting adaptive immunity (61, 62). Due to the wide distribution of the VDR in cells and tissues the biologic action of VitD extends into many systems including the adaptive and the innate immune system (63-65). Since the VDR is expressed in various immune cells, including B and T-lymphocytes as well as antigen presenting cells, is believed to have roles in the improvement of immune function and reduction of inflammation (66). Moreover, the discovery that VitD induces antimicrobial peptide gene expression explains, in part, the 'antibiotic' effect of VitD and has greatly renewed interest in the ability of VitD to improve immune function. Subsequent work indicates that this regulation is biologically important for the response of the innate immune system to infection and that VitD deficiency may lead to suboptimal responses toward bacterial and viral infections (67).

3.3. Sources of Vitamin D

VitD is known as a sunshine vitamin, which is produced either in the skin by exposure to solar ultraviolet B (UVB) radiation or coming from exogenous sources such as diet and/or supplements (68).

3.3.1. Sun exposure

More than 90% of VitD derives from sun exposure and only 10% via dietary sources (69, 70).

VitD is mainly acquired from endogenous production in the skin, upon exposure to solar ultraviolet B (UVB) radiation. UVB rays (290–315 nm) enters the skin and converts 7-dehydrocholesterol (7-DHC) into previtamin D_3 and then to vitamin D_3 (71). Exposure to sunlight, provides 10.000 to 20.000IU in the period of June to October when 30% of the body surface area is exposed to sunlight for 30 min a day (72). UVB rays are most efficient in producing VitD when the sun is most perpendicular to the earth’s surface between 10 AM

and 3 PM. Very little UVB reaches the earth's surface in the early morning or late afternoon hours or even less in the rest of the year. On the other hand, prolonged sun exposure does not result in the production of excess quantities of $VitD_3$ to cause intoxication because sunlight destroys any pre-$VitD_3$ or $VitD_3$ by converting it into inactive photoproducts (70, 73).

Studies have shown that children, especially infants, may require less sun exposure than adults to produce adequate VitD concentrations because of greater surface area to volume ratio (74). A study in 1985 found that 30 minutes of sun exposure of infants in diapers or 2 hours fully clothed without a hat maintained weekly 25(OH)D concentrations of 11ng/mL (75).

However, the global consensus recommendations (76) claim no safe threshold of UV exposure that allows for sufficient VitD synthesis across the population without increasing skin cancer risk, therefore, there are no recommendations available to validate the appropriate duration of sun exposure for infants and young children. Nevertheless, the American Academy of Pediatrics recommends that only children younger than 6 months should be kept out of direct sunlight and protected with clothing and hats (77).

3.3.2. Foods and supplements

Exogenous sources of VitD include foods naturally rich in VitD, VitD fortified foods and VitD supplements.

Dietary sources contain either $VitD_3$ or D_2 but very few food sources naturally contain significant amounts of VitD (Table 1). $VitD_3$ is found in oily fish (salmon, sardines, mackerel and herring), fish liver oils, egg yolk, and some animal products, such as, butter, red meat, liver, fat, and kidney whilst $VitD_2$ can be obtained in small quantities from wild mushrooms (78-80). Therefore, diet alone may provide the most up to 100 to 200IU of VitD per day (81). In exclusively BF infants, VitD concentration reflects that of the mothers. If a mother is VitD deplete, that contributes to VitD deficiency in her infant who also lack sun exposure (82).

VitD food fortification in inadequately sun exposed people seems to be the most appropriate way of improving the status in the general population in order to meet dietary VitD recommendations, and this has already been introduced by some countries.

The infant formula is generally fortified in all countries and in some others the foods, including, milk, orange juice, butter and other products in order to help meet needs. However, the fortification policies vary widely. United States and Canada fortify milk and other products like yogurts and cheese while some European countries fortify also cereals and margarine (Table 1) (73).

Table 1. Dietary Sources of VitD_2 and D_3

Sources		**Vitamin D content (D_2 or D_3)**
Natural sources		
Egg yolk		About 20 IU D_3 or D_2
Salmon	Fresh, wild (3.5oz)	About 600-1000 IU D_3 or D_2
	Fresh, farmed (3.5oz)	About 100-250 IU D_3 or D_2
	Canned (3.5oz)	About 300-600 IU D_3 or D_2
Sardines, canned (3.5oz)		About 300 IU D_3
Mackerel, canned (3.5oz)		About 250 IU D_3
Tuna, canned (3.6 oz)		About 230 IU D_3
Cod liver oil (1tsp)		About 400- 1000 IU D_3
Shiitake mushrooms	Fresh (3.5oz)	About 100 IU D_2
	Sun-dried (3.5oz)	About 1600 IU D_2
Exposure to UVB (0.5 minimal erythemal dose)		About 3000 IU D_3
Fortified foods	**Vitamin D content**	
Breakfast cereals	About 100 IU/serving, usually D_3	
Fortified milk (8 oz)	About 100 IU D_3	
Infant formulas (8 oz)	About 100 IU D_3	
Fortified orange juice (8 oz)	About 100 IU D_3	
Fortified yogurts (8 oz)	About 100 IU usually D_3	
Fortified butter (3.5 oz)	About 50 IU usually D_3	
Fortified margarine (3.5 oz)	About 430 IU usually D_3	
Fortified cheeses (3 oz)	About 100 IU usually D_3	
[(3.5oz=98g), (3.6oz=100.8g), (8oz= 224g), (1tsp=5ml)]		

In Greece most foods are not enriched in VitD, unlike the baby food products that systematically are enriched, as required by relevant legislation. Except from infant formula milk, some margarines, cereals, and some yoghurts are fortified. Even though fortification for some of the other milks had started the previous years, the content is very low 200-300IU per liter while in the United Kingdom is 400IU per quarter (83), and butter is supplemented with 20IU/10g on average (14).

In the USA and Canada cow's milk is fortified while they permit voluntary fortification of other products. Fluid milk, milk alternatives and ready-to-eat-cereals are the major contributors to VitD intake in the USA, while milk and margarine are the major fortified foods in Canada (84). Food fortification is a way to increase the VitD supply to the population although, some authors suggest that the current level of fortification in the USA and Canada is not effective in reaching the required levels of VitD intake (84). In Australia, some foods like margarine is fortified with VitD, whilst some others like milk and milk products are voluntarily fortified (85). In Finland VitD fortification of fluid milks, margarines/fat spreads is on a voluntary, and not mandatory basis, but most companies comply with the option to fortify, resulting in a systematic (mass) VitD fortification (86, 87).

Supplementation with oral VitD has been shown to significantly improve VitD intake across a variety of age, race, ethnic and gender groups, as well as improving VitD status. Oral VitD supplements in different doses are widely available in most countries (88).

3.4. Vitamin D status and reference values

Although $1,25(OH)_2D$ is the active metabolite of VitD, it is not used for determining VitD status. This is due to the fact that the half-life of 25(OH)D is two to three weeks much longer than that of $1,25(OH)_2D$ and $1,25(OH)_2D$ is usually normal or even elevated in patients with VitD deficiency (89). Serum 25(OH)D is the best way for determining individual VitD status as it reflects VitD inputs both from cutaneous synthesis and dietary intake. Also, adequate 25(OH)D levels in the circulation are needed as to cross the cell membrane since a lot of cells like those of the immune system have the ability to produce locally $1,25(OH)_2D$ and act in a autocrine function (90) (Figure 2).

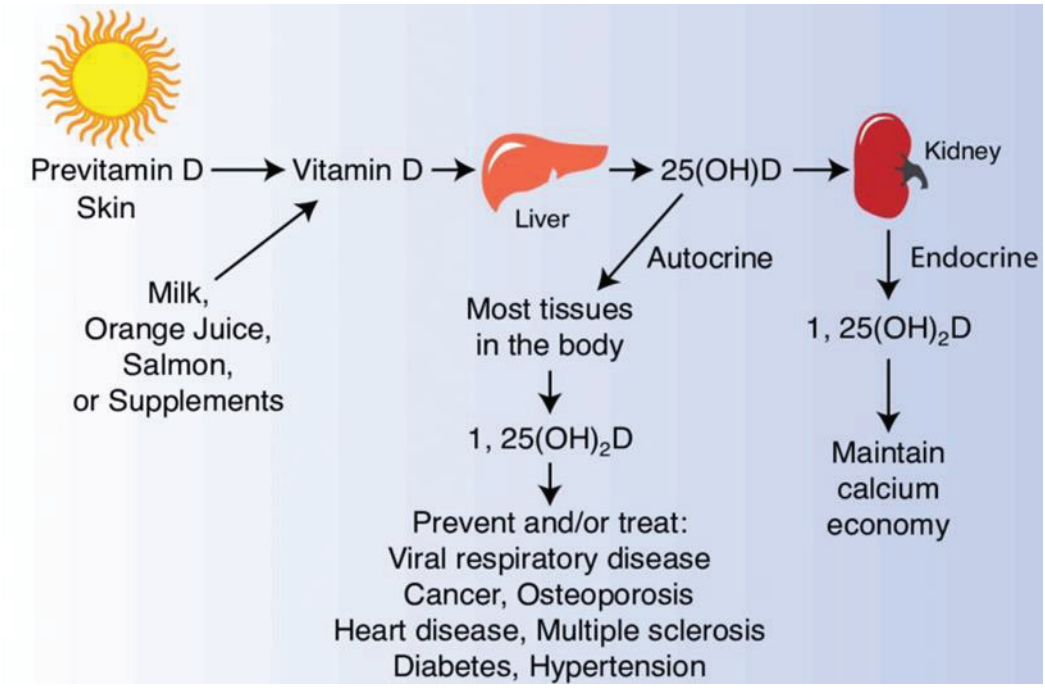

Figure 2. Autocrine and endocrine functions of VitD [adapted from (91)].

However, the reference standards used are not clearly defined. There appears to be a wide variability between lower and upper limits of healthy serum 25(OH)D levels and that is the reason why there is not an international consensus on the optimal concentrations in adults and children.

Internationally, definitions of VitD sufficiency, insufficiency and deficiency vary across different guidelines and there is a lack of consensus on the cut-off points and definitions (sufficiency/adequacy, insufficiency, deficiency, severe deficiency) as summarized in table 2. Moreover, the interpretation of serum 25(OH)D levels is further complicated by the variability in results obtained using different assay methods (80).

Table 2. International guidelines for definitions of VitD status. Societies and Organizations from 2008 until 2019.

Society/ Organization	**25(OH)D Thresholds**
Lawson Wilkins Pediatric Endocrine Society (78)	<5ng/ml: Severe Deficiency <5-14 ng/ml: Deficiency 15- 19 ng/ml: Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
Institute of Medicine (IOM) (92)	- : Severe Deficiency <12 ng/ml: Deficiency 12-20 ng/ml: Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
The Endocrine Society (93)	- : Severe Deficiency <20 ng/ml: Deficiency 21-29 ng/ml: Insufficiency ≥30 ng/ml: Sufficiency/Adequacy
French Society of Pediatrics (94)	- : Severe Deficiency <20 ng/ml: Deficiency - : Insufficiency ≥20 ng/mL: Sufficiency/Adequacy
British Pediatric and Adolescent Bone Group (95)	- : Severe Deficiency <10 ng/ml: Deficiency 10-19 ng/ml: Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
German Nutrition Society (96)	- : Severe Deficiency - : Deficiency - : Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
The Spanish Association of Pediatrics (97)	- : Severe Deficiency <20ng/ml: Deficiency - : Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHN) (98)	<10ng/ml: Severe Deficiency <20ng/ml: Deficiency - : Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
Central Europe (99)	- : Severe Deficiency <20ng/ml: Deficiency 20-29ng/ml: Insufficiency ≥30 ng/ml: Sufficiency/Adequacy

Position Statement- Australia/New Zealand (100)	<5ng/ml: Severe Deficiency 5-11ng/ml: Deficiency 12-19ng/ml: Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
American Academy of Pediatrics (AAP) (101)	- : Severe Deficiency <20ng/ml: Deficiency - : Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
Scientific Advisory Committee on Nutrition (SACN) (80)	- : Severe Deficiency - : Deficiency - : Insufficiency ≥10 ng/ml: Sufficiency/Adequacy
International pediatric consensus recommendations on Rickets (76)	- : Severe Deficiency <12ng/ml: Deficiency 12-19ng/ml: Insufficiency ≥20 ng/ml: Sufficiency/Adequacy
Italian Society of Preventive and Social Pediatrics (102)	<10ng/ml: Severe Deficiency <20ng/ml: Deficiency 20-29ng/ml: Insufficiency ≥30 ng/ml: Sufficiency/Adequacy
Hellenic Endocrinology Society (103)	<12ng/ml: Severe Deficiency 12-20ng/ml: Deficiency - : Insufficiency ≥20 ng/ml: Sufficiency/Adequacy

The UK Scientific Advisory Committee on Nutrition (SACN) considers the 25(OH)D concentration of all individual should not fall below 10ng/mL at any time of the year (80). Levels below that threshold, increases the risk of poor musculoskeletal health based on the majority of reported cases of symptomatic VitD deficiency in children. The IOM recommendations advise that a serum 25(OH)D level of 20ng/mL would cover the requirements of 97.5% of the population even under conditions of minimal sun exposure (104) therefore, it claims < 20ng/ml as VitD insufficiency.

