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Department of Midwifery

Τίτλος εργασίας

**Συμπλήρωμα Βιταμίνης D κατά τη διάρκεια
της κύησης σε Ελληνίδες μητέρες**

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Diploma Thesis

Title

**Vitamin D supplementation during
pregnancy in Greek population**

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**ΠΑΝΕΠΙΣΤΗΜΙΟ ΔΥΤΙΚΗΣ
ΑΤΤΙΚΗΣ ΣΧΟΛΗ
ΤΜΗΜΑ**

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Μέλη Εξεταστικής Επιτροπής συμπεριλαμβανομένου και του Εισηγητή

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Η κάτωθι υπογεγραμμένη Τσιάρα Ελένη του Νικολάου, με αριθμό μητρώου 18039 φοιτήτρια του Πανεπιστημίου Δυτικής Αττικής της Σχολής ΣΕΥΠ του Τμήματος Μαιευτικής, δηλώνω υπεύθυνα ότι:

«Είμαι συγγραφέας αυτής της μεταπτυχιακής εργασίας και ότι κάθε βοήθεια την οποία είχα για την προετοιμασία της είναι πλήρως αναγνωρισμένη και αναφέρεται στην εργασία.

Επίσης, οι όποιες πηγές από τις οποίες έκανα χρήση δεδομένων, ιδεών ή λέξεων, είτε ακριβώς είτε παραφρασμένες, αναφέρονται στο σύνολό τους, με πλήρη αναφορά στους συγγραφείς, τον εκδοτικό οίκο ή το περιοδικό, συμπεριλαμβανομένων και των πηγών που ενδεχομένως χρησιμοποιήθηκαν από το διαδίκτυο.

Επίσης, βεβαιώνω ότι αυτή η εργασία έχει συγγραφεί από εμένα αποκλειστικά και αποτελεί προϊόν πνευματικής ιδιοκτησίας τόσο δικής μου, όσο και του Ιδρύματος.

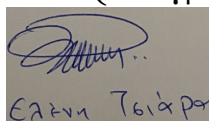
Παράβαση της ανωτέρω ακαδημαϊκής μου ευθύνης αποτελεί ουσιώδη λόγο για την ανάκληση του πτυχίου μου».

** Επιθυμώ την απαγόρευση πρόσβασης στο πλήρες κείμενο της εργασίας μου μέχρι 6 μήνες και έπειτα από αίτηση μου στη Βιβλιοθήκη και έγκριση του επιβλέποντα καθηγητή*

Η Δηλούσα

* Ονοματεπώνυμο / Ιδιότητα

Ελένη Τσιάρα / Μαία
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Ελένη Τσιάρα

Ψηφιακή Υπογραφή Επιβλέποντα

*** Σε εξαιρετικές περιπτώσεις και μετά από αιτιολόγηση και έγκριση του επιβλέποντα, προβλέπεται χρονικός περιορισμός πρόσβασης (embargo) 6-12 μήνες. Στην περίπτωση αυτή θα πρέπει να υπογράψει ψηφιακά ο/η επιβλέπων/ουσα καθηγητής/τρια, για να γνωστοποιεί ότι είναι ενημερωμένος/η και συναινεί. Οι λόγοι χρονικού αποκλεισμού πρόσβασης περιγράφονται αναλυτικά στις πολιτικές του **I.A. (σελ. 6):****

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Abstract

Background: Vitamin D deficiency or insufficiency is thought to be common among pregnant women. Recently, a special attention has been paid to gestational hypovitaminosis D in relation to pregnancy outcomes. There is little information on vitamin D intake in pregnancy and lactation and few studies on clinical outcomes. Vitamin D supplementation during pregnancy has been suggested as an intervention to protect against adverse gestational outcomes.

Objective: The aim of this study is to determine the necessary vitamin D supplementation in a cohort of Greek pregnant women. The main goals of this study are: a) to analyze and correlate data of pregnant women with different therapeutic protocols for vitamin D supplementation during pregnancy and relation to vitamin D insufficiency or deficiency of the newborn.

Methods: All pregnant women from a private clinic of a maternity hospital in Athens were randomized and included in the study. A single blind randomized design was used in order to define three groups of pregnant women who received different vitamin D₃ supplementation after the 12th week of pregnancy (Group A<8400 IU per week; Group B<12800 IU per week; Group C>16800 IU per week). Maternal vitamin D₃(OH-VitD₃) levels were recorded on the 3rd month of gestation and the 1st postpartum day. Newborns' vitamin levels (OH-VitD₃) were measured on the 3rd day of their life.

Results: In total 192 pregnant women and 194 newborns were involved in the study. Newborns absorb Vitamin D₃ from their mother. There is a significant proportion of pregnant women who have vitamin D deficiency or insufficiency both on the 3rd month of gestation (91%) and on the delivery date (80%). Only 15% of newborns demonstrated low vitamin D levels at birth. Vitamin D supplementation with a dose of 16800 iu weekly from the 3rd month of pregnancy seems to have a significant effect on fetal vitamin D levels, resulting to no infant with vitamin D deficiency (less than 15ng/ml). In our survey there is no indication of adverse effect of Vitamin D on newborn's weight or premature labor. Premature labor is not associated with higher maternal levels of vitamin D.

Conclusion: Vitamin D supplementation during pregnancy is safe and provides an efficient therapeutic modality to prevent maternal and neonatal vitamin D deficiency or insufficiency.

Keywords: Pregnancy, Vitamin D insufficiency, Vitamin D deficiency, Vitamin D supplementation

Περίληψη

Εισαγωγή: Η έλλειψη ή ανεπάρκεια βιταμίνης D πιστεύεται ότι είναι συχνή μεταξύ των εγκύων γυναικών. Πρόσφατα, έχει δοθεί ιδιαίτερη προσοχή στην υποβιταμίνωση D της κύησης και η σχέση αυτής με τα αποτελέσματα της εγκυμοσύνης. Υπάρχουν λίγες πληροφορίες σχετικά με την πρόσληψη βιταμίνης D3 κατά την εγκυμοσύνη και τη γαλουχία και λίγες μελέτες για τα κλινικά αποτελέσματα. Η λήψη συμπληρωμάτων βιταμίνης D3 κατά τη διάρκεια της εγκυμοσύνης έχει προταθεί ως θεραπευτική παρέμβαση για την προστασία από δυσμενή έκβαση της κύησης.

Σκοπός: Σκοπός της παρούσας μελέτης είναι να προσδιοριστεί η απαραίτητη δοσολογία του συμπληρωμάτων βιταμίνης D3 σε μια ομάδα Ελληνίδων εγκύων. Οι κύριοι στόχοι αυτής της μελέτης είναι: α) η καταγραφή των δημογραφικών δεδομένων και του γενικού ιστορικού των εγκύων γυναικών που συμμετέχουν στη μελέτη, β) ο προσδιορισμός της αποτελεσματικότητας και της ασφάλειας διαφορετικών θεραπευτικών πρωτοκόλλων για τη λήψη συμπληρωμάτων βιταμίνης D3 κατά τη διάρκεια της εγκυμοσύνης και γ) για να διαπιστωθεί οποιαδήποτε νοσηρότητα στη μητέρα ή το έμβρυο που σχετίζεται με έλλειψη ή ανεπάρκεια βιταμίνης D3.

Μεθοδολογία: Στη μελέτη συμπεριλήφθηκαν όλες οι έγκυες γυναίκες από την κλινική σε κεντρικό μαιευτήριο στην Αθήνα. Ένας απλός, τυφλός, τυχαιοποιημένος σχεδιασμός χρησιμοποιήθηκε για να οριστούν τρεις ομάδες εγκύων γυναικών που έλαβαν διαφορετικά συμπληρώματα βιταμίνης D3 μετά τη 12^η εβδομάδα της εγκυμοσύνης (Group A<8400 IU εβδομαδιαίως, Group B<12800 IU εβδομαδιαίως, Group C>16800 IU εβδομαδιαίως). Τα επίπεδα βιταμίνης D3 (OH-VitD3) στον ορό του αίματος της μητέρας καταγράφηκαν στον 3ο μήνα της κύησης και μία ημέρα μετά τοκετό. Τα επίπεδα της βιταμίνης D3 (OH-VitD3) στον ορό του αίματος των νεογνών μετρήθηκαν την 3^η ημέρα μετά τη γέννησή τους.

Αποτελέσματα: Συνολικά στη μελέτη συμμετείχαν 192 έγκυες γυναίκες και 194 νεογνά. Τα νεογνά απορροφούν τη βιταμίνη D από τη μητέρα τους. Υπάρχει ένα σημαντικό ποσοστό εγκύων που έχουν έλλειψη ή ανεπάρκεια βιταμίνης D3 τόσο στον 3ο μήνα της κύησης (91%) όσο και κατά την ημερομηνία τοκετού (80%). Μόνο το 15% των νεογνών παρουσίασαν χαμηλά επίπεδα βιταμίνης D3 (OH-VitD3) κατά τη γέννηση. Τα συμπληρώματα βιταμίνης D3 με δοσολογία 16800 iu εβδομαδιαία από τον 3ο μήνα της εγκυμοσύνης φαίνεται να έχουν σημαντική επίδραση στα επίπεδα βιταμίνης D3 (OH-VitD3) του εμβρύου, με αποτέλεσμα να μην υπάρχει έλλειψη βιταμίνης D3 στα νεογνά. Στην έρευνά μας δεν υπάρχει καμία ένδειξη δυσμενούς επίδρασης της βιταμίνης D3 στο βάρος του νεογνού ή στον πρόωρο τοκετό. Ο πρόωρος τοκετός δε συσχετίζεται με υψηλότερα επίπεδα βιταμίνης D3 στη μητέρα.

Συμπέρασμα: Η λήψη συμπληρωμάτων βιταμίνης D3 κατά τη διάρκεια της εγκυμοσύνης είναι ασφαλής και παρέχει μια αποτελεσματική θεραπευτική μέθοδο για την πρόληψη της μητρικής και νεογνικής έλλειψης ή ανεπάρκειας βιταμίνης D3.

Λέξεις κλειδιά: Εγκυμοσύνη, ανεπάρκεια βιταμίνης D, έλλειψη βιταμίνης D, συμπληρώματα βιταμίνης D

1. Introduction

The implication of vitamin D in multiple biological routes such as immunomodulation, cellular proliferation and differentiation led to extensive research regarding the determination of the optimal serum levels and the clinical consequences of low or high blood concentrations. Serum vitamin D levels are mainly expressed by 25(OH)D that determines the deficient, insufficient or sufficient vitamin D status. Based on the fact that vitamin D is found naturally only in limited food resources, such as egg yolk, salmon, and liver, maternal 25(OH)D levels depend mostly on the amount of sunlight exposure and on vitamin D supplementation.¹

During the last years, a special attention has been paid to gestational hypovitaminosis D in relation to pregnancy outcomes. Vitamin D has an increasingly recognized repertoire of nonclassical actions, such as promoting insulin action and secretion, immune modulation and lung development.^{2,3} It therefore has the potential to influence many factors in the developing fetus. Maternal vitamin D during pregnancy is of vital importance as 25(OH)D readily passes through the hemochorial placenta and determines fetal and neonatal vitamin D status. There is little information on vitamin D intake in pregnancy and lactation and few studies on clinical outcomes. Some have suggested that the requirement for vitamin D in these women may be up to 6000 iu/day and the ideal vitamin D regimen to prevent and treat vitamin D insufficiency in utero is unknown.^{4,5}

Pre-eclampsia and neonatal hypocalcemia are the most prevalent complications of maternal hypocalcemia and are clearly linked to substantial morbidity. A statistical association of glucose intolerance and hypovitaminosis D has been established. Maternal vitamin D is crucial for fetal bone development. Fetal lung development and neonatal immune conditions such as asthma may relate in part to maternal vitamin D levels.^{6,7}

In conclusion, hypovitaminosis D may be associated with hypertension, pre-eclampsia and increased caesarean section rates. There are no multicenter randomized control trials showing that vitamin D supplementation alters these putative risks.⁸

There are a few published studies on hypovitaminosis D in pregnant women and their newborns in Greece, but so far there is no unanimously accepted therapeutic protocol for this serious condition which carries a significant risk for fetal and newborn morbidity.⁹⁻¹¹

The aim of this study is to determine the necessary vitamin D supplementation in a cohort of Greek pregnant women. The main goals of my research are: a) to record the demographic data and the general history of pregnant women who

are involved in the study, b) to determine the efficacy and safety of different therapeutic protocols for vitamin D supplementation during pregnancy and c) to establish any comorbidity in the mother or fetus that is related to vitamin D insufficiency or deficiency.