In Greece, the Hellenic Endocrinology Society (HES) published in 2019 the "Therapeutic guidelines for the administration of VitD to the Greek population", which in accordance with IOM recommends that >20 ng/mL is a sufficient limit for children and adults (103).

3.5. Vitamin D toxication (VDT)

VDT can happen either exogenous (iatrogenic) or endogenous. Overdosing due to use of pharmaceutical products is the most frequent cause of exogenous VDT and is a rare event caused accidentally or by prolonged use (months) of VitD mega doses. Toxicity cannot happen by the abnormally high exposure of skin to the sun neither by consumption of foods. The human body can regulate the quantity of pre-VitD produced in the skin by UVB radiation and a diversified diet typically does not provide large amounts of VitD, and the fortification of food products with VitD is modest (105). Endogenous etiologies may develop from ectopic production of $1,25(OH)_2D$ in granulomatous diseases, such as sarcoidosis and tuberculosis, or in lymphoma (106). Although VDT is rare, it can be life threatening if not promptly identified.

The intake at which the dose of VitD supplement is toxic is not clear. Research supported that daily doses of VitD up to 10.000IU were safe in healthy males, and there was no evidence of hypercalcemia or hypercalciuria for 5 months (107, 108) nevertheless, because sunshine can provide an adult with VitD in an amount equivalent to daily oral consumption of 10.000IU, this is intuitively a safe dose (109).

Holick, suggested that doses of more than 10.000IU daily raise 25(OH)D levels more than 150ng/mL and are accompanied with severe hypercalcemia and hypercalciuria (73) and very low or undetectable parathyroid hormone (PTH) activity (105). Nevertheless, the Global Consensus Recommendations state that toxicity can occur when serum 25(OH)D is more than 100ng/mL, with hypercalciuria and suppressed PTH (76).

Despite controversies the available guidelines from IOM and Endocrine Society agree that 25(OH)D concentrations of 100ng/mL is considered to be the upper limit of normal and concentrations above 150ng/mL pose a significant risk of VDT and that VitD deficiency treatment regimens with use of very high doses need regular monitoring (93, 104, 110).

3.6. Dietary guidelines

3.6.1. General current guidelines for VitD intake

VitD supplementation in the first year of life is essential to ensure an adequate VitD status and to prevent nutritional rickets. Newborns and infants are poorly exposed to sunlight, as the AAP recommends that infants younger than 6 months of age should be

kept out of direct sunlight (111). The importance of VitD supplementation during the first year of life has been essential particularly in exclusively BF infants and during the winter season.

Some studies evaluated the effect of daily VitD supplementation at a dosage of 400IU in children during the first year of life and found that it was effective in maintaining serum 25(OH)D levels ≥30 ng/mL (112-115). Moreover, various International Scientific Societies agree to recommend VitD supplementation during the first year of life (76, 78, 94, 98-100, 102, 116). These Societies suggest that the administration of 400IU daily is safe and effective to ensure an adequate VitD status for all infants from birth to 12 months of age independently of their mode of feeding. Additionally, the Endocrine Society (USA) recommended a daily intake of 400–1000IU of VitD in children under 1 year of age at risk for VitD deficiency (93).

As nutritional rickets may develop during the entire pediatric age and VitD deficiency may negatively affect bone health, an adequate 25(OH)D status is also important for older children and adolescents (76, 80, 117).

The IOM dietary reference intakes (DRI) report, together with the European Food Safety Authority (EFSA) dietary reference values (DRV) report, can be regarded as the main nutritional VitD guidelines (104, 117). Similar recommendations have been made by other major health agencies (80, 96) and are listed in Table 3.

VitD guidelines have generally been based on beneficial effects of VitD on musculoskeletal health outcomes (rickets, osteomalacia, fractures, muscle weakness, falls) and occasionally on extra skeletal health outcomes such as pregnancy-related health outcomes or mortality (118). They are based on conditions of minimal or no endogenous VitD synthesis and referred to the general healthy population at different ages.

Table 3. Dietary reference values (DRV) and dietary reference intakes (DRI) for VitD at different ages from different Health Authorities

Country (Health Authority)	USA and Canada (IOM)		UK (SANC)	Europe (EFSA)	Germany, Australia and Switzerland (DACH)	Nordic European Countries (NORDEN)
DRV/DRI	**EAR**	**RDA**	**RNI**	**AI**	**AI**	**RI**
25(OH)D in ng/mL (nmol/L)	16 (40)	20 (50)	10 (25)	20 (50)	20 (50)	20 (50)
Age group	**Vitamin D intakes International units per day (40 IU=1μg)**					
0-6 months	400		340-400		400	
7-12 months	400		340-400	400	400	400
1-5 years	400	600	400	600	800	400
6-17 years	400	600	400	600	800	400
18-69 years	400	600	400	600	800	400
70-74 years	400	800	400	600	800	400
75 years and older	400	800	400	600	800	800
Pregnancy	400	600	400	600	800	400
Lactation	400	600	400	600	800	400

EAR: estimated average requirements, RDA: recommended dietary allowance, RNI: reference nutrient intake, AI: adequate intake, RI: recommended intake

3.6.2. Current guidelines for vitamin D intake in Greece

Greece is a country with abundance of solar radiation, which is a key factor in the synthesis of VitD, while at the same time the consumption of foods with VitD content is present in the Greek-Mediterranean diet. However, research showed that a high percentage of the country's population is insufficient/deficient in VitD (14, 15, 83, 119, 120).

In Greece, the Hellenic Endocrinology Society (HES) has recently published recommendations for clinical care and practice. Concerning the children, it suggests routine screening of 25(OH)D only in the high-risk population. For the prevention of VitD deficiency, it is recommended to all infants, aged 0-12 months, to receive a daily dose of 400IU. Also, for ages > 1 year, the estimated needs are at least 600IU/day coming from endogenous synthesis, food and/or supplements (103). They also emphasize the importance of foods enrichment with calcium and VitD (eg cereals, juices, margarine, etc) and conclude on how invaluable is informing the population about the need for a balanced diet and exercise, as well as safe exposure to the sun.

The exact duration of VitD supplementation has not been established. Since growth velocity is high in the first 2 years, supplementation should be offered to all children younger than 2 years (92, 121)

CHAPTER 4. VITAMIN D DEFICIENCY

4.1. Definition of vitamin D sufficiency/deficiency

The serum concentration that constitutes VitD deficiency is controversial and not well supported by clinical trials, especially in the paediatric population. However, several authors and societies define VitD deficiency as a serum level of 25(OH)D ≤20ng/mL, since this level meets the needs of 97% of the population with regard to bone health (73, 92, 93). Severe VitD deficiency is defined as <10ng/mL 25(OH)D, because below this cut-off the risk of rickets is high (104, 122). The bone-centric guidelines recommend a target 25(OH)D concentration of 20ng/mL, and age-dependent daily VitD doses of 400-800IU. The guidelines focused on pleiotropic effects of VitD recommend a target 25(OH)D concentration of 30ng/mL, and age, body weight, disease-status, and ethnicity dependent VitD doses ranging between 400 and 2000IU/day (123).

4.2. Prevalence of vitamin D deficiency among infants and children

Although VitD is very easy to obtain, the changes of evolution and modernization, clothing, obesity, environment pollution, excessive use of sunscreens, increasing hours spent indoors, has resulted in reduced VitD synthesis. Poor VitD status is increasing and it is estimated that 1 billion people worldwide, of all age groups, suffers from VitD insufficiency or deficiency (73). A large observational study has suggested that 40% of European population (children, teenagers and adults) are VDD, and 13% are severely deficient (124).

Moreover, there has been concern with the evidence that the prevalence of VitD deficiency among infants, toddlers and young infants, across ethnicity and season, is considerably high in many parts of the world (125-128), even in countries with abundant sunshine (15, 83, 129). Among infants, exclusive BF (with or without supplementation) in the setting of maternal VitD deficiency, reduced dietary consumption, and decreased exposure to sunlight are the leading causes of VitD deficiency.

A large study in the United States showed that 9% of the general pediatric population had VitD deficiency and a high percentage of 61% were VitD insufficient (127). The most recent study in infants was from Uttarakhand, a northern state in India. The prevalence of VitD deficiency was 74% in infants and 85.5% in mothers. Nearly half of the infants and mothers had severe VitD deficiency and maternal VitD deficiency was shown to be an important risk factor for the deficiency (130).

In North-Western Greece, VitD deficiency (<20ng/mL) was found at a high rate in exclusively BF infants aged 1 to 6 months especially in the winter season (15). Similarly, in another study, from the sunny region of Athens, the results showed a remarkable rate of deficiency in the group of mother-neonate pairs, even in those who delivered in summer and autumn (14). Another study in the northwest of Greece, reported that a significant percentage of children and adolescents were in high risk for VitD deficiency mostly during winter (83).

4.3. Causes of Vitamin D deficiency

The major cause of VitD deficiency is inadequate exposure to sunlight. Also, there are many factors which affect the extent of cutaneous production of VitD, such as, skin pigmentation, time in sun, seasonality, latitude, time of the day, clothing habits, atmospheric conditions, pollution level, age, use of sunscreen, and outdoor activities (70, 72, 89, 104, 131) (Figure 3).

Additionally, there are factors that affect the amount of circulating VitD. For example, in obesity the release of the synthesized VitD from its cells is hindered making it unavailable in the circulation (132). Medical conditions such as malabsorption disorders, (cystic fibrosis or Crohn's disease), liver disease, and kidney disease, and certain medications like phenobarbital, valproic acid, and ketoconazole, can also hamper VitD circulating levels (81) (Figure 3).

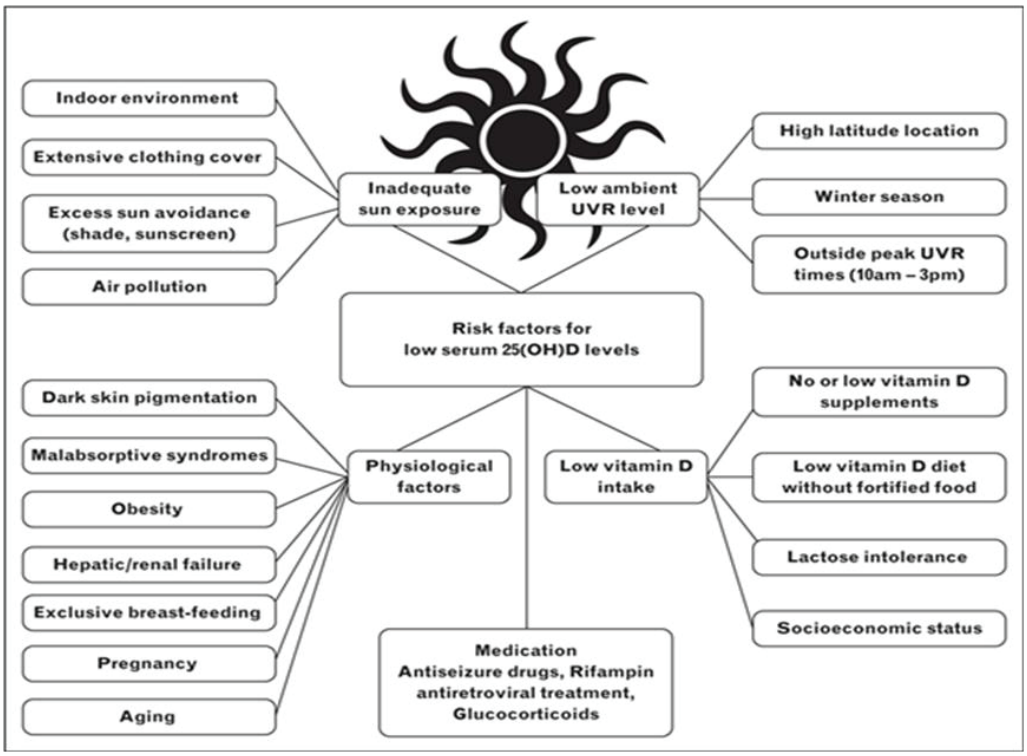

Figure 3. Causes of VitD deficiency, adapted from [(133)].

In infants and young children, most common risk factors associated with VitD deficiency are being born to a VitD deficient mother, dark skin color (ethnicity), prolonged BF without supplements, chronic illnesses or hospitalization and excessive use of sunscreen (74). Moreover, a US study claims that a significant number of infants may be born with VitD deficiency/insufficiency especially if their mothers are non-white race/ethnicity, younger age, and with a history of multiple pregnancies (134).

4.4. Clinical consequences of Vitamin D deficiency in Children

4.4.1. Skeletal effects -Rickets

It is well known that VitD deficiency in the developing skeleton of infants/children is related to rickets, while in adults is related to osteomalacia. In a VitD deficient child, the intestinal absorption of calcium and phosphorus is decreased. The parathyroid glands recognize the low serum concentrations of calcium and release PTH to increase the serum calcium back into a normal range. PTH increases the calcium reabsorption and the phosphorus excretion in the kidneys, while it stimulates bone resorption thus decreasing bone mineralization. When VitD deficiency continues for weeks to months, osteomalacia, stunted growth and rickets may develop (78, 135).

Rickets frequently occurs among children in malnourished populations living in resource-limited countries, however, it can also happen in children in resource-rich countries if exposure to sunlight is limited, if the use of supplements and fortified foods is not sufficient, and in children with chronic illnesses. Children with rickets commonly present with symptoms of bony deformity and muscle weakness. Irritability and impaired growth may also be present. Severe VitD deficiency may give rise to hypocalcemic seizures or tetany, especially in the neonatal period (135).

4.4.2. Non skeletal effects

The past decades it has been reported that long term VitD deficiency starting from fetal life is implicated in disorders other than those of the skeletal system. Many observational studies as well as randomized control trials (RCTs), Systematic reviews and meta-analyses (MAs) have been carried out exploring that relationship (72, 136, 137).

The demonstration that the VitD receptor is expressed in virtually all cells of the body have led to widespread utilization of VitD supplementation for the prevention and treatment of numerous disorders.

Studies in different populations from around the world have described associations between low serum of 25(OH)D and a large number of diseases, including cardiovascular diseases, malignancies, diabetes, obesity, infections, neuropsychiatric, and autoimmune diseases (124, 138-141).

Although VitD has been extensively studied in the forementioned diseases and there are indications that low serum 25(OH)D concentrations might be linked to several diseases, solid conclusions cannot be drawn.

An umbrella review provided comprehensive summary of the published literature in relation to the role of VitD in human diseases and health related traits and concluded that firm universal conclusions about its benefits cannot be drawn. (142). Similarly, a recent systematic review (2017) concluded that results from RCTs and meta-analysis of RCTs, only provide limited support for such effects, as most studies have failed to document significant effects. Nevertheless, few meta-analyses have suggested beneficial effect on respiratory tract infections (n=3), depression (n=1), blood pressure (n=2) and on mortality (n=8) in response to VitD supplementation (143). Hence, more large well-designed clinical trials and dose-response data to test the effects of VitD on chronic disease outcomes including autoimmunity, obesity, diabetes mellitus, hypertension, and heart disease still are needed.

4.4.3. Susceptibility to infections

VitD is believed to have a major role in the improvement of immune function and reduction of inflammation. There are now clear data that its active metabolite $1,25(OH)_2D$ is a hormone that regulates gene expression in multiple signaling pathways apart from those impacting bone mineral density. $1,25(OH)_2D$ has been documented to mediate in the innate and adaptive immune systems and triggers effective antimicrobial pathways against bacterial, viral and fungal pathogens in the cells of the innate immune system (57).

Given its increasingly recognized role as an immunomodulator, studies have begun to explore the relationship between VitD deficiency and the incidence and severity of infections in children and adults. An epidemiologic study has demonstrated a link between VitD

deficiency and increased incidence of respiratory infections (144). A systematic review and meta-analysis reports that VitD deficiency seems to be associated with higher mortality in acute and critically ill children (145).

However, the current body of evidence for association between poor VitD status and susceptibility to infections in the infant age is still insufficient and even more for the 25(OH)D concentrations needed for protection, if any.

4.5. Breastfeeding and vitamin D

Fetuses and newborn's serum 25(OH)D levels are dependent on mother's 25(OH)D status. After birth they depend on breast milk, and/or supplements as sources of VitD for the first few months of life, except those on formula.

As the VitD content of human breast milk is dependent on maternal 25(OH)D status, which is often inadequate, and sun exposure may be restricted for infants, they are particularly vulnerable to VitD deficiency (2). Although it is clear and almost irrefutable that human breast milk is the best nutritive substance for infants during the first year, there has been raised concern about the adequacy in providing enough VitD, especially for infants who are exclusively BF for a prolonged period.

In general, the mean antirachitic activity of human milk in healthy lactating women, unsupplemented or supplemented with VitD, ranges from 10 to 80IU/L (81, 146-148). Henderson (2005) has reported that the intake of the infant is low even on a VitD sufficient mother (15-50IU/L). Exclusively BF infants consuming an average of 750ml daily milk from mothers, from that the intake of VitD comes up to only 10 to 40IU/d in the absence of sun exposure or supplements (149). As BM contains relatively small amounts of VitD, it is therefore, difficult for a BF infant to obtain an adequate intake of 400IU daily, as recommended by the IOM (92) and the problem gets bigger especially when the mother is also VitD deficient (150). That is in agreement with several studies claiming that in exclusively BF infants non- supplementation is an independent predictor of VitD deficiency (151-153).

Other studies that report also high prevalence of VitD deficiency in exclusively BF infants have aroused public concern of whether supplements should be for BF infants or for the lactating mother, in order to minimize the associated health consequences (78, 115, 149,

154). However, the lactating mother requires high doses of VitD to be able to transfer adequate amount to the infant (115). Recent studies suggest that maternal intake of high doses of VitD (4000 to 6400IU/d) may achieve 25(OH)D concentrations in breast milk to provide sufficient VitD supplementation for BF infants even if both mother and infant are limited in sunlight exposure (115, 148). Those doses are much higher than the recommended daily allowance of 600IU suggested by the IOM for lactating women and also significantly higher than the amount of VitD found in standard prenatal or multivitamins which typically provide 400 to 600IU per dose (92).

Studies from US and Europe and various International Scientific Societies suggest that supplementation of BF infants with 400IU daily of VitD meets their requirements and is sufficient to prevent VitD deficiency when sun exposure is limited (93, 115, 121, 148, 154-156).

CHAPTER 5. INFECTIONS IN INFANTS AND YOUNG CHILDREN-CORRELATION WITH VITAMIN D

5.1. Respiratory tract infections (RTIs)

RTIs are considered as a serious cause of morbidity and mortality in early childhood and are a major public health issue in both developed and developing countries (157). Although bacteria and fungi can cause respiratory infections, viruses are the leading cause of infections in infants and young children. The most common viruses among infants and children with RTIs are respiratory syncytial virus (RSV), influenza virus A, influenza virus B, parainfluenza viruses, adenoviruses and coronaviruses (158).

Although TB was the prototypical disease linked with low 25(OH)D concentrations, studies that followed attempted to find an association between infants with upper respiratory tract infections (URTIs), lower respiratory tract infections (LRTIs) and 25(OH)D concentrations but so far, with no unequivocal results.

Some studies agreed in that decreased serum 25(OH)D concentrations were prevalent among most infants and children-patients with RTIs and VitD deficiency was associated with increased rates of RTIs (159-161). This may be a proof that VitD deficiency/ insufficiency, at least in part, contributes to the pathogenesis of several infectious diseases of the respiratory tract in childhood. Also, from the available evidence, normal to high serum 25(OH)D status appears to have some beneficial influence on the incidence and severity of some, but not all types of RTIs and it could be an effective and inexpensive way of prophylaxis (162-164).

Moreover, in some studies, an association was also found between infants with LRTI, cord blood and maternal 25(OH)D concentrations (165-168). These studies show a strong reverse association between cord blood 25(OH)D levels and LRTI presentation within the first years of life. Since cord blood 25(OH)D concentrations are dependent on the mother's concentrations the suggestion is that supplementation during pregnancy would result to higher maternal levels and consequently would have beneficial effects in the prevention and severity of LRTIs in early childhood.

VitD may also have an impact on the continued spread of severe acute respiratory syndrome-coronavirus (SARS-CoV-2), and COVID-19 disease. The risk of morbidity and

mortality was found to be higher for elderly individuals and those with comorbid conditions such as hypertension and diabetes mellitus (169). Nonetheless, children, including very young children, can also develop COVID-19, and sometimes with no symptoms.

With the remarkable potential of VitD, several researchers proposed VitD supplementation could possible play a role in the prevention and/or treatment of COVID-19, by modulating the immune response to the virus both in the adult and pediatric population (169-173).

The National Institute for Health and Care Excellence (NICE), in collaboration with Public Health England and the Scientific Advisory Committee on Nutrition (SACN), published an updated rapid review of recent studies on VitD and COVID-19 for adults, young people and children in hospitals and community settings (174). Their recommendations support the current government advice, for everyone to take VitD supplements (400IU), particularly during winter and spring and in the COVID-19 pandemic, when people may have stayed indoors more than usual. They also agreed that low VitD status was associated with more severe outcomes from COVID-19, which was reinforced by the resent research, in adults and children. In those studies, they found that decreased serum 25(OH)D level could worsen clinical outcomes of COVID-19 patients, while increased serum 25(OH)D level could either mitigate worst outcome or improve clinical outcomes (175-177).

5.2. Gastrointestinal infections-diarrhea

Diarrhea is one of the most common diseases affecting children under the age of five years in developing countries and it is the second most common cause of morbidity and mortality due to an infectious cause in this age group (178). Worldwide, 3-5 billion cases of acute gastroenteritis and nearly 2 million deaths occur each year in children under 5 years (179). Rotavirus gastroenteritis occurs almost exclusively in infants and children, with nearly every child having been infected by the age of 5 years (180) and with majority of serious infections to occur between the age of 4 and 24 months (181). Globally, there is insufficient data on the association between VitD levels and diarrhea in children.

5.3. Urinary tract infection (UTI)

Urinary tract infection is one of the most common infections in infants during the first year of life and the second most common bacterial infection (after RTIs) in childhood. Escherichia coli is the pathogen mostly found in primary cases. Other common uropathogens include Klebsiella, Proteus, Enterobacter, Citrobacter, Staphylococcus saprophyticus, and Enterococcus (182, 183). UTIs occur in 3% to 5% of girls and 1% of boys (184). To date, several studies in children have revealed a link between Vitamin D deficiency and UTI but with no established evidence.

CHAPTER 6. PURPOSE OF THIS STUDY

6.1. Purpose/objective

VitD is known that in addition to skeletal has also many non- skeletal functions like in the immune system. One of the main causes of hospitalization for children are infectious diseases. RTIs are the first reason for hospitalization and intensive care unit admission among infants and young children. Gastroenteritis and urinary tract infections are also common diseases affecting young children and significant causes of hospitalization.

The objective of this study was to examine whether there was any relationship between VitD status and the incidence of RTIs, gastrointestinal and urinary tract infections in Greek children aged less than 2 years by determining their 25(OH)D serum levels on admission to the hospital. In addition, to explore the plausible effects of BF, season and VitD supplementation on the susceptibility to these infections.

CHAPTER 7. PATIENTS AND METHODS

7.1. Patients

The study group included 449 infants and young children [239 male (53.2%), 210 females (46.8%)] aged 28 days to 24 months who were admitted to the Department of Pediatrics of the University Hospital of Ioannina between March 2017 and December 2019. 316 patients with symptoms of common infections (fever, breathing difficulties, cough, vomiting, diarrhea) and 133 clinically healthy infants. They were divided into 5 groups: Group1 with upper RTIs (n=95) such as otitis media, rhinosinusitis, pharyngotonsillitis, influenza, group 2 with lower RTIs (n=99) such as pneumonia and bronchiolitis, group 3 with gastroenteritis (n= 61), group 4 with UTI (n= 61) and group 5 with healthy controls (n= 133). Controls were selected to be matched by age and gender with the patients from infants who attended the hospital pediatric clinic for other reasons such as weight gain deficit, scheduled follow up, pharmaceutical intoxication, nicotine poisoning, court ordered foster children and had no active infection. Exclusion criteria for all groups were skeletal and congenital diseases, genetic syndromes, malabsorptive disorders and prematurity (<36weeks of gestation).

Clinical characteristics like age, gender, mode of delivery, gestational age, birth weight, definitive ICD10 diagnosis, type of nutrition (BF, formula and mixed feeding), supplementation or not with VitD as well as season of admission were recorded after discussion with parents and filling out structured questionnaires.