2. Material & Methods

Background

The central task of the research is to examine some of the effects of Vitamin D supplementation during pregnancy. Can medication on mother with Vitamin D supplement have positive effect on newborn's Vitamin D? Are there any external factors that affect Vitamin D in infants? In order to answer the above questions, a protocol with Vitamin D supplementation was introduced.

Hospital where the protocol was tested

In order to examine the effect of different dose of Vitamin D on newborn, we observe the behavior of pregnant women served in a major Maternity & Gynecological Hospital (<https://www.mitera.gr/en/maieytiki-gynaikologiki-kliniki/>) for a duration of 28 months (January 2019 till April 2021).

Once a pregnant woman's record is filed on the secretary's office, a random number from a digital clock classify them, totally random, into one out of three groups. The group member A, B or C is logged on the register of the survey. This way our sample of pregnant women were blindly categorized into three distinct groups, almost of identical size.

Mitera Hospital has a robust GDPR protocol. All participant, but one, consent to participate in the administration of the medicinal product (<https://www.mitera.gr/en/personal-data-protection-policy/>).

The administration of Vitamin D is on the standard treatment during pregnancy and covered by the confidentiality agreement each pregnant had signed before admitted in the hospital.

No personal or sensible data were entered into the survey register.

Factors considered to record on the survey

The following measures were considered as exploratory factors for the observed value of infantile Vitamin D.

1. Mother Profile

- Mother's blood group; in case Vitamin D is absorbed differently depending on mothers' blood group
- Number of previous labors.
- Mother's age

2. Labor Conditions

- Week of labor
- Effect of Anticoagulant Treatment
- Effect of thyroid disease
- Twins

3. Newborn's Profile

- Gender
- Infant's weight; more as an important exogenous variable, since it is interesting to examine a causative relation between Vitamin D and newborn's weight.

Pretest of the protocol

Relevant data from UK show that there is evidence for applying a similar protocol in Greece, without any negative effects.

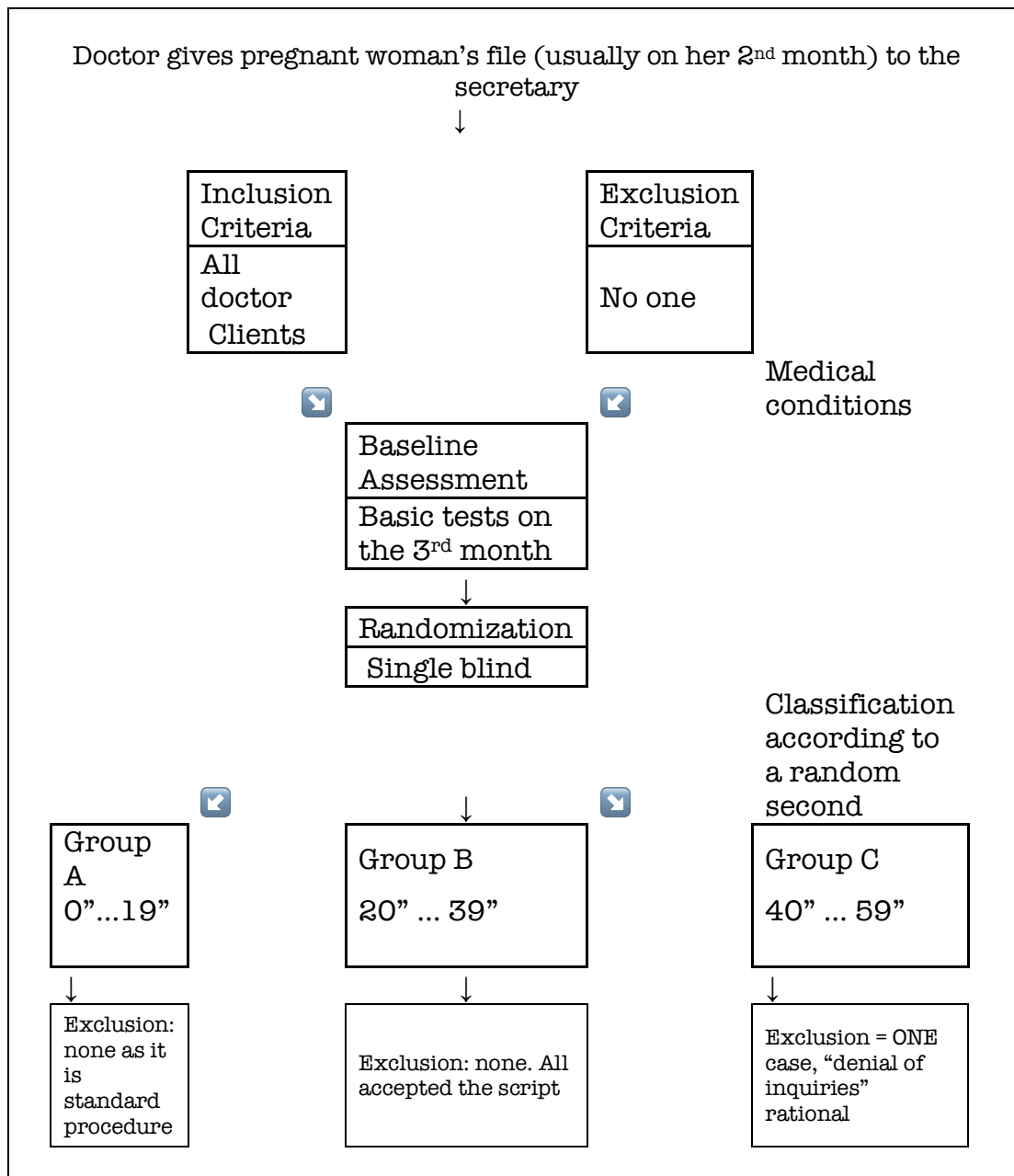
From the early 2010's occasional usage of Vitamin D on mothers with deficient values, less than 15 ng/ml, in MITERA hospital demonstrated encouraging measurements on children with no side effects. This event encouraged us to formalize and systemically apply the treatment in this current survey.

Experimental Design

All pregnant women of one Consultant's clinic in a main maternity hospital in Athens were included in the study. The clinic is well established in Athens for several years and it includes women of different age from many parts of the country. Because of its reputation, women expect to have high quality of service provision and therefore the administration of a vitamin-D is regarded as good clinical practice and was widely accepted and welcomed.

Greek population has high exposure to sun annually. However, several studies indicate that a significant percentage of the population has vitamin-D insufficiency or deficiency.⁹⁻¹¹

A single blind randomized design was used in order to define three groups.



Medical treatment of subject belonging on each of the three groups

	3 rd month	...	9 th month	9 th month
	Mother Vitamin D		Mother Vitamin D	Newborn Vitamin D
Group A Random numbers 0..19	Not measured	→ Standard treatment , i.e. 8400 iu weekly	Measured	Measured
Group B Random numbers 20..39	Measured	→ 12800 iu weekly	Measured	Measured
Group C Random numbers 40..59	Measured	→ 16800 iu weekly	Measured	Measured

Comments

Script for group A: “You will take vitamin D that is proven to be good for the baby”

Script for group B: “You will take vitamin D that is proven to be good for the baby”

Script for group C: “You will take vitamin D that is proven to be good for the baby”

Pregnant Vitamin on 3rd month is not measured on GROUP A as this was the standard operational procedure in hospital

Pregnant Vitamin on 3rd month is prescribed on GROUP B and C in order mother to have the planned quantity.

Absorption is defined as “Mother’s Vitamin D level in 9th month” minus “Mother’s Vitamin D level in 3rd month of pregnancy”

Sample Size determination

In order to determine the sample size, alpha and beta parameters will be used according to the established statistical analysis.

Alpha = 0.05 : The probability of a type-I error -- finding a difference when a difference does not exist. Most medical literature uses an alpha cut-off of 5% (0.05) -- indicating a 5% chance that a significant difference is actually due to chance and is not a true difference.

Beta = 0.20: The probability of a type-II error -- not detecting a difference when one actually exists, indicating a 20% chance that a significant difference is missed.

Beta is directly related to study power, $\text{Power} = 1 - \beta = 80\%$

From the pilot measurements, the standard deviation of vitamin D in children is around 10.

From a discussion with the medical team, it was considered to be very interesting if the medication group could have 5 more units of vitamin D than the control group.

Adding these numbers, an initial sample of 60 women is estimated to be adequate in each group.

Z value (alpha parameter)	1,95
Z value (beta parameter)	0,84
Vitamin D standard deviation on newborn	10
Effect, difference between group means	5
==> Estimated Sample size	60

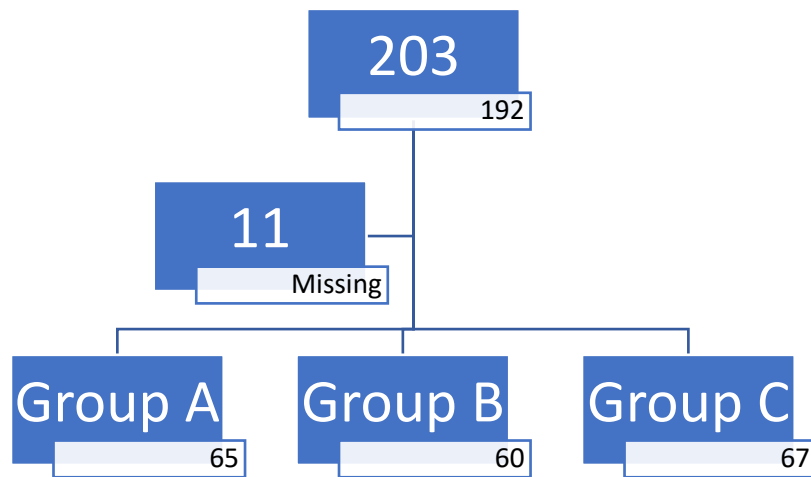
This is considered a sample size where we could detect a statistical difference of 5 units or more between groups.¹²

The sample

Pregnant women were split randomly (in a blind way, according to a random number determined by the second of a clock at the time of the file in the secretary's office into three different groups)

Sequence id		Random number for group	Assignment	Date time of entrance in clinic	Consent (Verbal on add on	Specific Response to script
1		20	Group A	5/1/2019	12/1/2019	
2		51	Group B	8/1/2019	21/1/2019	
3		48	Group C	12/1/2019	14/1/2019	
...			
40	...	45	Group B	12/10/2020	DENIED	*
...			
192		15	Group A	20/3/2021	23/3/2021	
193		98	Group C	20/3/2021	23/3/2021	

* Case 40 was the only one excluded as she refused participating in the study



Flowchart of the sampling

Subjects not participating in the study

Since there was only one woman who did not participate in the study, it was feasible to proceed to a second phase. The data (Vitamin-D levels and baby's weight) were communicated verbally to pregnant women and were in the anticipated range of group A (control group). Thus, the excluded case was not involved in the validity of the findings.