Blood specimens were collected from all subjects on admission for their routine test and a small sample of serum after centrifugation was stored at -20 ° C until assaying.

7.2. Methods

The concentrations of 25(OH)D in serum were determined by chemiluminescence microparticle immunoassay (CMIA) on the Abbott Analyser i1000 SR. It identifies both the 25(OH)D3 and 25(OH)D2 molecules and the sensitivity of the method is 2.2 ng/mL.

Serum 25(OH)D concentrations ≥20ng/mL were regarded as sufficient and <20ng/mL as deficient. The definition of VitD concentrations was based on the recommendation of the US Institute of Medicine (IOM) for VitD (104).

7.3. Statistical Analysis

Means and standard deviations (SD) were used to describe the scale variables included in the study, such as age and 25(OH)D concentrations. The categorical ones such as diagnosis and VitD deficiency were described with the use of frequencies and percentages. Univariate analysis included the use of the Pearson Chi square statistics to examine associations between diagnosis, and gender, mode of delivery, season and way of nutrition. A multinomial logistic regression model was applied to assess the simultaneous effect of these variables on diagnosis. Differences in the 25(OH)D values by diagnosis, season and type of nutrition were assessed applying Analysis of Variance followed by multiple comparisons under the Tukey's HSD criterion. Statistical significance was set at 0.05 in all cases and the analysis was carried out with the use of the SPSS v.23.0 software.

CHAPTER 8. RESULTS

8.1. Characteristics of patients and controls

The clinical characteristics of all subjects are shown in Table 4. Only in 8 subjects, data for the delivery mode are missing due to lack of communication.

Patients with URTI. Fifty were male and 45 females. The delivery mode was caesarean section for 46 infants (49.5%) and vaginal birth for 47 (50.5%). Their mean age was 12.5 months (± 8.1 SD), their mean gestational age 38.5 weeks (± 1.1 SD) and their mean birth weight was 3137 g (± 404 SD).

Patients with LRTI. Sixty-one were male and 38 females. The delivery mode was caesarean section for 55 infants (56.7%) and vaginal birth for 42 (43.3%). Their mean age 9.6 months (± 7.6 SD), their mean gestational age 38.4 weeks (± 1.1 SD) and their mean birth weight was 3161 g (± 402 SD).

Patients with Gastroenteritis. Thirty-five were male and 26 females. The delivery mode was caesarean section for 34 infants (57.6%) and vaginal birth for 25 (42.4%). Their mean age 11.8 months (± 7.5 SD), their mean gestational age 38.6 weeks (± 1.1 SD) and their mean birth weight was 3186 g (± 424 SD).

Patients with UTI. Twenty-five were male and 36 females. The delivery mode was caesarean section for 36 infants (59.0%) and vaginal birth for 25 (41.0%). Their mean age was 7.8 months (± 6.1 SD), their mean gestational age 38.5 weeks (± 1.3 SD) and their mean birth weight was 3121 g (± 457 SD).

Controls. Sixty-eight were male and 65 females. The delivery mode was caesarean section for 77 infants (58.8%) and vaginal birth for 54 (41.2%). Their mean age 9.9 months (± 7.2 SD), their mean gestational age 38.3 weeks (± 1.1 SD) and their mean birth weight was 3128 g (± 463 SD).

Overall, there were no significant differences in the characteristics among the four study groups (URTI, LRTI, Gastroenteritis, UTI) or with the controls with respect to gender,

(p=0.129) delivery mode (p=0.368), gestational age (p=0.676) and birth weight (p=0.898). With respect to age a difference was found only between the URTI and UTI groups (p=0.001), with that of URTI being higher (Table 4).

Table 4. Clinical characteristics of the infants with upper and lower respiratory infections, gastroenteritis, urinary tract infections and controls

	URTI	LRTI	Gastroenteritis	UTI	Controls	p
Total number	95	99	61	61	133	
Age (months, mean ± SD)	12.5 ± 8.1[a]	9.6 ± 7.6	11.8 ± 7.5	7.8 ± 6.1[b]	9.9 ± 7.2	0.001*
Males (%)	50 (52.6%)	61 (61.6%)	35 (57.4%)	25 (41.0%)	68 (51.1%)	0.129
Females (%)	45 (47.3%)	38 (38.3%)	26 (42.6%)	36 (59.0%)	65 (48.9%)	
Delivery Mode: Caesarian section	46 (49.5%)	55 (56.7%)	34 (57.6%)	36 (59.0%)	77 (58.8%)	0.368
Vaginal birth	47 (50.5%)	42 (43.3%)	25 (42.4%)	25 (41.0%)	54 (41.2%)	
Gestational age (Weeks, mean ± SD) (median)	38.5 ± 1.1 (38)	38.4 ± 1.1 (38)	38.6 ± 1.1 (38)	38.5 ± 1.3 (38)	38.3 ± 1.1 (38)	0.676
Birth Weight (g, mean ± SD) (median)	3137 ± 404 (3100)	3161 ± 402 (3150)	3186 ± 424 (3150)	3121 ± 457 (3130)	3128 ± 463 (3100)	0.898

a vs b p=0.001: Comparisons of the age between the URTI and UTI infected infants

Also, no differences were observed in the mean circulating levels of 25(OH)D between the infection groups and controls for the year round as shown in Table 5. The concentrations were 33.4 ± 19.6, 30.0 ± 15.2, 31.6 ± 15.7 and 32.3 ± 12.7 ng/ml for the infection groups URTI, LRTI, gastroenteritis and UTI and 33.3 ± 16.3 ng/ml for the controls (p=0.559). The rates of vitamin D deficiency did not differ between the groups either (p=0.589).

Table 5. The mean circulating levels of 25(OH)D in all groups and the rates of vitamin D deficiency through the year

Diagnosis N	25(OH)D (ng/ml) mean ± SD (Median)	Vitamin D deficiency (≤ 20ng/ml) n (%)
URTI **(95)**	33.4 ± 19.6 (29.5)	23/95 (24.2%)
LRTI **(99)**	30.0 ± 15.2 (27.9)	24/99 (24.2%)
Gastroenteritis **(61)**	31.6 ± 15.7 (28.3)	11/61 (18%)
UTI **(61)**	32.3 ± 12.7 (32.6)	8/61 (13.1%)
Controls **(133)**	33.3 ± 16.3 (31)	25/133 (18.8%)
p	0.559	0.589

8.2. The relationship of the infection rates with respect to vitamin D status and season

The distribution of the infants from each group (URTI, LRTI, Gastroenteritis, UTI and controls) in the two seasons, their mean serum levels of 25(OH)D in the two seasons and the rates of vitamin D deficiency are shown in Tables 6 and 7. As shown in Table 6 the distribution of infants in the two seasons was different (p=0.000). Different also was the frequency of VitD deficiency between groups (p=0.002) (Table 7). However, the mean circulating levels did not differ between the 5 groups either for the summer/autumn period (p=0.880) or for the winter/spring season (0.376) (Table 6).

Patients with URTI. Forty-one patients (43.2%) were admitted in comparable rates during the winter/spring period and 54 (56.8%) in summer/autumn and their distribution did not differ from those of controls (p=0.529) (Table 6). The 25(OH)D levels between the two seasons were also comparable (31.3 ± 17.3 vs 35.0 ± 21.3 ng/ml, (p=0.358) (Table 6). The frequency of vitamin D deficiency between seasons did not differ either. Only 11 children (26.8%) were deficient during the winter/spring period vs 12 (22.2%) in summer/autumn (p=0.604) (Table 7).

Patients with LRTI. The admission rates in the two seasons were significantly different, being higher in winter/spring 75.8% vs 24.2% in summer/autumn and the distribution was also different from controls (p=0000) (Table 6). However, the 25(OH)D mean levels did not show any significant difference between the two periods (29.6 ± 16.4 vs 31.3 ± 10.6 ng/ml, p=0.638 (Table 6). A tendency for more children to be vitamin D deficient in the winter/spring (28%) than in the summer/autumn period (12.5 %) was observed but again it was not statistically significant (p=0.123) (Table 7).

Patients with Gastroenteritis. Twenty-two patients (36.1%) were admitted during the winter/spring period and 39 (63.9%) during summer/autumn. Although they were more infants admitted in the hospital with this infection in the summer/autumn period their distribution between seasons did not differ from those of controls (p=0.142) (Table 6). However, their 25(OH)D levels were found significantly different between the two periods. But they were lower in the winter/spring season (25.0 ± 10.3) compared to 35.3 ± 17.1 ng/ml in the summer/autumn (p=0.014) (Table 6). However, the rates of vitamin D deficiency between seasons did not differ. Only 6 children (27.3%) were deficient during the winter/spring period vs 5 (12.8%) in summer/autumn (p=0.909) (Table 7).

Patients with UTI. Thirty-six patients (59.0%) were admitted during the winter/spring period and 25 (41.0%) during summer/autumn with no significant difference between the two seasons p= 0.133 from controls (Table 6). The 25(OH)D levels between the two seasons were comparable (30.8 ± 14.0 vs 34.5 ± 10.6 ng/ml, (p=0.277) (Table 6). The rates of vitamin D deficiency between seasons did not differ. Only 7 children (19.4%) were vitamin D deficient in the winter/spring vs 1 (4.0%) in summer/autumn period (p=0.265) (Table 7).

Controls. Distribution of children selected as controls were similar between the two time periods (winter/spring and summer/autumn, 47.3% vs 52.7%). Also, the 25(OH)D levels were comparable between the two periods (33.1 ± 18.9 vs 33.6 ± 13.7 ng/ml, p=0.854) (Table 6), but there was a trend of vitamin D deficiency to be at a higher rate in the winter/spring period (25.4% vs 12.9%, p=0.065) (Table 7).

Table 6. The means ± SD (median) of 25(OH)D concentrations in the circulation of the infants with infections and controls with respect to season

Diagnosis (N)	N (%)	p	p between all groups	Season	25(OH)D (ng/ml) (mean ± SD) (Median)	p for 25(OH)D between seasons
URTI (95)	41 (43.2%)	0.529	0.000*	Winter/Spring	31.3 ± 17.3 (29.1)	0.358
	54 (56.8%)			Summer/Autumn	35.0 ± 21.3 (33.3)	
LRTI (99)	75 (75.8%)	0.000*		Winter/Spring	29.6 ± 16.4 (26.6)	0.638
	24 (24.2%)			Summer/Autumn	31.3 ± 10.6 (28.8)	
Gastroenteritis (61)	22 (36.1%)	0.142		Winter/Spring	25.0 ± 10.3 (25.3)	0.014*
	39 (63.9%)			Summer/Autumn	35.3 ± 17.1 (31.4)	
UTI (61)	36 (59.0%)	0.133		Winter/Spring	30.8 ± 14.0 (31.1)	0.277
	25 (41.0%)			Summer/Autumn	34.5 ± 10.6 (32.7)	
Controls (133)	63 (47.3%)			Winter/spring	33.1 ± 18.9 (30)	0.854
	70 (52.7%)			Summer/autumn	33.6 ± 13.7 (33.1)	

p=0.376: Comparison of serum 25(OH)D levels between all 5 groups for the winter/spring season

p=0.880: Comparison of serum 25(OH)D levels between all 5 groups for the summer/autumn season

Table 7. The rates of vitamin D deficiency with respect to season

Diagnosis (N)	Season	Vitamin D deficiency 25(OH)D ≤ 20ng/ml	p between all groups	p For Vit-DD between seasons
URTI (95)	Winter/Spring	N=11 (26.8%)	0.002*	0.604
	Summer/Autumn	N=12 (22.2%)		
LRTI (99)	Winter/Spring	N=21 (28.0%)		0.123
	Summer/Autumn	N=3 (12.5%)		
Gastroenteritis (61)	Winter/Spring	N=6 (27.3%)		0.909
	Summer/Autumn	N=5 (12.8%)		
UTI (61)	Winter/Spring	N=7 (19.4%)		0.265
	Summer/Autumn	N=1 (4.0%)		
Controls (133)	Winter/spring	N=16 (25.4%)		0.065
	Summer/autumn	N=9 (12.9%)		

8.3. The impact of nutrition mode on the infection rates

Another factor tested was how the feeding type (BF, bottle and mixed) affected the 25(OH)D circulating levels which in turn might have a role in the infection rates. As shown in Table 8 the type of feeding did not differ between all groups (p=0.130). However, there was a trend for significance in the duration of exclusive BF time (months) between the groups (p=0.060). The longer was in the URTI and Gastroenteritis groups with median times 10 and 7 months respectively and shorter in the LRTI, UTI and controls (4 months). At the same time the percentages of infants on exclusive BF were similar for all 5 groups (29.5% URTI, 28.3% LRTI, 28.3% Gastroenteritis, 34.4% UTI and 23.3% Controls).