Response rate was almost 100%, as a result of the script convincing. The role of Vitamins in human health is widely accepted in Greece with culture in farming sector and traditional Mediterranean diet. Vitamin D is a nutrition supplement without significant side effects.

The sampling distribution of group is as follows:

Group	Count
A	65
B	60
C	67
Total	192

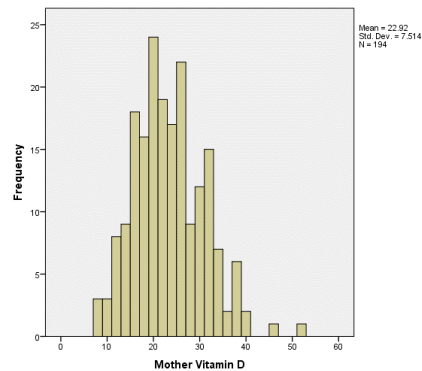
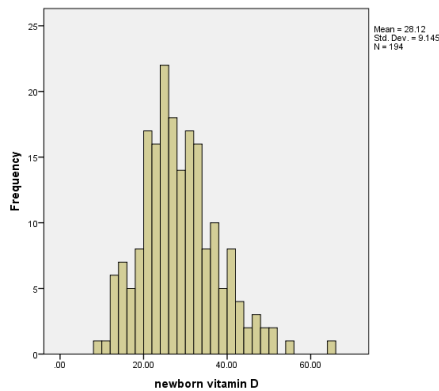
In group C, two women carried twins; there were 69 newborns in this group, adding to a total of 194 newborns.

Measurement and bias procedures

Blind categorization of women in three groups eliminates the effect of any subconscious factor. Validity deals with the levels of Vitamin D measured. Since this is done in a qualified lab, it is taken as granted.

Two different staff members recorded each woman's data collected in the survey. In case of discrepancy between the two measurements a third measurement was repeated in order validity to be assured.

The distribution of Vitamin D either for newborn or for mother is almost normal,



letting us to conclude that the measure of central tendency is a valid, consistent proxy of the underlying real population measure. If the distribution was not normal, as observed, but uniform (that is a flat line), we would then have a random, unreliable, measurement process.

Clinical sensitivity was tested internally with the scientific staff of the clinic in order to examine the extent. Most important issues were measured. The process is clearly understood by mothers and by the technical staff.

Handling of missing Data

In very few cases, 4 in total, there were missing measurements of newborn's Vitamin D levels, since for operational reasons, it was impossible to get a valid observed value.

Most women were willing to participate as medication with Vitamin D is a standard medical procedure.

All statistical procedures used have the option to take into account of the missing values.

Demographic Data

The following table demonstrates the distribution of the sample used in the survey

	Average	Standard Deviation
Mother's Age (years)	33,7	4,9
Newborn's weight (grammars)	3005,9	336,7

Early Labor	N
0 No	181
1 Yes	11
Total	192

Number of Labors	N
1st	119
2nd	61
3rd	11
4th	1
Total	192

Blood type	N
A(-)	8
A(+)	66
AB(+)	7
B(-)	1
B(+)	23
O(-)	14
O(+)	73
Total	192

Extra Medication	N
0 no medicine	149
1 Medicines included in the factors examined	43
Total	192

Gender of newborn	N
Girl	89
Boy	105
Total	194

Main research issues and statistical hypotheses

→ Do Mothers and Infants have high or low values of Vitamin D?

Hypothesis 1: There are many mothers and children with low level of Vitamin D

Statistical method used to test the hypothesis: Frequency tables of percentage of vitamin D on mother and infant

→ What is the degree of Relation of mother and infant Vitamin D level?

Hypothesis 2: There is relation of mother's and child's Vitamin D level

Statistical method used to test the hypothesis: Correlation analysis

→ Is there a significant improvement on vitamin D levels of group C and B over group A?

Hypothesis 3a: Infants in Group B have more vitamin D than these in group A

Hypothesis 3b: Infants in Group C have more vitamin D than these in group A

Hypothesis 3c: Infants in Group C have more vitamin D than these in group B

Statistical method used to test the hypothesis: T-test of difference of mean will reveal if the extra amount of vitamin D is worth being administered

In accordance with every observational study, cross tabulation of grouping variable with other factors will determine if there are any interesting differences.

Are there any factors considered in the study that can affect Vitamin-D levels?

Effect of Anticoagulant Treatment / Effect of thyroid disease / Insulin

A multiple regression analysis on infantile vitamin D will determine the most important factors that simultaneously affect positively or negatively the infant.

In summary:

#	Hypothesis	Tool to determine the effect of hypothesis
1	There are many infants with low vit-D level /Less than benchmark 15ng/ml	Frequency tables
	There are many mothers with low vit-D level /Less than benchmark 20ng/ml	Frequency tables
2	Increase in the dose of medication in mother will increase child's vit-D level	Correlation Multiple Regression
3a	Group B differ from group A	T-test
3b	Group C differ from group A	T-test
3c	Group B differ from group C	T-test
4	Effect of Factors considered to include on survey on group and infant's vit-D	Correlation for continuous Anova and general linear tests for categorical

3. RESULTS - Statistical Analysis

Issue 1. Increase of Vitamin D level

In total, the increase of Vitamin D levels from the initial measurement till the moment of birth is highly significant

TABLE 3. 1 Differences in pregnant Vitamin

T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval	
					Lower	Upper
-4.786	126.534	.000	-5.819	1.216	-8.225	-3.413

*Equal variances not assumed

Table 3.2 shows the mean, median and standard deviation for the increase of vitamin D levels across group B and C

TABLE 3. 2 Percentage of increase in Vitamin D level in group B and C

	Total	Group		
		A	B	C
Median	26.9		15.2	35.4
Mean	47.7		36.4	57.3
Standard Deviation	79.5		76.5	81.3

The increase of the measured value of Vitamin D on women at the time of labor is almost double in group C than in group B.

The variability of the increase is relatively high since the personal characteristics of each pregnant woman has a prominent role. This fact does not allow us to conclude that the increase of Vitamin D in group C is higher than of B {ANOVA F value = 2.210, Sig. = 0.140 (>0.05)}

Issue 2. Infantile Weight and Vitamin D level between groups

TABLE 3. 3 Birth weight

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
A	65	2970.38	300.060	37.218	2896.03	3044.74
B	60	3046.33	357.354	46.134	2954.02	3138.65
C	69	3004.13	351.586	42.326	2919.67	3088.59
Total	194	3005.88	336.722	24.175	2958.19	3053.56

Mean value of birth weight is the same across all groups (F stat = .793 p.value Sig.= .454)

TABLE 3. 4 Newborn Vitamin D

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
A	65	24.030	77.901	0.9662	22.100	25.961
B	60	26.283	76.625	0.9892	24.303	28.262
C	69	33.552	89.582	1.0784	31.413	35.717
Total	194	28.118	91.448	0.6565	26.823	29.413

TABLE 3. 5 Pairwise comparisons between groups of Newborn Vitamin D

Group		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I)	(J)				Lower Bound	Upper Bound
A	B	-2,25256	1,46588	0,378	-5,793	1,2879
	C	-9,53445*	1,41529	0,000	-12,9527	-6,1162
B	A	2,25256	1,46588	0,378	-1,2879	5,793
	C	-7,28188*	1,44534	0,00	-10,7727	-3,791
C	A	9,53445*	1,41529	0,000	6,1162	12,9527
	B	7,28188*	1,44534	0,000	3,791	10,7727

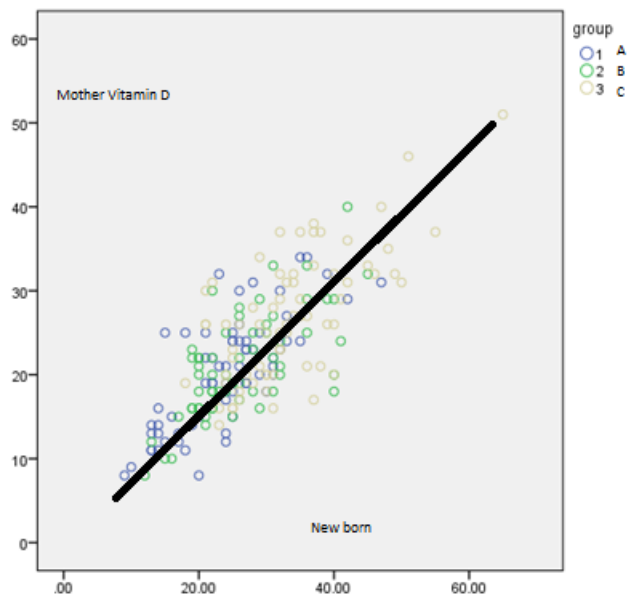
(Bonferroni method) Group C differs from both A and B, while B and A are quite undistinguishable

Issue 3. Relation of mother's and infant's Vitamin D level

The correlation of mother and infant Vitamin D level at the same moment is high and statistically significant.

TABLE 3. 6 Correlation of mother's and infant's Vitamin D level

		NEONATALVITD1
MotherToNewbornVITD	Pearson Correlation	.755**
	Sig. (2-tailed)	.000
	N	194



Since the two human beings are related to the same environment, one can detect that there is one-way relation; an increase on mother's vitamin D level will lead to a significant increase on newborn's vitamin D level.

Issue 4. Effect of Anticoagulation Treatment

TABLE 3. 7 Effect of Anticoagulation Treatment

	Anticoagulant Needed		Anticoagulant No needed		Total	
Group	Absorption	Newborn Vitamin D	Absorption	Newborn Vitamin D	Absorption	Newborn Vitamin D
A		28.000		23.038		24.030
B	12.417	26.000	28.019	26.314	26.432	26.283
C	119.040	33.667	61.772	33.537	74.222	33.565
Total	88.576	30.147	45.053	27.687	52.194	28.118

Absorption rate $[(\text{Final} - \text{Initial}) / \text{Initial}]$ is greater in group C than in group B when anticoagulants are used.

Additionally, newborn's vitamin D level is slightly higher when anticoagulants are administered in pregnancy.

Factor	F-value	Sig
<i>Absorption</i>	3.374	0.069
Newborn vitamin D	2.039	0.155

Thus, anticoagulation treatment seems to affect mother's absorption mechanism leading to significantly higher Vitamin D levels in newborns.

Issue 5. Effect of thyroid disease

There was no control group/mechanism for the effect of thyroid disease during the course of the pregnancy. The majority of samples requiring treatment for thyroid disease were observed on group C; as a consequence, it is inappropriate to make any statistical comparison.

Only from observational point of view, F statistics value and significance p-value were recorded.