Also, the serum levels of 25(OH)D did not differ between patient and control groups with respect to feeding type (p=0.668 for BF, p=0.266 for bottle and p=0.415 for mixed). This may be due to the vitamin D supplementation strategy suggested for all infants.

Patients with URTI. Twenty-eight patients (29.5%) were exclusively BF, 59 (62.1%) were bottle fed and 8 (8.4%) were on mixed feeding. The mean 25(OH)D levels between the three types of feeding were comparable with no significant differences between them (35.1 ± 26.8 vs 33.0 ± 15.9 vs 29.8 ± 16.2 ng/ml, p=0.785) (Table 8). The mean time of exclusively BF was 9.56 ± 7.09 months.

Patients with LRTI. Twenty-eight patients (28.3%) were exclusively BF, 68 (68.7%) were bottle fed and 3 (3%) were on mixed feeding. The mean 25(OH)D levels did not show any significant difference between the three types of feeding either (28.7 ± 22.4 vs 30.6 ± 11.3 vs 28.0 ± 9.28 ng/ml, p=0.840) (Table 8). The mean time of exclusively BF was 6.54 ± 5.98 months.

Patients with Gastroenteritis. Eleven patients (28.3%) were exclusively BF, 42 (68.7%) were bottle fed and 3 (3.0%) were on mixed feeding. The mean 25(OH)D levels between the three types of feeding were comparable with no significant differences between them (30.8 ± 24.6 vs 30.3 ± 12.1 vs 39.1 ± 18.0 ng/ml, p=0.358) (Table 8). The mean time of exclusively BF was 7.91 ± 5.8 months.

Patients with UTI. Twenty-one patients (34.4%) were exclusively BF, 32 (52.5%) were bottle fed and 8 (13.1%) were on mixed feeding. The mean 25(OH)D levels did not show any significant difference between the three types of feeding either (28.0 ± 14.5 vs 35.3 ± 11.5 vs 31.8 ± 9.8 ng/ml, p=0.124) (Table 8). The mean time of exclusively BF was 4.81 ± 5.82 months.

Controls. Thirty-one children (23.3%) were exclusively BF, 88 (66.1%) were bottle fed and 14 (10.5%) were on mixed feeding. The mean 25(OH)D levels between the three types of feeding were different (p=0.029). The difference was significant between the BF and mixed infants p=0.043 with the latter being lower (BF=27.3 ± 17.4 ng/ml, vs mixed=40.1 ± 15.9) (Table 8). The mean time of exclusively BF in this group was 5.61 ± 5.58 months.

Table 8. The mean 25(OH)D levels in all five groups with respect to their feeding type and the duration in months of exclusive B/F.

Diagnosis (n)	Type of feeding	n (%)	P between all groups	25(OH)D (ng/ml) (mean ± SD) (Median)	p†	Months of BF Mean ± SD (Median)	p††
URTI (95)	B/F	28 (29.5%)	0.130	35.1 ± 26.8 (33.3)	0.785	9.56 ± 7.09 (10)	0.060
	Bottle	59 (62.1%)		33.0 ± 15.9 (30.1)			
	Mixed	8 (8.4%)		29.8 ± 16.2 (25.4)			
LRTI (99)	B/F	28 (28.3%)		28.7 ± 22.4 (21)	0.840	6.54 ± 5.98 (4)	
	Bottle	68 (68.7%)		30.6 ± 11.3 (28.8)			
	Mixed	3 (3.0%)		28.0 ± 9.28 (26.6)			
Gastroenteritis (61)	B/F	11 (28.3%)		30.8 ± 24.6 (27.0)	0.358	7.91 ± 5.8 (7)	
	Bottle	42 (68.7%)		30.3 ± 12.1 (28.1)			
	Mixed	3 (3.0%)		39.1 ± 18.0 (40.0)			
UTI (61)	B/F	21 (34.4%)		28.0 ± 14.5 (27.3)	0.124	4.81 ± 5.82 (4)	
	Bottle	32 (52.5%)		35.3 ± 11.5 (33.7)			
	Mixed	8 (13.1%)		31.8 ± 9.8 (33.2)			
Controls (133)	B/F	31 (23.3%)		27.3 ± 17.4 (24.8)	0.029 (Mixed vs BF 0.043)	5.61 ± 5.58 (4)	
	Bottle	88 (66.1%)		34.4 ± 15.5 (31.6)			
	Mixed	14 (10.5%)		40.1 ± 15.9 (37.8)			

Comparisons of the 25(OH)D concentrations between all groups with respect to feeding type: *p=0.668 for BF, p=0.266 for bottle and p=0.415 for mixed*

† p for 25(OH)D levels between type of feeding

† †p for months of BF between all groups

8.4. The impact of vitamin D supplementation on the circulating 25(OH)D levels

The impact of vitamin D supplementation on the 25(OH)D levels in patients and controls are shown in Table 9. In all 5 groups the supplemented infants were over twice the non-supplemented and did not differ between them (p=0.118). But there were differences in the circulating 25(OH)D levels except in the UTI and the trend in the LRTI group.

Patients with URTI. Fifty-two (54.7%) patients were supplemented with vitamin D and 43 (45.3%) not. Hence the mean serum 25(OH)D levels in the supplemented infants were significantly higher compared to the rest (38.8 ± 21.9 vs 26.8 ± 14.0 ng/ml p=0.002) (Table 9).

Patients with LRTI. Similar rates of vitamin D supplementation were found in this group of patients too. Fifty-four (54.5%) patients were supplemented with vitamin D and 45 (45.5%) not. Here however, the 25(OH)D mean levels were not significantly different among the two groups, but they had a trend to be higher in the supplemented group (32.5 ± 12.8 vs 27.0 ± 17.2 ng/ml p=0.074) (Table 9).

Patients with Gastroenteritis. In this group the supplemented patients were nearly twice as many. Thirty-eight (62.3%) patients were supplemented with vitamin D and 23 (37.7%) not. As expected, the 25(OH)D mean levels were significantly higher in the supplemented group (34.9 ± 16.1 vs 26.0 ± 13.6 ng/ml p=0.032) (Table 9).

Patients with UTI. In this patient group again, the supplemented ones were more than twice than the rest. Forty-five (73.8%) patients were supplemented with vitamin D and 16 (26.2%) not. However, the 25(OH)D mean levels were comparable in the two groups with no significant difference (33.1 ± 11.9 vs 30.3 ± 15.0 ng/ml p=0.453) (Table 9).

Controls. In the control group the rates between supplemented infants were not much different. Seventy-seven children (57.8%) were supplemented and 56 (42.2%) not. But the serum levels of 25(OH)D in the supplemented infants were significantly higher than in the non- supplemented (36.7 ± 17.6 vs 28.8 ± 13.3 ng/ml p=0.006) (Table 9).

Table 9. The numbers and percentages of infants supplemented or not with vitamin D, and their 25(OH)D circulating levels (Means ± SD).

Diagnosis (n)	Vit-D intake	n (%)	p between all groups	25(OH)D (ng/ml) (mean ± SD) (Median)	p†
URTI (95)	No	43 (45.3%)	0.118	26.8 ± 14.0 (25.8)	0.002*
	Yes	52 (54.7%)		38.8 ± 21.9 (34.2)	
LRTI (99)	No	45 (45.5%)		27.0 ± 17.2 (25.4)	0.074
	Yes	54 (54.5%)		32.5 ± 12.8 (28.9)	
Gastroenteritis (61)	No	23 (37.7%)		26.0 ± 13.6 (24.7)	0.032*
	Yes	38 (62.3%)		34.9 ± 16.1 (31.2)	
UTI (61)	No	16 (26.2%)		30.3 ± 15.0 (31.9)	0.453
	Yes	45 (73.8%)		33.1 ± 11.9 (32.7)	
Controls (133)	No	56 (42.2%)		28.8 ± 13.3 (28.2)	0.006*
	Yes	77 (57.8%)		36.7 ± 17.6 (35.7)	

† p for 25(OH)D levels between supplemented and not supplemented groups

CHAPTER 9. DISCUSSION AND CONCLUSIONS

9.1. Discussion

9.1.1. Vitamin D status and infections

VitD deficiency has been suggested to be associated with a susceptibility to infections (13). Despite the interest and several studies, the results are still unequivocal. In the present study, we found comparable mean circulating levels of 25(OH)D in all infection groups and controls. Also, the incidence of VitD deficiency, as defined by 25(OH)D levels <20 ng/ml, was not much different in the groups of children with common childhood infections aged 1 month to 2 years than the controls. It is the first study in Greece evaluating the association between serum 25(OH)D levels and infections in this age group. Sick infants admitted with either lower or upper RTIs, Gastroenteritis, UTI and healthy controls had similar VitD levels through the year.

RTIs: In the two groups of infants with RTIs we did not find any differences in their mean circulating levels of VitD. In agreement with our results McNally et al., (185) found no significant difference in the VitD levels between the LRTI group and controls. However, they reported that the children admitted to the pediatric intensive care unit had significantly lower levels than either controls or of the less severe ones treated in the general pediatric department. The authors suggested that LRT disease severity might be associated with VitD due to its immunomodulatory properties. Another study from Canada (186) showed that for infants less than 25 months old and with a 25(OH)D threshold of 32 ng/ml, there was no association with the risk of hospitalization for acute LRTI (mainly bronchiolitis). In addition, the 25(OH)D concentrations were similar in cases and controls in agreement with our results. Another study from Turkey did not find significant correlation between VitD levels and LRTI in terms of disease and severity (187), while here the rates of VitD deficiency/insufficiency were high in both case and control groups in contrast to the Canadian one.

However, there are several studies showing low VitD levels to be associated with URTI and LRTI and that VitD deficiency is a significant risk for RTIs (160, 188, 189).

Roth et al (2010) reported an association of VitD deficiency and LRTI in early childhood and that unadjusted odds of LRTI were halved for each increase of 10 nmol/L in 25(OH)D (160). Garg et al. (2016) observed that the rates of LRTIs were much higher in the VitD

deficient and insufficient children under 5 years old compared to the VitD sufficient ones (189). Wayse et al. (2004), (188) also observed for children under 5 years of age that the odds ratio for severe LRTI was significantly lower when 25(OH)D levels were >22.5 nmol/L and with exclusive BF within the first months.

In our study we found no difference between either the two infection groups or with controls in the rates of VitD deficiency (25(OH)D <20 ng/mL).

Gastroenteritis: Some studies carried out in Columbia, Egypt, Pakistan and Saudi Arabia showed VitD deficiency to be associated with an increased incidence of diarrhea/ recurrent diarrhea in infants and school-aged children (190-193). Also a prospective study in Turkey enrolled children less than 4 years, and found that serum VitD levels were significantly lower in the patient group than in the healthy control group ($p<0.001$) showing that low VitD may be a potential risk factor for rotaviral diarrhea for children of this age (194).

In contrast, in our study we found no difference between this infection group and controls in the 25(OH)D circulating levels as well as in the rates of VitD deficiency through the year. Similarly a study in Tanzania although reported a high prevalence of VitD deficiency in children under the age of five, with a majority 53.7% being VitD deficient, 34.0% insufficient and only 12.2% sufficient, VitD deficiency was not found to be specifically associated with diarrhea (195).

UTI: In our study we found no difference on serum 25(OH)D between UTI patients and control group. Our findings are in accordance with a most recent study on children, between 2 and 14 years old, where again failed to detect any meaningful association between UTI and VitD level (196). However, in the same study a prevalence of infection was reported higher in girls than boys, which is a known risk factor, and agrees with our observations as more than half of our patients (59%) were female too.

This is in accordance with some studies that found significantly lower 25 (OH)D levels in children with UTI compared to controls and supports that VitD deficiency is a risk factor for UTI and especially in girls (12, 197-199).

Also, in a Swedish study the authors reported that 21% of the children in their study had VitD deficiency or insufficiency despite prescribed daily VitD supplementation during the

first two years of life. Thus, they concluded that inadequate VitD levels may be a risk factor for the first UTI in girls (200). In our study the rate of infants with VitD deficiency came up to about 13% but it did not differ from controls.

9.1.2. Impact of season

On examining whether the season had a role in the incidence of infections and in relation to VitD levels we found that the rates of LRTIs were significantly higher in the winter/spring compared to the summer/autumn months (p=0.000). Our results are in line with other studies. Outbrakes of respiratory infections reach a peak in the winter months, when the weather is cold, the people get crowded indoors and there is a decrease of sunlight (the main source of VitD), which is one of the factors for VitD deficiency. The nadir is in the summer months when the weather is hot, people get outdoors and get more sunlight as well as ventilate their homes (13, 201, 202). However, we did not find any difference in the 25(OH)D levels between seasons.