TABLE 3. 8 Effect of thyroid disease on vit-D levels

Factor	F-value	Sig
Mother vitamin D	0.195	0.659
<i>Absorption</i>	<i>0.02 !!!</i>	<i>0.963</i>
Newborn vitamin D	0.232	0.631

Issue 6. Levels of Vitamin D at the moment of labor

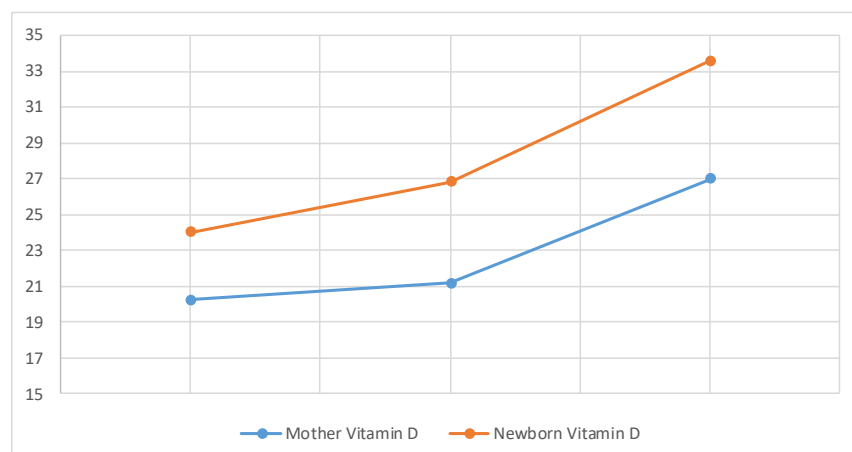
TABLE 3. 9 Observed Values of Vitamin D

	Mother Vitamin D	Newborn Vitamin D	Mother Vitamin D	Newborn Vitamin D
	Mean	Mean	Std. Dev.	Std. Dev.
A	20.22	24.030	6.733	77.901
B	21.17	26.283	6.206	76.625
C	26.99	33.565	7.595	89.582
Total	22.92	28.118	7.514	91.448

There is an effect of grouping variable on the vitamin levels at the time of birth

Group differ	F-value	Sig
Mother	18.902	.000
New born	24.874	.000

TABLE 3. 10 - Figure of Observed Values of Vitamin D



Issue 7. Mother's Vitamin D level <30ng/ml on first visit

	Frequency	Percent
Mother first visit under 30ng/ml	117	91.4
No	11	8.6

		Group		Total
		B	C	
Mother first visit under 30ng/ml	NO	8.5%	8.7%	8.6%
	YES	91.5%	91.3%	91.4%
Total		100.0%	100.0%	100.0%

Issue 8. Mother's Vitamin D <20ng/ml on the first visit

More than 50% of mothers had Vitamin D level <20ng/ml on their first visit

TABLE 3. 11 Vitamin D level<20ng/ml during first visit

Mother first visit <20ng/ml	Frequency	Percent
YES	75	58.6
NO	53	41.4

Group B and C differ, but not significantly on the initial value

TABLE 3. 12 Vitamin D level <20ng/ml on the first visit (per group)

Mother first visit <20ng/ml	Group		Total
	B	C	
NO	33.9%	47.8%	41.4%
YES	66.1%	52.2%	58.6%
Total	100.0%	100.0%	100.0%

Issue 9. Newborn's Vitamin <15ng/ml

TABLE 3. 13 Newborn's Vitamin D<15ng/ml

Newborn's level <15ng/ml	Frequency	Percent
YES	12	6.2
NO	182	93.8

TABLE 3. 14 Newborn's Vitamin D<15ng/ml (per group)

Newborn's level <15ng/ml	Group			Total
	1	2	3	
NO	84.6%	96.7%	100.0%	93.8%
YES	15.4%	3.3%		6.2%
Total	100.0%	100.0%	100.0%	100.0%

Issue 10. Newborn's Vitamin D <20ng/ml

TABLE 3. 15 Newborn's Vitamin D<20ng/ml

Newborn's level <20ng/ml	Frequency	Valid Percent
NO	28	14.4
YES	166	85.6

TABLE 3. 16 Newborn's Vitamin D<20ng/ml (per group)

Newborn's level <20ng/ml	Group			Total
	1	2	3	
NO	72.3%	85.0%	98.6%	85.6%
YES	27.7%	15.0%	1.4%	14.4%
Total	100.0%	100.0%	100.0%	100.0%

Issue 11. Newborn's Vitamin D >20ng/ml

TABLE 3. 17 Vitamin D of new born over 20

Newborn's level >20ng/ml	Frequency	Valid Percent
YES	166	85.6
NO	28	14.4

TABLE 3. 18 Vitamin D of new born over 20

Newborn's level >20ng/ml	Group			Total
	1	2	3	
NO	27.7%	15.0%	1.4%	14.4%
YES	72.3%	85.0%	98.6%	85.6%
Total	100.0%	100.0%	100.0%	100.0%

Issue 12. Mother's Vitamin D <30ng/ml in labor

TABLE 3. 19 Vitamin D level <30ng/ml in labor

Mother in labor with vit-D <30ng/ml	Frequency	Percent
YES	156	80.4
NO	38	19.6

TABLE 3. 20 Vitamin D level <30ng/ml in labor (per group)

Mother in labor with vit-D <30ng/ml	Group			Total
	1	2	3	
NO	12.3%	8.3%	36.2%	19.6%
YES	87.7%	91.7%	63.8%	80.4%
Total	100.0%	100.0%	100.0%	100.0%

Issue 13. Mother's Vitamin D <20ng/ml in labor

TABLE 3. 21 Vitamin D level <20ng/ml in labor

Mother in labor with vit-D <20ng/ml	Frequency	Valid Percent
YES	68	35.1
NO	126	64.9

TABLE 3. 22 Vitamin D level <20ng/ml in labor (per group)

Mother in labor with vit-D <20ng/ml	Group			Total
	1	2	3	
NO	53.8%	56.7%	82.6%	64.9%
YES	46.2%	43.3%	17.4%	35.1%
TOTAL	100.0%	100.0%	100.0%	100.0%

Issue 14. Mother's Vitamin D >30ng/ml in labor

TABLE 3. 23 Vitamin D level >30ng/ml in labor

Mother in labor with vit-D >30ng/ml	Frequency	Percent
YES	38	19.6
NO	156	80.4

TABLE 3. 24 Vitamin D level >30ng/ml in labor (per group)

Mother in labor with vit-D >30ng/ml	Group			Total
	1	2	3	
NO	87.7%	91.7%	63.8%	80.4%
YES	12.3%	8.3%	36.2%	19.6%
TOTAL	100.0%	100.0%	100.0%	100.0%

Overall, due to the inherent correlation of mother's and newborn's vitamin D levels, all the aforementioned mentioned statistical results are summarized in the following table.

TABLE 3. 25 Consolidated table of observation percentages

Issue		YES	NO	% yes
7	Mother first visit <30ng/ml	117	11	91%
8	Mother first visit <20ng/ml	75	53	59%
9	Newborn <15ng/ml	12	182	6%
10	Newborn <20ng/ml	28	166	14%
11	Newborn >20ng/ml	166	28	86%
14	Mother in labor >30ng/ml	38	156	20%
12	Mother in labor <30ng/ml	156	38	80%
13	Mother in labor <20ng/ml	68	126	35%

TABLE 3. 26 Consolidated table of observation percentages (by group)

	A	B	C	Asymp. Sig.	A	B	C
	count				%		
				(2-sided)	65	60	69
Mother first visit under 30		54	63	.965		90%	91%
Mother first visit under 20		39	36	.111		65%	52%
Mother in labor under 20	30	26	12	.001	46%	43%	17%
Mother in labor under 30	57	55	44	.000	88%	92%	64%
Newborn under 15	10	2	0	.001	15%	3%	0%
Newborn under 20	18	9	1	.000	28%	15%	1%

Issue 15. More significant predictors of infant's Vit-D level

A stepwise multiple regression analysis was run in order to find which factor plays the most prominent role in newborn's Vitamin D level.

The following table 3.27 displays the Regression coefficient in predicting newborn's Vitamin D level.

TABLE 3. 27 Regression coefficient in predicting newborn's Vitamin D level

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant term)	11.223	2.118		5.298	.000
Vitamin D of mother in labor	.739	.093	.614	7.917	.000
Absorption	.174	.070	.191	2.465	.015

The goodness of fit test (R-Square) is high (0.566)

There is high correlation between newborn's vitamin D level and exploratory variables like the mother's gestational age and the number of labors that she had lifelong (two related measures).

TABLE 3. 28 Correlation of newborn's vitamin D level with exploratory variables (most important)

	Pearson Correlation	Sig. (2-tailed)	N
	Newborn	Vitamin	D
Gestational age	.187	.009	194
Count of different labors	-.118	.102	194

- Women giving birth to infants at higher gestational age, result in infants with higher levels of vitamin D.
- The first labor newborns showed higher levels vitamin D.
- All other factors have a relatively smaller effect and significance.

Issue 16. Premature delivery and treatment with Vit-D supplements

Due to random sampling frame, on group B, only one premature birth was recorded. This fact it is making impossible to draw any meaningful conclusions. Additionally, in group C there is a very small number of premature childbirths.

TABLE 3. 29 *Premature delivery and Vitamin D*

Premature delivery on group C	N	Mean value of Vitamin D on newborn	Std. Deviation	Std. Error Mean
NO - Normal	60	33,60	9,50	1,23
YES- Early Childbirth	9	33,33	4,12	1,37

T-value (Equal variances not assumed) = 0,15 and Sig. (2-tailed) = 0,89. Thus, there is no higher level of Vitamin D in premature newborns in comparison with normal births.

Statistical Analysis of the effect of various factors on newborn's vitamin D level

Relation of newborn's Vitamin D across gender (0: girls, 1:boys)

There is no evidence that boys and girls behave differently within the three treatment groups. Neither gender nor gender by group interaction are significant.

Report

NEONATALVITD1 newborn vitamin D

gender	group	Mean	N	Std. Deviation
0	1 A	22.7419	31	6.54201
	2 B	26.5417	24	8.82181
	3 C	33.3529	34	9.69812
	Total	27.8202	89	9.56861
1	1 A	25.2059	34	8.70660
	2 B	26.1111	36	6.91077
	3 C	33.7714	35	8.31401
	Total	28.3714	105	8.80806
Total	1 A	24.0308	65	7.79016
	2 B	26.2833	60	7.66257
	3 C	33.5652	69	8.95820
	Total	28.1186	194	9.14485

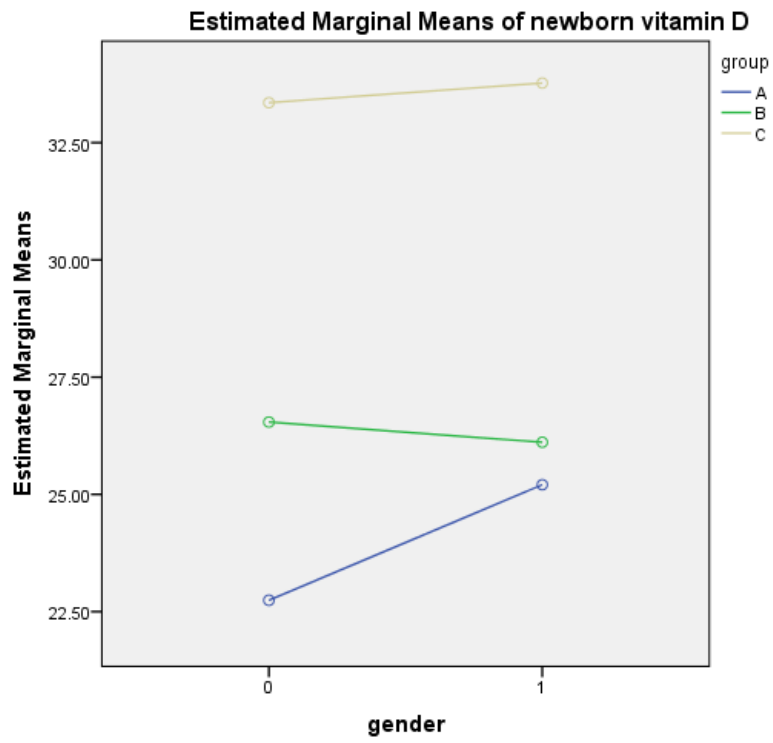
		F	Sig.
NEONATALVITD1 newborn vitamin D * gender	Between Groups	1.174	.677
	Within Groups		
	Total		

Tests of Between-Subjects Effects

Dependent Variable: NEONATALVITD1 newborn vitamin D

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3439.329 ^a	5	687.866	10.182	.000
Intercept	148768.064	1	148768.064	2202.072	.000
gender	31.792	1	31.792	.471	.494
group	3343.392	2	1671.696	24.745	.000
gender * group	69.061	2	34.531	.511	.601
Error	12700.944	188	67.558		
Total	169527.000	194			
Corrected Total	16140.273	193			

a. R Squared = .213 (Adjusted R Squared = .192)



Relation of newborn's Vitamin D level and type of labor

(0: Natural labor, 1: caesarean section)

There is no evidence that C-section operation affects newborn's Vitamin D level on all three treatment groups. Neither gender nor gender by group interaction are significant.