Many epidemiologic studies have demonstrated strong associations between seasonal variations in serum 25(OH)D and the incidence of infectious diseases in newborns and young infants. A trial in Iowa found that VitD deficiency was much more common among BF infants during winter than in summer (152). An earlier study from Greece found that the incidence of infants born with very low 25(OH)D levels was higher in the winter, even though quite a few had also low levels in the summer months (15).

No seasonal variation we found either in the circulating 25(OH)D levels of the infants with RTIs and UTI even though there was a significant difference in the rate of LRT infections. This is probably attributed to the fact that more than half of the infants in all groups were supplemented with VitD. The last years in Greece it is recommended to supplement infants from the day of birth irrespective of the feeding method. The current recommendation from the American Academy of Pediatrics is that all infants and children should have a minimum daily intake of 400IU of VitD beginning soon after birth. The same recommendation is given by many health professionals including the Hellenic Endocrinology Society in Greece (103). This also may be an explanation as to why the infection rates in our study were found to be independent of VitD status.

A significant difference was found only in the serum 25(OH)D in the infants with Gastroenteritis (p=0.014) were 25(OH)D in summer/autumn was significantly higher than winter/spring. But unexpectedly the incidence of Gastroenteritis was higher in the summer/autumn season than in winter/spring.

In general, rotavirus is known to cause diarrheal illness throughout the year but predominantly during winter months especially in countries with temperate climates however, several studies have shown higher rotavirus gastroenteritis incidence during the period between January and June (203) Moreover, a study with children less than 5years old, hospitalized with acute gastroenteritis, found that regarding the monthly distribution of cases, the highest number was clearly observed during the warm period especially between July and August (92 and 91cases, respectively) of hospitalized children (204) something that agrees with our results.

9.1.3 Impact of breast feeding

Another factor suggested to have a protective effect against infections is the breastfeeding (BF). Studies and health organizations support that exclusive BF and its duration is associated with reductions in the risk of common infections during the first years of life and beyond infancy (30, 205-209). In addition, they claim that, longer exclusive BF results in fewer infectious episodes and hospitalizations during infancy.

However, our analysis showed no such effect on the infection rates between all groups, with respect to the type of feeding that was comparable to controls. One explanation might be that the length of exclusive BF was not long enough, in most cases less than 6 months, while some studies claim it to be enough and others not.

In a Dutch study, infants who were breastfed exclusively until the age of 4 months, and partially thereafter, had lower risks for respiratory and gastrointestinal infections until the age of 6 months (30). On the contrary, in a large UK cohort, (208) infants who were exclusively BF for a period of <4 or 4-6 months and stopped BF by 6 months had a significantly increased risk of chest infection and diarrhea. In our study the infants on exclusive BF or mixed feeding were relatively small in numbers, while those on bottle were over half which again may obscure the results.

Studies also state that breastfed infants have lower levels of serum 25(OH)D, than formula milk feeding infants even when supplemented with 400IU of VitD daily (210, 211). In contrast our results showed that the serum levels of 25(OH)D did not differ between patients and control groups with respect to feeding type. It seems that 400IU daily (which is the recommended VitD supplementation strategy for all infants in Greece) is sufficient in raising serum 25(OH)D above the cut off limit of 20ng/ml, regardless of feeding type.

In Greece the recommended VitD supplementation strategy is in accordance with the AAP recommendations that any breast-fed or formula-fed infant should be supplemented with at least 400IU of VitD, at least until the infant is able to consume 1L per day of milk.

9.1.4. Vitamin D supplementation

Another factor examined in this study was whether VitD supplementation had any effect on the infections.

RTIs: Some randomized controlled trials evaluated the effect of one bolus or short-term high dose VitD supplementation in infants and children with LRTI (212-216). These studies did not provide strong evidence of benefits in preventing or reducing the time of recovery from the infections and that was despite the deficient states of the infants and children coming from underdeveloped countries and the significant increase in 25(OH)D levels after supplementation.

However, one study claimed a reduction in the risk of LRTIs in winter and it was in Mongolian schoolchildren with very low median 25(OH)D levels (<7 ng/ml) (216). Another study by Sarhan et al (2019) who studied infants less than 24 months old with bronchiolitis and VitD deficient/insufficient found that supplementation led to less time of hospitalization (217).

We did not examine the severity of the infections or the duration of hospitalization, but we did not see any difference in the rates of infection between those on VitD supplementation and those not. Despite the difference in their mean 25(OH)D levels that were significantly lower in the unsupplemented infants, the rates of infections were similar.

Gastroenteritis: In one study the hospitalized children with acute diarrhea in Dhaka, Bangladesh were randomized to daily supplementation with 1000IU VitD (n=27) or placebo (n=15) (218). Supplementation was not found to reduce stool weight or duration to clinical resolution. The small sample size and short duration of follow-up (5 days) make it difficult to draw conclusions from this study on the role of VitD supplementation. Similarly, in another study VitD supplementation with 1200IU daily in school-age children did not reduce the incidence of gastroenteritis, a secondary outcome of that trial (219).

A more sufficiently powered randomized controlled trial in young children was conducted in Kabul. Among children at high risk, 1 to 29 months of age, quarterly supplementation with 100.000IU of VitD had no effect on the risk for diarrheal illnesses (220). In our study also those who were infected were twice as many in the supplemented group despite their higher 25(OH)D levels. Hence it did not seem to have any effect.

UTI: For infants and young children results on VitD supplementation in UTIs are very scarce. A study suggested that VitD can have some beneficial effect on children with UTI through increasing cathelicidin level as detected in their urine. It was found that children with UTI and sufficient serum 25(OH)D levels had higher cathelicidin than the controls who also had sufficient 25(OH)D serum levels. On the contrary, UTI patients with VitD insufficiency were not found to increase urine cathelicidin levels which helps the body's defensive system. The authors concluded that supplementation with VitD may have an enhancing role on top of the conventional antibiotics prescribed for UTIs in children (221).

Contrary to the forementioned, other studies did not find any relationship between 25(OH)D and UTIs. A study in Egypt has evaluated the effect of VitD supplementation (400IU/daily) for 6 months on the immunity and risk of infections including UTI during the 1st year of life among 99 full term infants. Their results showed no differences in the frequency of UTI in infants supplemented with VitD and those who were not (222). Similarly, another study found that even a daily dose of 1000IU has not significant impact on preventing recurrent UTI in pediatric patients (223). Moreover, other studies believe that supplementation of VitD increases the risk of UTI in young children and its administration should be done with caution (224, 225).

These last studies are in line with our findings as we also observed that the supplemented infants with UTI were nearly 3 times more than the unsupplemented ones despite the fact that the circulating 25(OH)D levels were similar.

In our study, we found that the recommended dose used in Greece (400IU/d) was adequate in raising 25(OH)D levels in most cases to normal levels in all 5 groups. So, the mean serum 25(OH)D was higher in the supplemented infants compared with the non-supplemented ones except in the case of UTI where the levels were similar and independent of the supplementation. However, this difference in the VitD status did not affect the incidence of the infections.

Hence, so far, the evidence is very weak as to recommend routine VitD supplementation to infants and young children for preventing the incidence of common childhood infections. However, studies of longer duration and the dose of supplementation, that are lacking at present, may be of some help in defining or not any biological benefit.

9.2. Conclusions

A) The majority of infants and young children admitted to a general hospital in North-Western Greece are not VitD deficient due to the implementation of exogenous VitD intake (400IU/d) right from birth especially for the breastfed ones.

B) VitD was not different in the children with common infections from controls leading on a VitD independent effect on the infection rates.

C) No seasonal variation found in the circulating VitD levels of the infants with RTIs and UTIs even though there was a significant difference in the rate of LRT infections. A seasonal variation was found only in the circulating VitD levels of the infants with gastroenteritis. No difference was found in the rates of VitD deficiency between seasons in each group.

D) Breastfeeding again, did not seem to have any positive effect on the infection rates. However, the infants on exclusive BF were relatively small in numbers for applicable evaluations and also showing that in Greece the percentage of BF is very low. Moreover, the serum levels of 25(OH)D did not differ between patients and control groups with respect to feeding type, most likely because of the supplementation.

E) No difference was found in the rates of infection between VitD supplemented and unsupplemented infants. Nevertheless, the mean serum 25(OH)D levels were significantly higher in the supplemented infants compared with the non-supplemented ones.

However, adequacy of VitD should continue to be kept as a goal for keeping normal levels in these infants even in a sunny country like Greece due to its many well-known non-skeletal effects and especially its immunomodulatory functions.

ABSTRACT

Purpose: Childhood infections are one of the main reasons for hospitalization and intensive care unit admission among infants and young children. VitD is known to have except the skeletal, and non- skeletal functions especially in the immune system. The objective of this study was to determine any association between VitD status with the determination of serum 25 hydroxyvitamin D levels [25(OH)D] and common infections (respiratory tract infections, gastroenteritis and urinary tract infections) in hospitalized children aged less than 2 years. As a second objective was to explore the possible effects of BF, season and VitD supplementation on the susceptibility of infants to common infections.

Methods: Children under 2 years of age hospitalized during a 2-year period with common infections such as otitis media, bronchiolitis, pneumonia, gastroenteritis, and urinary tract infections were recruited. A group of age and gender-matched clinically healthy children were included as controls. Structured questionnaires were used to attain information concerning clinical characteristics like age, gender, gestational age, birth weight, mode of delivery and types of nutrition (BF, formula and mixed feeding) supplementation or not with VitD as well as season of admission.

Serum 25(OH)D concentrations were measured and were compared between groups and controls for exploring the first aim. Subsequently there was subgrouping with regard to VitD sufficiency and deficiency (≥ 20ng/ml or <20ng/ml), with respect to season, types of feeding and VitD supplementation. For the analysis of the data comparisons between groups were used a multinomial logistic regression model, for examining confounder factors. A p-value ≤ 0.05 was considered statistically significant.

Results: The study group consisted of 316 cases (95 URTIs, 99 LRTIs, 61 gastroenteritis and 61 UTIs) and 133 healthy controls.

There were no significant differences in the characteristics among the four study groups (URTI, LRTI, gastroenteritis, UTI) or with the controls with respect to gender, (p=0.129) delivery mode (p=0.368), gestational age (p=0.676) and birth weight (p=0.898). With respect to age a difference was found only between the URTI and UTI groups (p=0.001), with that of URTI being higher.

The circulating 25(OH)D (mean ± SD) levels were similar among URTI, LRTI, gastroenteritis and UTI patients and controls (33.4 ± 19.6, 30.0 ± 15.2, 31.6 ± 15.7, 32.3 ± 12.7 and 33.3 ± 16.3 ng/ml, respectively). The majority of the participants in all 5 groups had sufficient 25(OH)D levels: 72/95 (75.8%), 75/99 (75.8%), 50/61 (82%), 53/61 (86.9%) and 108/133 (81.2%) respectively.

With respect to season the mean circulating 25(OH)D levels did not differ between the 5 groups either for the summer/autumn period (p=0.880) or for the winter/spring season (p=0.376) even though there was a significant difference in the rate of infections (p=0.000).

A significant difference was found only in the serum 25(OH)D in the infants with gastroenteritis (p=0.014) where the 25(OH)D level in the summer/autumn period was significantly higher than that of the winter/spring (35.3 ± 17.1 vs 25.0 ± 10.3). But unexpectedly the incidence of gastroenteritis was higher in the summer/autumn (63.9%) than in the winter/spring period (36.1%). Also, in the patients with LRTIs the admission rates between the two seasons were significantly different, being higher in winter/spring 75.8% vs 24.2% in summer/autumn.

The frequency of VitD deficiency between seasons did not differ between all 5 groups. 11 children (26.8%) were deficient during the winter/spring period vs 12 (22.2%) in summer/autumn (p=0.604) in URTI group. In the LRTI group a tendency for more children to be deficient was found in the winter/spring (28%) than in the summer/autumn period (12.5 %) approaching statistical significance (p=0.123). Only 6 children (27.3%) were deficient during the winter/spring period vs 5 (12.8%) in summer/autumn (p=0.909) in gastroenteritis group and 7 (19.4%) deficient in the winter/spring vs 1 (4.0%) in the summer/autumn period (p=0.265) in the UTI group.

In the control group there was a trend of VitDD to be at a higher rate in the winter/spring period but again only approaching statistical significance (25.4% vs 12.9%, p=0.065).