Report

NEONATALVITD1 newborn vitamin D

C-Section group	Mean	N	Std. Deviation	
0	1 A	23.6364	55	8.19522
	2 B	26.3019	53	7.82890
	3 C	34.3860	57	9.33839
	Total	28.2061	165	9.63194
1	1 A	26.2000	10	4.73286
	2 B	26.1429	7	6.79285
	3 C	29.6667	12	5.66221
	Total	27.6207	29	5.72222
Total	1 A	24.0308	65	7.79016
	2 B	26.2833	60	7.66257
	3 C	33.5652	69	8.95820
	Total	28.1186	194	9.14485

	F	Sig.
NEONATALVITD1 newborn vitamin D * C- Section	101	.751
Between Groups (Combined)		
Within Groups		
Total		

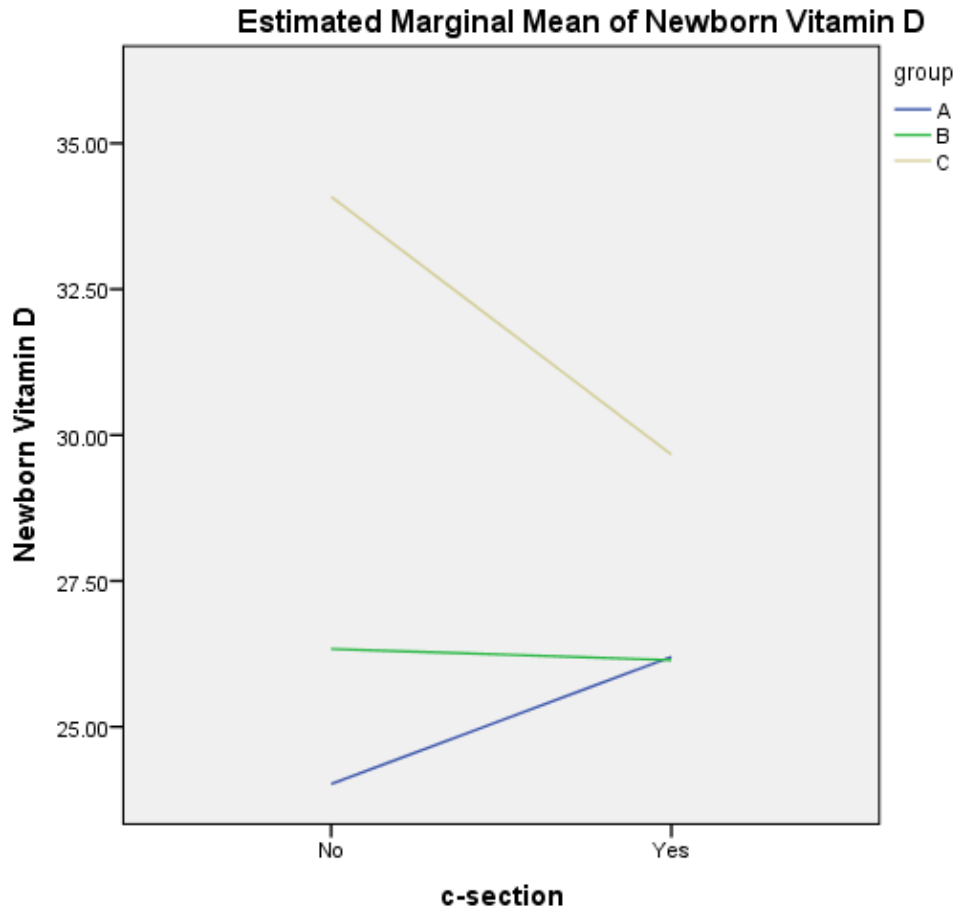
Tests of Between-Subjects Effects

Dependent Variable: NEONATALVITD1 newborn vitamin D

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3611.744 ^a	5	722.349	10.839	.000
Intercept	72657.746	1	72657.746	1090.284	.000
C-Section group	14.070	1	14.070	.211	.646
C-Section * group	1041.106	2	520.553	7.811	.001
Error	248.866	2	124.433	1.867	.157
Total	12528.530	188	66.641		
Corrected Total	169527.000	194			
	16140.273	193			

a. R Squared = .224 (Adjusted R Squared = .203)

There is a hint of interaction effect, since group C with caesarean operation has observed less Vitamin D levels than anticipated.



Relation of newborn's Vit-D levels with thyroid disease

Report

NEONATALVITD1 newborn vitamin D

Thyroid disease	group	Mean	N	Std. Deviation
0	1 A	23.9375	64	7.81507
	2 B	26.4068	59	7.66793
	3 C	33.8548	62	9.12364
	Total	28.0486	185	9.22737
1	1 A	30.0000	1	.
	2 B	19.0000	1	.
	3 C	31.0000	7	7.39369
	Total	29.5556	9	7.53510
Total	1 A	24.0308	65	7.79016
	2 B	26.2833	60	7.66257
	3 C	33.5652	69	8.95820
	Total	28.1186	194	9.14485

		F	Sig.
NEONATALVITD1 newborn vitamin D * thyroid disease	Between Groups (Combined)	232	.631
	Within Groups		
	Total		

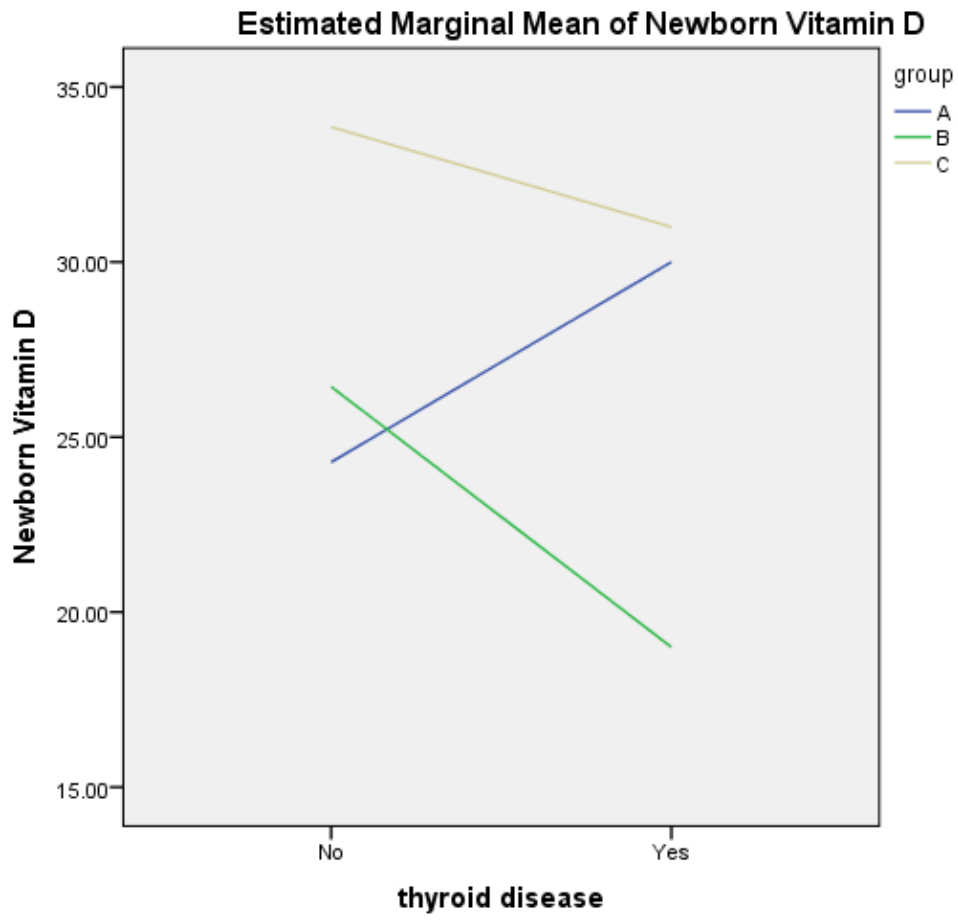
Tests of Between-Subjects Effects

Dependent Variable: NEONATALVITD1 newborn vitamin D

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3476.592 ^a	5	695.318	10.322	.000
Intercept	12302.354	1	12302.354	182.636	.000
Thyroid disease	8.046	1	8.046	.119	.730
group	381.209	2	190.604	2.830	.062
Thyroid disease * group	96.427	2	48.214	.716	.490
Error	12663.681	188	67.360		
Total	169527.000	194			
Corrected Total	16140.273	193			

a. R Squared = .215 (Adjusted R Squared = .195)

Whenever mothers suffer from thyroid disease, newborns in groups B and C have lower levels of vitamin D.



Relation of Vitamin D treatment and anticoagulation treatment

There is no significant relation despite the fact that anticoagulation therapy is related with higher level of Vitamin D in newborns.