The serum levels of 25(OH)D did not differ between patient and control groups with respect to feeding type (p=0.668 for BF, p=0.266 for bottle and p=0.415 for mixed). The percentages of infants on exclusive BF were similar for all 5 groups (29.5% URTI, 28.3% LRTI, 28.3% gastroenteritis, 34.4% UTI and 23.3% controls). BF was not found to have any effect on the infection rates between groups.

In all 5 groups the supplemented infants were over twice the non-supplemented and did not differ between them (p=0.118). Nevertheless, the serum levels of 25(OH)D in the supplemented infants were significantly higher than in the non-supplemented only in URTI (38.8 ± 21.9 vs 26.8 ± 14.0 ng/ml p=0.002), gastroenteritis (34.9 ± 16.1 vs 26.0 ± 13.6 ng/ml p=0.032) and control (36.7 ± 17.6 vs 28.8 ± 13.3 ng/ml p=0.006) groups. In the LRTI and UTI groups the 25(OH)D mean levels were not significantly different between supplemented and non-supplemented group (32.5 ± 12.8 vs 27.0 ± 17.2 ng/ml p=0.074 and 33.1 ± 11.9 vs 30.3 ± 15.0 ng/ml p=0.453 respectively).

Conclusions:

- The majority of infants and young children admitted to a general hospital in North-Western Greece are not VitD deficient probably due to the implementation of exogenous VitD intake (400IU/d) right from birth especially for the breastfed ones.
- VitD was not different in the children with common infections from controls suggesting a VitD independent effect on the infection rates examined.
- No differences were found in the serum levels of VitD in infants with infections and in controls between the two periods (seasons), except for gastroenteritis, while there was a significant difference in the incidence of infections and especially of the LRTIs where it was greater in winter/spring. In gastroenteritis, despite the higher 25(OH)D in summer/autumn, there was a higher incidence of disease than in winter/spring.
- Regarding comparisons between the frequency of infants with VitD deficiency or not, no differences were found between the infected and control groups.
- Exclusive BF does not seem to have the best effect on infection rates between groups. However, the number of infants under exclusive BF was relatively small to be adequately evaluated and also shows that in Greece the percentage of BF is very low. In addition, serum 25(OH)D levels did not differ between patients and controls with respect to the type of feeding, most likely due to VitD supplementation.
- No difference in infection rates was found between infants receiving and those not receiving VitD supplementation. Nevertheless, 25(OH)D levels were significantly higher in infants receiving supplementation.

ΠΕΡΙΛΗΨΗ

Σκοπός: Οι παιδικές λοιμώξεις αποτελούν το συχνότερο αίτιο εισαγωγής στο νοσοκομείο και σε μονάδες εντατικής θεραπείας σε βρέφη και μικρά παιδιά. Η βιταμίνη D όπως είναι γνωστό έχει εκτός από τις σκελετικές και έξω-σκελετικές δράσεις ειδικά στο ανοσοποιητικό σύστημα.

Κύριος σκοπός αυτής της μελέτης ήταν να διερευνηθεί όποια σχέση μπορεί να υπάρχει μεταξύ των επιπέδων της υδρόξυ-βιταμίνης D [25(OH)D] στον ορό και των κοινών λοιμώξεων (λοιμώξεις αναπνευστικού, γαστρεντερικού και ουροποιητικού συστήματος) σε παιδιά κάτω των 2 ετών κατά την εισαγωγή τους στο νοσοκομείο. Παράλληλα σε δεύτερο επίπεδο ήταν και η διερεύνηση πιθανών επιδράσεων του αποκλειστικού μητρικού θηλασμού (ΜΘ), της εποχής αλλά και της χορήγησης συμπληρώματος βιταμίνης D στην εμφάνιση των υπό μελέτη λοιμώξεων.

Μέθοδος: Βρέφη και μικρά παιδιά ηλικίας κάτω των 2 ετών νοσηλεύτηκαν κατά τη διάρκεια μιας περιόδου 2 ετών με κοινές παιδικές λοιμώξεις όπως ωτίτιδα, βρογχιολίτιδα, πνευμονία, γαστρεντερίτιδα και λοιμώξεις του ουροποιητικού συστήματος. Ως ομάδα ελέγχου (μάρτυρες) χρησιμοποιήθηκαν μια ομάδα κλινικά υγιών παιδιών παρόμοιας ηλικίας και φύλου. Χρησιμοποιήθηκαν δομημένα ερωτηματολόγια για την καταγραφή κλινικών χαρακτηριστικών όπως ηλικία, γένος, ηλικία κύησης, βάρος γέννησης, είδος τοκετού και είδος διατροφής (αποκλειστικός μητρικός θηλασμός, βρεφικό γάλα, μικτή διατροφή), συμπλήρωμα ή όχι βιταμίνης D, όπως επίσης και η εποχή κατά τη νοσηλεία. Οι συγκεντρώσεις της 25(OH)D στον ορό μετρήθηκαν και συγκρίθηκαν μεταξύ ασθενών και μαρτύρων. Στη συνέχεια, έγινε διερεύνηση της επάρκειας και ανεπάρκειας βιταμίνης D (≥ 20ng/mL ή <20ng/mL αντίστοιχα), καθώς επίσης και διερεύνηση της επίδρασης του ΜΘ, της εποχής αλλά και της χορήγησης συμπληρώματος βιταμίνης D στην εμφάνιση των υπό διερεύνηση λοιμώξεων. Για την ανάλυση των δεδομένων μεταξύ των ομάδων χρησιμοποιήθηκε ένα μοντέλο πολυεθνικής λογιστικής παλινδρόμησης και η τιμή του $p \leq 0,05$ θεωρήθηκε ως στατιστικά σημαντική.

Αποτελέσματα: Η ομάδα μελέτης αποτελούνταν από 316 ασθενείς (95 με λοίμωξη ανώτερου αναπνευστικού, 99 με λοίμωξη κατώτερου αναπνευστικού, 61 με γαστρεντερίτιδα, 61 με λοιμώξεις ουροποιητικού) και 133 υγιείς μάρτυρες.

Δεν υπήρχαν σημαντικές διαφορές στα χαρακτηριστικά μεταξύ των τεσσάρων ομάδων μελέτης (λοιμώξεις ανώτερου και κατώτερου αναπνευστικού, γαστρεντερίτιδα και λοιμώξεις ουροποιητικού) ή με τους μάρτυρες ως προς το φύλο, (p=0,129), είδος τοκετού (p=0,368), ηλικία κύησης (p=0,666) και βάρος γέννησης (p=0,898). Όσον αφορά την ηλικία, διαπιστώθηκε διαφορά μόνο μεταξύ των ομάδων με λοίμωξη του ανώτερου αναπνευστικού και λοιμώξεις του ουροποιητικού (p=0,001), με αυτή της πρώτης να είναι υψηλότερη.

Τα επίπεδα της μέσης τιμής της 25(OH)D (μέσος όρος ± SD) ήταν παρόμοια μεταξύ των ασθενών με λοίμωξη ανώτερου και κατώτερου αναπνευστικού, γαστρεντερίτιδας, λοιμώξεων ουροποιητικού και μάρτυρες (33,4 ± 19,6, 30,0 ± 15,2, 31,6 ± 15,7, 32,3 ± 12,7 και 33,3 ± 16,3 ng/mL, αντίστοιχα). Η πλειοψηφία των συμμετεχόντων και στις 5 ομάδες είχαν επαρκή επίπεδα 25(OH)D: 72/95 (75,8%), 75/99 (75,8%), 50/61 (82%), 53/61 (86,9%) και 108/133 (81,2%) αντίστοιχα.

Όσον αφορά την εποχή, ο μέσος όρος της 25(OH)D δεν διέφερε μεταξύ των 5 ομάδων ούτε κατά την περίοδο καλοκαίρι/φθινόπωρο (p=0,880) ούτε κατά την περίοδο χειμώνα/άνοιξη (p=0,376) παρόλο που βρέθηκε σημαντική διαφορά στη συχνότητα μεταξύ των λοιμώξεων (p=0,000).

Σημαντική διαφορά της 25(OH)D βρέθηκε μόνο στα βρέφη με γαστρεντερίτιδα (p=0,014) όπου η τιμή της 25(OH)D το καλοκαίρι/φθινόπωρο ήταν σημαντικά υψηλότερη από ότι το χειμώνα/άνοιξη (35,3 ± 17,1 έναντι 25,0 ± 10.3). Αν και, απροσδόκητα, η συχνότητα εμφάνισης γαστρεντερίτιδας ήταν υψηλότερη το καλοκαίρι/φθινόπωρο (63,9%) από ότι την περίοδο χειμώνα/άνοιξη (36,1%).

Επίσης, σε ασθενείς με λοίμωξη του κατώτερου αναπνευστικού τα ποσοστά εισαγωγής μεταξύ των δύο εποχών είχαν σημαντική διαφορά. Υψηλότερα το χειμώνα/άνοιξη 75,8% έναντι 24,2% το καλοκαίρι/φθινόπωρο.

Η ανεπάρκεια βιταμίνης D μεταξύ των εποχών δεν διέφερε μεταξύ των 5 ομάδων ξεχωριστά. 11 παιδιά (26,8%) είχαν ανεπάρκεια κατά την περίοδο χειμώνα/άνοιξη έναντι 12 (22,2%) το καλοκαίρι/φθινόπωρο (p=0,604) στην ομάδα με λοίμωξη ανώτερου αναπνευστικού. Στην ομάδα με λοίμωξη κατώτερου αναπνευστικού, βρέθηκε μία τάση για περισσότερα παιδιά να παρουσιάζουν ανεπάρκειας βιταμίνης D την περίοδο χειμώνα/άνοιξη (28%) από ό, τι την περίοδο καλοκαίρι/φθινόπωρο (12,5%), αλλά η διαφορά δεν ήταν στατιστικά σημαντική (p = 0,123). Στην ομάδα της γαστρεντερίτιδας

μόνο 6 παιδιά (27,3%) είχαν ανεπάρκεια κατά την περίοδο χειμώνα/άνοιξη έναντι 5 (12,8%) το καλοκαίρι/φθινόπωρο (p = 0,909) και στην ομάδα με λοιμώξεις ουροποιητικού μόνο 7 παιδιά (19,4%) είχαν ανεπάρκεια το χειμώνα/άνοιξη έναντι 1 (4,0%) την περίοδο καλοκαίρι/φθινόπωρο (p = 0,265). Στους μάρτυρες επίσης, υπήρχε μια τάση ανεπάρκειας την περίοδο χειμώνα/άνοιξη που όμως δεν έφτανε στατιστική σημαντικότητα (25,4% έναντι 12,9%, p=0,065).

Τα επίπεδα της 25(OH)D δεν διέφεραν μεταξύ των ομάδων ασθενών και μαρτύρων σε σχέση με το είδος διατροφής (p=0,668 για ΜΘ, p=0,266 για βρεφικό γάλα και p=0,415 για μικτή διατροφή). Τα ποσοστά βρεφών σε αποκλειστικό ΜΘ ήταν παρόμοια και για τις 5 ομάδες (29,5% στις λοιμώξεις ανώτερου αναπνευστικού, 28,3% στις λοιμώξεις κατώτερου αναπνευστικού, 28,3% στις γαστρεντερίτιδες, 34,4% στις λοιμώξεις ουροποιητικού και 23,3% στα βρέφη μάρτυρες). Ο ΜΘ δεν βρέθηκε να συσχετίζεται με την εμφάνιση των λοιμώξεων που εξετάστηκαν.

Σχετικά με την εξωγενή χορήγηση βιταμίνης D, τα βρέφη υπό συμπλήρωμα είχαν διπλάσια επίπεδα από αυτά που δεν λάμβαναν και δεν διέφεραν μεταξύ των ομάδων (p=0,118). Παρ 'όλα αυτά, μελετώντας ξεχωριστά την κάθε ομάδα βρέθηκε ότι στα βρέφη υπό συμπληρωματική αγωγή, τα επίπεδα της 25(OH)D ήταν σημαντικά υψηλότερα από ό,τι στα βρέφη χωρίς συμπλήρωμα στις ομάδες με λοίμωξη του ανώτερου αναπνευστικού (38,8 ± 21,9 έναντι 26,8 ± 14,0 ng/mL p=0,002), στη γαστρεντερίτιδα (34,9 ± 16,1 έναντι 26,0 ± 13,6 ng/mL p=0,032) και στους μάρτυρες (36,7 ± 17,6 έναντι 28,8 ± 13,3 ng/mL p=0,006). Στις υπόλοιπες δύο ομάδες με λοίμωξη του κατώτερου αναπνευστικού και του ουροποιητικού, δεν βρέθηκαν σημαντικές διαφορές στα επίπεδα της 25(OH)D (32,5 ± 12,8 έναντι 27,0 ± 17,2 ng/mL p=0,074 και 33,1 ± 11,9 έναντι 30,3 ± 15,0 ng/mL p=0,453 αντίστοιχα).

Συμπεράσματα:

- Η πλειοψηφία των βρεφών και των μικρών παιδιών που νοσηλεύτηκαν σε γενικό νοσοκομείο της Νοτιοδυτικής Ελλάδας δεν έχουν ανεπάρκεια βιταμίνης D πιθανώς λόγω της εξωγενούς πρόσληψης βιταμίνης D (400IU/ημ) από τις πρώτες μέρες γέννησή τους, ειδικά για αυτά που θηλάζουν αποκλειστικά.

- Η βιταμίνη D δεν διέφερε στα παιδιά με τις λοιμώξεις που μελετήσαμε από ότι στα παιδιά μάρτυρες που δείχνει ότι η βιταμίνη D δεν φαίνεται να έχει επίδραση στη συχνότητα αυτών των λοιμώξεων.

- Δεν βρέθηκαν διαφορές στα επίπεδα βιταμίνης D στον ορό των βρεφών με λοιμώξεις και στους μάρτυρες μεταξύ των δύο περιόδων (εποχές), εκτός της γαστρεντερίτιδας, ενώ υπήρξε σημαντική διαφορά στη συχνότητα των λοιμώξεων και ειδικότερα του κατώτερου αναπνευστικού όπου ήταν πιο μεγάλη την περίοδο χειμώνα/άνοιξη. Στη γαστρεντερίτιδα παρά την υψηλότερη 25(OH)D το καλοκαίρι/φθινόπωρο υπήρξε και ψηλότερη συχνότητα νόσησης απ' ότι το χειμώνα/άνοιξη.

- Σχετικά με συγκρίσεις μεταξύ της συχνότητας των παιδιών με ανεπάρκεια βιταμίνης D ή μη, δε βρέθηκαν διαφορές μεταξύ των ομάδων ασθενών και μαρτύρων.

- Ο αποκλειστικός ΜΘ δεν φαίνεται να επηρεάζει προς το καλύτερο τα ποσοστά των λοιμώξεων μεταξύ των ομάδων. Ωστόσο ο αριθμός των βρεφών υπό αποκλειστικό ΜΘ ήταν σχετικά μικρός για να μπορέσει να αξιολογηθεί επαρκώς και να αμφισβητήσει τα πλεονεκτήματα του ΜΘ επίσης, δείχνει ότι στην Ελλάδα το ποσοστό ΜΘ είναι πολύ χαμηλό. Επιπλέον, τα επίπεδα της 25(OH)D στον ορό δεν διέφεραν μεταξύ των ασθενών και μαρτύρων σε σχέση με το είδος της σίτισης πιθανότατα λόγω λήψης συμπληρώματος βιταμίνης D.

- Δεν βρέθηκε διαφορά στα ποσοστά λοίμωξης μεταξύ παιδιών που λάμβαναν και αυτών που δεν λάμβαναν συμπλήρωμα βιταμίνης D. Παρ 'όλα αυτά, τα επίπεδα 25(OH)D ήταν σημαντικά υψηλότερα στα παιδιά που λάμβαναν συμπλήρωμα.

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ΠΑΡΑΡΤΗΜΑ

	Ημερομηνία:
	Θάλαμος :
	Αρ. αρχείου:
	Ημ. Εισαγωγής:

ΕΡΩΤΗΜΑΤΟΛΟΓΙΟ

Όνομα (Name):	**Γένος ☐ Α ☐ Θ** **Ημ. Γενν (DOB):**		**Εθνικότητα:** **Ηλικία:**
Ηλικία γέννησης (weeks) :	**Τοκετός: ☐ ΦΤ ☐ ΚΤ**	**Βάρος Γενν:**	**Τόπος διαμονής:**
Θηλασμός:	**Ξένο γάλα:**	**Μικτή διατροφή:**	**Στερεές τροφές:**
Σχόλια για θηλασμό :			
Ο παιδίατρος κατά την έξοδο από το μαιευτήριο έδωσε οδηγία για λήψη βιτ-D? ☐ Ναι ☐ Όχι **Ποσότητα/ημέρα:**			
Λήψη βιτ-D τώρα: ☐ Ναι ☐ Όχι	**Ποσότητα/ημέρα:**		**Σκεύασμα:** D3 fix
Συμπτώματα εισαγωγής στο νοσοκ:			
Αγωγή που λαμβάνει:			
Τελική Διάγνωση (ICD-10):			

ΑΤΟΜΙΚΟ ΙΣΤΟΡΙΚΟ ΥΓΕΙΑΣ

ΕΜΒΟΛΙΑ

Ηπατίτιδας Β (Hep B)	**Δόσεις:**	**Ιλαράς/Παρωτίτιδας/Ερυθράς (MMR)**	**Δόσεις:**
Διφθερίτιδας/Τετάνου/Κοκκύτη(DTaP)	**Δόσεις:**	**Ανεμοευλογιάς (Var)**	**Δόσεις:**
Πολυομυελίτιδας (IPV)	**Δόσεις:**	**Ηπατίτιδας Α (Hep A)**	**Δόσεις:**
Αιμοφίλου Ινφλ. Β	**Δόσεις:**	**Φυματινοαντίδρασης (Mantoux)**	**Δόσεις:**
Μηνιγγιτιδόκοκκου C (MCC)	**Δόσεις:**	**Φυματίωσης (BCG)**	**Δόσεις:**
Πνευμονιόκοκκου (PCV)	**Δόσεις:**	**Μηνιγγιτιδόκοκκου (Β)**	**Δόσεις:**

Παιδικές Ασθένειες: ☐ Ανεμευλογιά ☐ Ερυθρά ☐ Ιλαρά ☐ Οστρακιά ☐ Κοκκύτης ☐ Παρωτίτιδα ☐ Πολιομυελίτιδα

Άλλο Πρόβλημα υγείας (Άσθμα/Αλλεργίες/Αναιμία):

Λήψη φαρμάκων/ βιταμίνες/ εισπνεόμενα:

Έκθεση στον ήλιο: ☐ ΝΑΙ ☐ ΟΧΙ **Πόσες φορές/ημέρα :** **Διάρκεια:**

Εισαγωγή για άλλο λόγο στο νοσοκομείο ☐ ΝΑΙ ☐ ΟΧΙ

Ηλικία	Λόγος Εισαγωγής	Νοσοκομείο

Λήψη βιτ-D : **Επίπεδα : 25(OH)D**
Ημ/νία λήψης : **1,25 (OH)D**

Επιμελητής που παρακολουθεί το παιδί:

Ειδικευόμενος :

ΟΙΚΟΓΕΝΕΙΑΚΟ ΙΣΤΟΡΙΚΟ ΥΓΕΙΑΣ

Ονομ/μο Μητέρας:		**Ηλικία:**	**Εθνικότητα :**
Επίπεδο μόρφωσης : ☐ PhD ☐ MSc ☐ ΠΕ ☐ ΤΕ ☐ ΔΕ ☐ΥΕ ☐ ΆΛΛΟ			
Εργάζεστε: ☐ ΝΑΙ ☐ ΟΧΙ	**Επάγγελμα:**		
Εγκυμοσύνες:	**Αριθμός Τέκνων:**		
Θηλάζετε τώρα: ☐ ΝΑΙ ☐ ΟΧΙ			
Λήψη βιτ- D στην εγκυμοσύνη:			
Κάπνισμα: ☐ ΝΑΙ ☐ ΟΧΙ **Αριθμός/ημέρα**		**Κάπνισμα κατά την εγκυμοσύνη: ☐ ΝΑΙ ☐ ΟΧΙ**	
Προβλήματα υγείας:			
Λήψη φαρμάκων:			
Ονομ/μο Πατέρα:		**Ηλικία:**	**Εθνικότητα :**
Επίπεδο μόρφωσης : ☐ PhD ☐ MSc ☐ ΠΕ ☐ ΤΕ ☐ ΔΕ ☐ΥΕ ☐ ΆΛΛΟ			
Εργάζεστε: ☐ ΝΑΙ ☐ ΟΧΙ	**Επάγγελμα:**		
Κάπνισμα: ☐ ΝΑΙ ☐ ΟΧΙ	**Αριθμός/ημέρα**		
Προβλήματα υγείας:			

<u>Αδέλφια</u>

Γένος	**Ηλικία**	
☐ Α ☐ Θ		
☐ Α ☐ Θ		
☐ Α ☐ Θ		

Τηλ επικοινωνίας:

ΕΓΓΡΑΦΟ ΣΥΓΚΑΤΑΘΕΣΗΣ ΓΙΑ ΣΥΜΜΕΤΟΧΗ ΣΕ ΕΡΕΥΝΗΤΙΚΗ ΜΕΛΕΤΗ

ΙΔΡΥΜΑ: ΠΓΝΙ (Παιδιατρική Κλινική)

ΤΙΤΛΟΣ ΜΕΛΕΤΗΣ: Συσχέτιση του μητρικού θηλασμού και των επιπέδων βιταμίνης D με την προδιάθεση για λοιμώξεις στη βρεφική ηλικία

ΕΙΣΑΓΩΓΗ

Σας ενημερώνουμε με σκοπό να λάβει μέρος το παιδί σας σε μία επιδημιολογική μελέτη της Παιδιατρικής Κλινικής του ΠΓΝΙ. Αρχικά σας γνωστοποιούμε ότι η μελέτη είναι εθελοντική. Σε κάθε περίπτωση το παιδί σας δε θα χάσει κανένα από τα δικαιώματά του σε ό,τι αφορά την ιατρική του περίθαλψη. Πριν αποφασίσετε να λάβετε μέρος παρακαλούμε να συζητήσετε μαζί μας οποιαδήποτε απορία σας.

ΣΚΟΠΟΣ ΤΗΣ ΜΕΛΕΤΗΣ

Κατά τη διάρκεια της βρεφικής ηλικίας οι λοιμώξεις αποτελούν το συχνότερο αίτιο εισαγωγής σε νοσοκομείο. Πρωταρχική σημασία για την προστασία του βρέφους αλλά και την ομαλή ανοσοποιητική ωρίμανση έχει ο μητρικός θηλασμός. Άλλοι ενισχυτικοί παράγοντες είναι η εφαρμογή εμβολιασμών, η σωστή υγιεινή, η επαρκής διατροφή μετά τη διακοπή του θηλασμού και τα επίπεδα βιταμίνης D. Τα τελευταία χρόνια έχει αναδειχθεί η ευεργετική επίδραση της βιταμίνης D στην ωρίμανση αλλά και ενίσχυση της αμυντικής λειτουργίας των βρεφών.

Σκοπός της μελέτης είναι: α) η καταγραφή των λοιμώξεων σε βρέφη που θα νοσηλευτούν κατά τη διάρκεια 2 ετών στην Παιδιατρική Κλινική, β)η καταγραφή του είδους της βρεφικής διατροφής, γ) η καταγραφή της προληπτικής χορήγησής της, δ)η εκτίμηση των επιπέδων της βιταμίνης D στο αίμα, ε) η συσχέτιση των επιπέδων βιταμίνης D με την εμφάνιση λοιμώξεων αλλά και με το είδος της λοίμωξης, στ) η συσχέτιση των επιπέδων βιταμίνης D με τον μητρικό θηλασμό και τη χορήγησή της, ζ) η καταγραφή της ενημέρωσης και ευαισθητοποίησης των γονέων αλλά και των παιδιάτρων για τη σημασία του θηλασμού και της βιταμίνης D.

Τα αποτελέσματα της μελέτης πρόκειται να δημοσιευτούν σε ιατρικά περιοδικά ή/και σε επιστημονικά συνέδρια, χωρίς να κατονομάζονται τα άτομα που συμμετείχαν.

ΣΥΓΚΑΤΑΘΕΣΗ ΓΟΝΕΩΝ/ΚΗΔΕΜΟΝΩΝ

Ο υπογεγραμμένος γονέας/κηδεμόνας……..δηλώνω υπεύθυνα ότι ενημερώθηκα πλήρως από τη μαία ……σε ότι αφορά τη σκοπιμότητα της παρούσας μελέτης στην οποία θα λάβει μέρος το παιδί μου……..και δίνω τη συγκατάθεσή μου να συμμετέχει σε αυτή.

Ημερομηνία……/……/……..…… Υπογραφή γονέα/κηδεμόνα

Όνομα ολογράφως της μαίας που λαμβάνει τη συγκατάθεση

Υπογραφή