Report

NEONATALVITD1 newborn vitamin D

Anticoagulation group	Mean	N	Std. Deviation
0			
1 A	23.0385	52	7.78627
2 B	26.3148	54	7.63019
3 C	33.5370	54	8.06510
Total	27.6875	160	8.93667
1			
1 A	28.0000	13	6.68331
2 B	26.0000	6	8.69483
3 C	33.6667	15	11.98014
Total	30.1471	34	9.95789
Total			
1 A	24.0308	65	7.79016
2 B	26.2833	60	7.66257
3 C	33.5652	69	8.95820
Total	28.1186	194	9.14485

	F	Sig.
NEONATALVITD1 newborn vitamin D * Anticoagulation	2.039	.155
Between Groups (Combined)		
Within Groups		
Total		

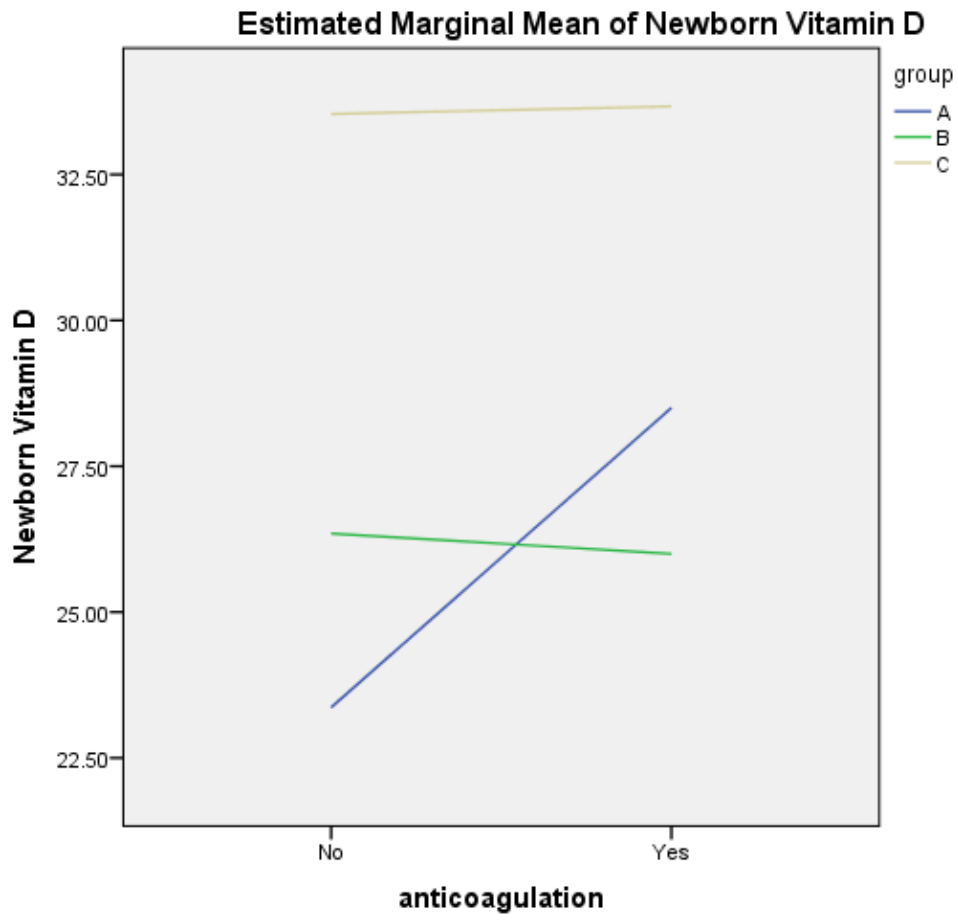
Tests of Between-Subjects Effects

Dependent Variable: NEONATALVITD1 newborn vitamin D

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3591.943 ^a	5	718.389	10.763	.000
Intercept	79366.334	1	79366.334	1189.072	.000
Anticoagulation group	62.243	1	62.243	.933	.335
Anticoagulation * group	1672.063	2	836.032	12.525	.000
Error	160.732	2	80.366	1.204	.302
Total	12548.330	188	66.746		
Corrected Total	169527.000	194			
	16140.273	193			

a. R Squared = .223 (Adjusted R Squared = .202)

In case of administrating anticoagulants on control group there is a tendency for higher levels of Vitamin D in infants compared to vitamin D levels of infants belonging to group B.



Relation of newborn's Vitamin D level with Insulin dependent mother

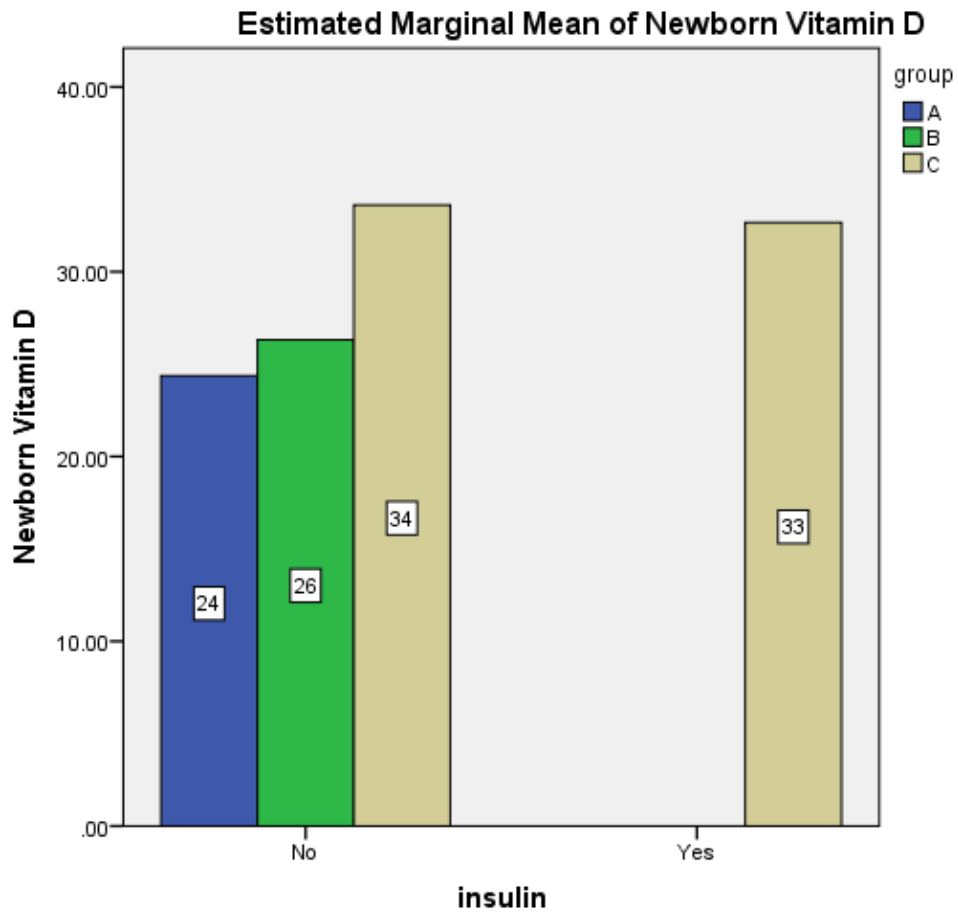
Only three mothers required administration of insulin, and they were all assigned on group C. Therefore, any statistical analysis is invalid.

Report

NEONATALVITD1 newborn vitamin D

Insulin	group	Mean	N	Std. Deviation
0	1 A	24.0308	65	7.79016
	2 B	26.2833	60	7.66257
	3 C	33.6061	66	9.12289
	Total	28.0471	191	9.18597
1	3 C	32.6667	3	4.72582
	Total	32.6667	3	4.72582
Total	1 A	24.0308	65	7.79016
	2 B	26.2833	60	7.66257
	3 C	33.5652	69	8.95820
	Total	28.1186	194	9.14485

		F	Sig.
NEONATALVITD1 newborn vitamin D * Insulin	Between Groups (Combined)	1.753	.387
	Within Groups		
	Total		



Relation of newborn's Vitamin D level with premature labor

There is no significant effect of vitamin D administration on premature labor.

Report

NEONATALVITD1 newborn vitamin D

premature group	Mean	N	Std. Deviation
0			
1 A	23.8710	62	7.94341
2 B	26.3898	59	7.68343
3 C	33.6000	60	9.49612
Total	27.9171	181	9.33445
1			
1 A	27.3333	3	.57735
2 B	20.0000	1	.
3 C	33.3333	9	4.12311
Total	30.9231	13	5.37683
Total			
1 A	24.0308	65	7.79016
2 B	26.2833	60	7.66257
3 C	33.5652	69	8.95820
Total	28.1186	194	9.14485

	F	Sig.
NEONATALVITD1 newborn vitamin D * premature	1.313	.253
Between Groups (Combined)		
Within Groups		
Total		

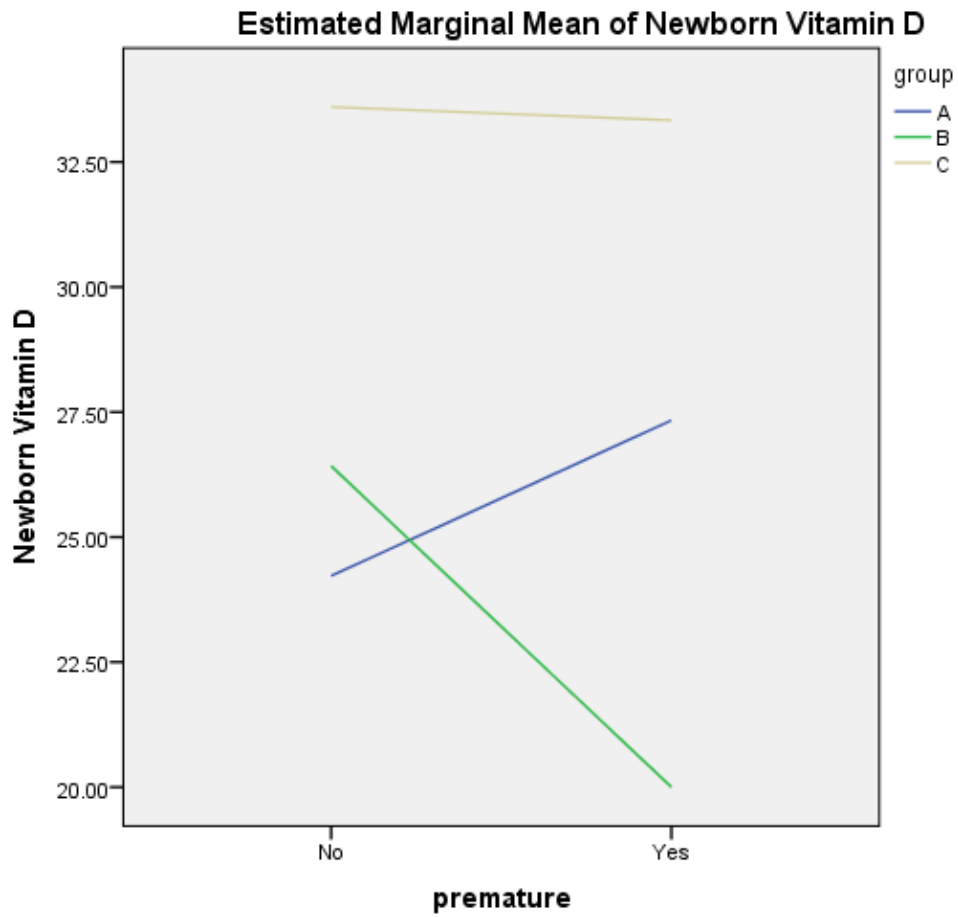
Tests of Between-Subjects Effects

Dependent Variable: NEONATALVITD1 newborn vitamin D

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3410.205 ^a	5	682.041	10.073	.000
Intercept	18116.370	1	18116.370	267.546	.000
premature	6.828	1	6.828	.101	.751
group	758.597	2	379.298	5.602	.004
premature * group	74.807	2	37.404	.552	.577
Error	12730.068	188	67.713		
Total	169527.000	194			
Corrected Total	16140.273	193			

a. R Squared = .211 (Adjusted R Squared = .190)

In groups A & C with a minimum sample size, the hypothesis that premature labor ("premature") has different levels of vitamin D in newborns compared to normal population is rejected.



Relation of newborn's Vitamin D level with first labor

Although there is not a significant effect of the first labor on newborn's Vitamin D level, all groups seem to have slightly lower level of newborn's vitamin D in the case of an experienced mother with more than one labor as compared to pregnant women on their first labor. An explanation of this, may be the confounding effect of age; inevitably the first labor is at smaller age with a younger body.

Report

NEONATALVITD1 newborn vitamin D

1 st labor	group	Mean	N	Std. Deviation
.00	1 A	23.2174	23	6.70791
	2 B	25.5500	20	7.22186
	3 C	31.3333	30	7.35472
	Total	27.1918	73	7.88926
1.00	1 A	24.4762	42	8.36729
	2 B	26.6500	40	7.93742
	3 C	35.2821	39	9.76554
	Total	28.6777	121	9.81429
Total	1 A	24.0308	65	7.79016
	2 B	26.2833	60	7.66257
	3 C	33.5652	69	8.95820
	Total	28.1186	194	9.14485

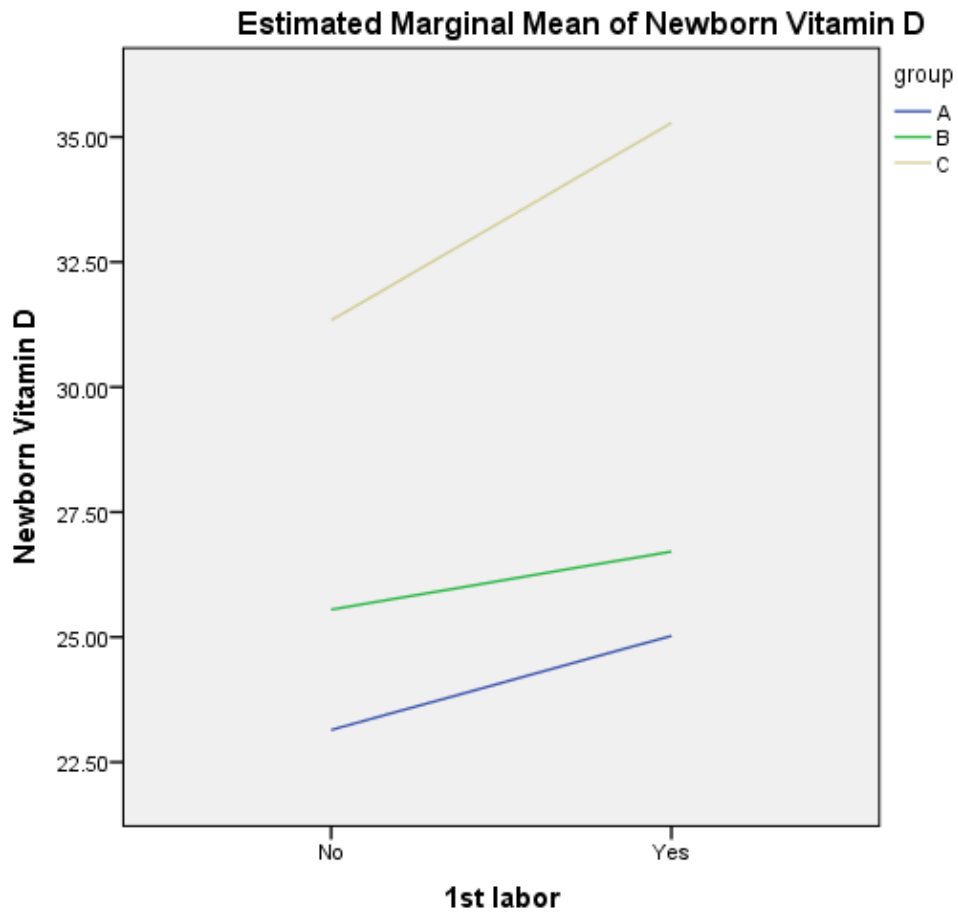
		F	Sig.
NEONATALVITD1 newborn vitamin D * 1 st labor	Between Groups (Combined)	1.203	.274
	Within Groups		
	Total		

Tests of Between-Subjects Effects

Dependent Variable: NEONATALVITD1 newborn vitamin D

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3639.270 ^a	5	727.854	10.946	.000
Intercept	137756.835	1	137756.835	2071.697	.000
1 st labor	197.676	1	197.676	2.973	.086
group	3127.930	2	1563.965	23.520	.000
1 st labor * group	81.130	2	40.565	.610	.544
Error	12501.003	188	66.495		
Total	169527.000	194			
Corrected Total	16140.273	193			

a. R Squared = .225 (Adjusted R Squared = .205)



Relation of Vitamin D supplementation with newborn's vitamin D levels

The administration of vitamin D supplementation has an interesting, almost significant, effect on group A subjects. The mean value on newborns' Vitamin D level is much higher in group C compared to group A. Future work can be done to quantify the ideal medical treatment during pregnancy.

Report

NEONATALVITD1 newborn vitamin D

medication group	Mean	N	Std. Deviation
1 A	22.9020	51	7.80065
2 B	26.4528	53	7.63485
3 C	33.9130	46	8.37145
Total	27.5333	150	9.06921
1 A	28.1429	14	6.44333
2 B	25.0000	7	8.36660
3 C	32.8696	23	10.19494
Total	30.1136	44	9.22387
1 A	24.0308	65	7.79016
2 B	26.2833	60	7.66257
3 C	33.5652	69	8.95820
Total	28.1186	194	9.14485

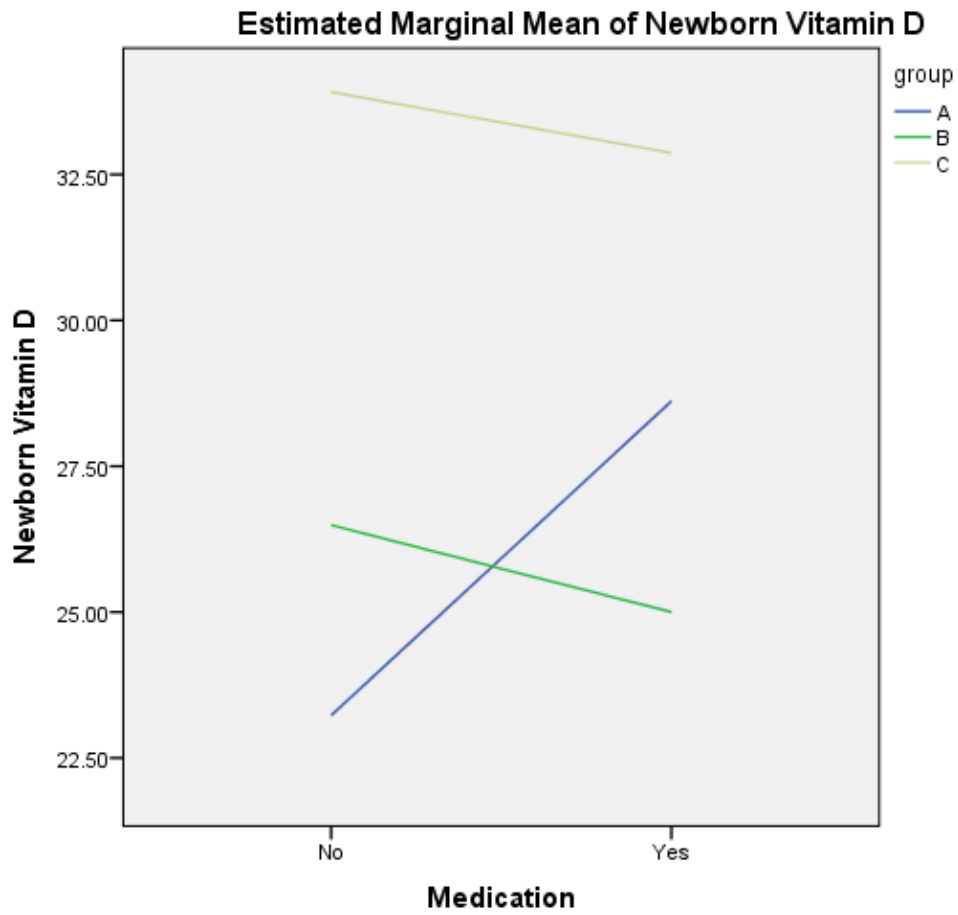
		F	Sig.
NEONATALVITD1 newborn vitamin D * medication	Between Groups (Combined)	2.733	.100
	Within Groups		
	Total		

Tests of Between-Subjects Effects

Dependent Variable: NEONATALVITD1 newborn vitamin D

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3666.656 ^a	5	733.331	11.053	.000
Intercept	90118.583	1	90118.583	1358.250	.000
medication	23.690	1	23.690	.357	.551
group	1969.422	2	984.711	14.841	.000
medication * group	298.791	2	149.395	2.252	.108
Error	12473.617	188	66.349		
Total	169527.000	194			
Corrected Total	16140.273	193			

a. R Squared = .227 (Adjusted R Squared = .207)



Relation of newborn's vitamin D level with infant's weight

In all three groups there is no linear correlation between newborn's Vitamin D levels and its weight.

Correlations

		Weight ΒΑΡΟΣ	NEONATALVITD 1 newborn vitamin D
Weight	Pearson Correlation	1	.011
	Sig. (2-tailed)		.883
	N	194	194

Correlations

group			NEONATALVITD 1 newborn vitamin D
1 A	Weight	Pearson Correlation	-.060
		Sig. (2-tailed)	.633
		N	65
2 B	Weight	Pearson Correlation	.068
		Sig. (2-tailed)	.606
		N	60
3 C	Weight	Pearson Correlation	.000
		Sig. (2-tailed)	.997
		N	69

Relation of newborn's Vitamin D level with initial mother's Vitamin D level before treatment

Clearly, the observed Vitamin D of the infant is unrelated with his/her initial mother's measurement.

Correlations

		NEONATALVITD l newborn vitamin D
Mother1stvitD_A Mother1stvitD	Pearson Correlation	-.044
	Sig. (2-tailed)	.625
	N	128

Correlations

group	MAMA1stvitD_A MAMA1stvitD	NEONATALVITD l newborn vitamin D
2 B	Pearson Correlation	-.124
	Sig. (2-tailed)	.351
	N	59
3 C	Pearson Correlation	-.040
	Sig. (2-tailed)	.747
	N	69

a. Cannot be computed because at least one of the variables is constant.

Relation of newborn's Vitamin D level with its mother's Vitamin D level at the same time

The tie in the measurements of Vitamin D levels for the mother and the newborn at the time of labor shows a correlation of almost 0.8; this is similar to as the two bodies were the same.

Correlations

		NEONATALVITD l newborn vitamin D
Mother to Newborn VITD	Pearson Correlation	.755**
	Sig. (2-tailed)	.000
	N	194

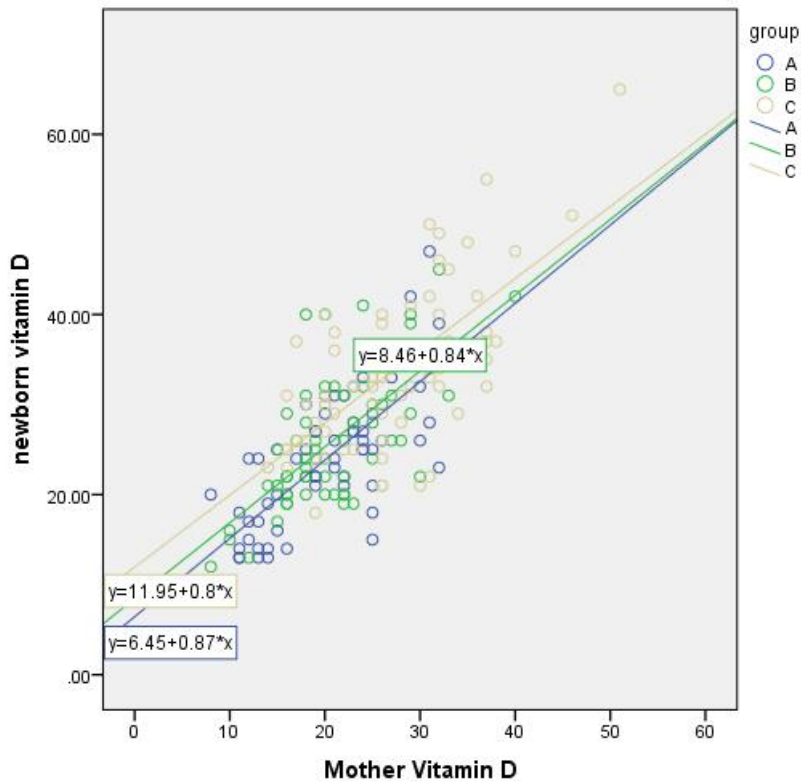
** . Correlation is significant at the 0.01 level (2-tailed).

Correlations

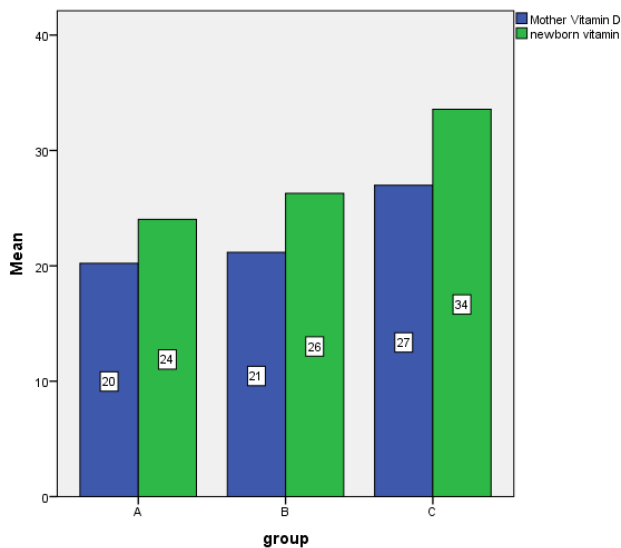
group		NEONATALVITD l newborn vitamin D	
1 A	Mother to Newborn VITD	Pearson Correlation	.752**
		Sig. (2-tailed)	.000
		N	65
2 B	Mother to Newborn VITD	Pearson Correlation	.682**
		Sig. (2-tailed)	.000
		N	60
3 C	Mother to Newborn VITD	Pearson Correlation	.679**
		Sig. (2-tailed)	.000
		N	69

** . Correlation is significant at the 0.01 level (2-tailed).

The regression lines that link mother's vitamin D level to her newborn are almost identical in the three groups, with the difference of the constant term indicating the starting level.



Clearly, the relation is almost identical in all groups, and the administration dose of vit-D supplementation has a parallel effect, which is greater and more efficient in group C.



Vit-D supplementation dosage and Vit-D levels on mother & child at the moment of birth and their difference

Group	Dose	Mother	Child	Baby-mother
A	8,4	20	24	4
B	12,8	21	26	5
C	16,8	27	34	7

On group C, there is a proportional higher increase in mother and newborn's vitamin D levels

Relation of newborn's Vitamin D level with mother's absorption ability

The rate that mother absorbs Vitamin D is the key component to newborn's vitamin D since it is highly correlated (Correlation = 0.591 & p-value <0,01). This effect is statistically identical in all groups. If a mother absorbs more vitamin D then her child will have proportionally high level of vitamin D.

Correlations

		NEONATALVITD l newborn vitamin D
absorption	Pearson Correlation	.591**
	Sig. (2-tailed)	.000
	N	128

** . Correlation is significant at the 0.01 level (2-tailed).

Correlations

Group		NEONATALVITD l newborn vitamin D
2 B	absorption	
	Pearson Correlation	.576**
	Sig. (2-tailed)	.000
3 C	absorption	
	Pearson Correlation	.546**
	Sig. (2-tailed)	.000
	N	69

** . Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Relation of newborn's Vitamin D level with increase of mother's Vitamin D level from 3rd month of gestation till delivery

In accordance with the previous findings, the higher the percentage of increase of Vitamin D levels in mother's measurements on 9th month versus the 3rd month of pregnancy, the higher the vitamin D levels on a newborn. If a pregnant woman has Vitamin D insufficiency/deficiency, and following Vitamin D supplementation during pregnancy increased her Vitamin D levels, then the child will benefit from this medication.

Correlations

		NEONATALVITD l newborn vitamin D
%increase	Pearson Correlation	.501**
	Sig. (2-tailed)	.000
	N	128

** . Correlation is significant at the 0.01 level (2-tailed).

Correlations

group		percincrease	NEONATALVITD l newborn vitamin D
2 B	%increase		
	Pearson Correlation	1	.511**
	Sig. (2-tailed)		.000
	N	59	59
3 C	%increase		
	Pearson Correlation	1	.486**
	Sig. (2-tailed)		.000
	N	69	69

** . Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

The table that follows summarizes the main statistical findings

	Issue	Finding
→	There are many newborns with low level /Less than benchmark 15ng/ml	A lot of newborn / mother have vit-D deficiency and few scarcity
	There are many mothers with low levels /Less than benchmark 20ng/ml	
	Vitamin D of Mother first visit under 30ng/ml	All (91%), no group differences
	Vitamin D of mother on the first visit under 20ng/ml	>half (58%) of cases no group differences
	Vitamin D of newborn under 15ng/ml	1/20 (6%), on group C none
	Vitamin D of newborn under 20ng/ml	1/7 (14%) on group C none
	Vitamin D of newborn over 20ng/ml	6/7 (85%), on group C all
	Vitamin D of mother on labor under 30ng/ml	4/5 (80%) on group C less (3/5 60%)
	Vitamin D of mother on labor under 20ng/ml	1/3 (35%) on group C 1/5 (17%)
	Vitamin D of mother on labor over 30ng/ml	1/5 (20%) on group C 2/5 (40%)
→	Increase of Vitamin D	On group C more than on group B
	Group B differ from group A	No difference
	Group C differ from group A	Yes, statistically different
	Group C differ from group B	Yes, statistically different
→	Relation of mother and newborn Vitamin D measurement	Strong Positive, significant
	Increase in the dose of medication in mother will increase child Vitamin D	Yes, Very significant
	More significant predictors of newborn Vitamin D	Amount of Vitamin on mother and absorbed quantity, Age of mother
→	Relation of newborn's Vitamin D across gender	Not significant
	Relation of newborn's Vitamin D and caesarean operation	Not significant
	Group C with caesarean operation seem to have lower Vitamin D values than anticipated	
	Relation of Vitamin D with thyroid disease	Not significant
	When there is thyroid disease, medication group B and C have lower values of vitamin D	

	Relation of Vitamin D treatment with Anticoagulation Treatment	Not significant
	In case of Anticoagulant Treatment on control group there is a tendency for more Vitamin D, as if infants' mother belongs to group B	
	Relation of Vitamin D with Insulin	Rare
	Relation of Vitamin D treatment with premature labor :	Not significant
	Not effect of Vitamin D dose on premature labor Same value of Vitamin D on normal infants and on early childbirth infants	
	Relation of Vitamin D treatment with first labor	Not significant
	First labor has slightly bigger values of Vitamin D	Possible age confounding effect
	Relation of Vitamin D treatment with pharmacy treatment	Slightly significant (on 90% confidence)
	On group A medical treatment has more Vitamin D than pregnant without	
	Relation of newborn's Vitamin D with infantile weight	Unrelated
	Observed newborn's Vitamin D level and initial mother's measurement	Unrelated
	Relation of newborn's Vitamin D with its mother Vitamin D at the same time	Strongly related
	The tie on the measurements of Vitamin D on mother and newborn at the moment of labor is as the two bodies were the same. There is a propotional increase in mother's and newborn's vitamin D level relative to the treatment dose	$P < 0.000$
	Relation of newborn's Vitamin D with absorption from mother	Strongly related $P < 0.000$
	Relation of newborn's Vitamin D with increase of mother's Vitamin D from 3 rd month to labour	Strongly related
	If a mother requires Vitamin D supplementation, and during pregnancy increased her vit-D levels, then the newborn will benefit from this medication	$P < 0.000$

- Group C seems to differ significantly from the other two groups of the study demonstrating higher effect on the measured Vitamin D levels in newborns.
- In many categories of statistical analysis, Group B seems to have similar results with Group A.
- Vitamin D absorption is an important factor and is clearly related to women's clinical features.
- First labor from younger women seem to require higher dose of Vitamin D supplementation.
- Premature birth is not associated with higher values of vitamin D.

4. Discussion

Since a strong relationship between 25(OH)D concentrations in mother-newborn pair has been established, inadequate maternal 25(OH)D levels during gestation would have a significant impact on fetal and neonatal vitamin D status. It has been proven that deficient vitamin D status during pregnancy will result in a deficient fetus, while vitamin D stores will be diminished approximately at the 8th week of life. Gestational vitamin D insufficiency has now been linked to a range of disorders, such as osteoporotic bone disease, altered brain development and adult mental health, autoimmune disease, asthma and food allergies. In some cases, profound maternal vitamin D deficiency presents as neonatal rickets. Furthermore, recent findings suggested that the increased prevalence of vitamin D deficiency/insufficiency during pregnancy would have consequences for the fetus and offspring bone health in later life.¹³⁻¹⁹ Currently, a level of at least 30 ng/ml 25(OH)D is considered as sufficient, values between 29 and 20 ng/ml as insufficiency and levels less than 20 ng/ml as deficiency in adults. In newborns a value below 20 ng/ml is considered as insufficiency while levels below 15 ng/ml as deficiency.²⁰

The aim of this study is to measure vitamin D₃ levels (OH-VitD₃) in pregnant women and their infants and also to determine external factors that can affect vitamin D absorption. The primary question that has to be answered is whether vitamin D supplementation therapy during pregnancy has any effect on newborns' vitamin D levels. The vitamin D supplementation which was administered was different in each group and covered by the confidentiality agreement that every pregnant woman signed before entering the hospital. No personal or sensible data were incorporated on the register. During this study we had to determine the efficacy and safety of different treatment protocols for vitamin D supplementation in pregnancy and to ascertain any comorbidity in the mother or fetus that is linked to vitamin D insufficiency or deficiency. Data from UK showed that there is evidence for applying a similar protocol in Greece without any negative effects.⁸

Our findings are consistent with randomized controlled studies from big tertiary referral units in the Western world.²⁰⁻²² The main findings of our study are the following:

1. *Newborns absorb Vitamin D from their mother ($P < 0.000$).^{2,3}*
2. *If a mother on the 3rd gestational month has vitamin D value less than 30ng/ml, medication with 12800 iu weekly shall have proportional effect on infant's vitamin D measurement.*
3. *Vitamin D supplementation with a dose of 16800 iu weekly from the 3rd month of pregnancy seems to have a significant effect on fetal vitamin D levels, resulting probably to no infant with vitamin D deficiency (less than 15ng/ml).*
4. *Midwives and doctors can monitor and adjust the medication, since there is not a constant absorption rate in all mothers, considered other*

medication mother is in need for. Subject variability of mothers is important for vitamin D absorption.

- 5. In our survey there is no indication of adverse effect of Vitamin D on newborn's weight or premature labor.^{5,6,17}*
- 6. Group C seems to be distinguished from the other two groups of the study, with higher effect on the measured Vitamin D level of newborns. In many statistical categories, Group B seems to be indifferent from Group A.*
- 7. Premature labor / birth is not associated with higher maternal levels of vitamin D.¹⁷*

In conclusion, vitamin D supplementation during pregnancy is safe and provides an efficient therapeutic modality to prevent neonatal vitamin D deficiency/insufficiency. There is still necessity for further randomized controlled studies in order to determine a universally accepted treatment protocol for vitamin D supplementation in gravidity.

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