MINISTRY OF MINISTRY OF

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**HANOI MEDICAL UNIVERSITY**



**LE QUANG TOAN**

**STUDY OF RALATIONSHIPS BETWEEN PLASMA 25-HYDROXYVITAMIN D CONCENTRATION AND INSULIN RESISTANCE, AND EFFECTS OF VITAMIN D SUPPLEMENTATION ON INSULIN RESISTANCE IN GESTATIONAL DIABETES MELLITUS**

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LIST OF PUBLICATIONS

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2. Le Quang Toan, Do Trung Quan, Nguyen Van Tien (2014). Relationships between vitamin D and insulin resistance in women with gestational diabetes mellitus. *Journal of Medical Research,* 91 (6), 31 – 37.

**ABREVIATIONS**

|  |  |
| --- | --- |
| 1,25(OH)2D | 1,25-dihydroxyvitamin D |
| 25(OH)D | 25-hydroxyvitamin D |
| BMI | Body Mass Index |
| DM/GDM | Diabetes Mellitus/Gestational Diabetes Mellitus |
| GW | Gestation week |
| FPG/PG | Fasting Plasma glucose/Plasma glucose |
| HbA1c | Glycosylated Hemoglobin A1c |
| HOMA | Homeostasis Model Assessment  |
| HOMA2-IR | Insulin resistance assessed by HOMA2 |
| HOMA2-IR-Cp | HOMA2-IR calculated by FPG and C-peptide |
| HOMA2-IR-In | HOMA2-IR calculated by FPG and insulin |
| IR | Insulin resistance |
| NGDM | Non-Gestational Diabetes Mellitus |
| OGTT | Oral glucose tolerance test |

**INTRODUCTION**

 Vitamin D insufficiency is very common in the world and pregnant women are at high risk of this condition. The condition is also very common in Vietnam with prevalence ranging from 52,0 to 60,0% in women. Numerous roles of vitamin D, other than classical ones, have recently been discovered, including relationships to insulin resistance (IR) in gestational diabetes mellitus (GDM).

 Prevalence of GDM has been rapidly increasing recently in the world as well as in Vietnam, reaching 20.3% in a recent study in a large city. GDM can cause numerous consequences for the mother and the fetus if not timely and effectively diagnosed and managed. Two principle pathological factors of GDM are islet beta cell dysfunction and IR. Until present, all the oral hypoglycemic agents have been not approved for use in pregnant women. Therefore, research on factors that are related to and capable of reducing IR in GDM has scientific and practical importance.

 Plasma 25-hydroxyvitamin D (25-(OH)D) level was inversely correlated to IR and vitamin D supplementation compared to placebo or higher vitamin D doses to lower ones reduced IR and improved blood glucose in pregnant women with and without GDM in a number of studies.

 However, those studies included both pregnant women with and those without GDM, and both those with and those without vitamin D deficiency. Therefore, to study the relationships separately in only pregnant women with both GDM and vitamin D deficiency is necessary. On the other hand, until now relationships between vitamin D and IR in GDM have not been studied in Vietnam.

 For these reasons we conducted the research ***"Study of relationships between plasma 25-hydroxyvitamin D with insulin resistance and effects of vitamin D supplementation on insulin resistance in gestational diabetes mellitus"*** with the following objectives:

1. *To determine the prevalence of vitamin D insufficiency in pregnant women at the National Hospital of Gynecology & Obstetrics and the National Hospital of Endocrinology.*
2. *To explore relationships between plasma 25-hydroxyvitamin D concentration in women with gestational diabetes mellitus*
3. *To initially examine effects of vitamin D supplementation on insulin resistance in women with gestational diabetes mellitus*

***New scientific findings and practical contributions***

- The finding of the prevalence of vitamin D insufficiency in women with GDM serves the base for making recommendations on vitamin D insufficiency screening and vitamin D supplementation for this population.

- The thesis confirmed the inverse relationship between plasma 25(OH)D level and IR in women with GDM, and the superiority of a higher dose vitamin D supplementation compared with a lower dose in reducing IR increase in the period from the middle to the end of gestation. This finding serves the basis for recommending vitamin D supplementation for women with GDM and vitamin D insufficiency as well as the basis for further research on vitamin D supplementation effects in GDM prevention and its adjuvant treatment of this condition.

***Thesis structure***

 The thesis has 116 pages (excluding the references and appendix), 4 chapters, 27 tables, 12 charts, 6 figures and 143 references. Introduction 2 pages, literature review 36 pages, Subjects and methods 16 pages, Study results 26 pages, Discussions 34 pages, Conclusions 2 pages and Suggestions 1 page.

**Chapter 1. LITERATURE REVIEW**

**1.1. Review of vitamin D**

***1.1.1. Chemical nature and metabolism of vitamin D***

 Vitamin D exists in two forms, Cholecalciferol (Vitamin D3) and Ergocalciferol (Vitamin D2). Vitamin D is converted to 25(OH)D in liver by the first hydroxylation and in kidneys by the second one to 1,25(OH)2D that is biologically active and therefore is considered a hormone.

***1.1.2. Vitamin D status assessment***

 Plasma 52(OH)D concentration is selected as the indicator of vitamin D status because it is directly related to its intake, has longest plasma half-life and is not affected by regulating factors compared with vitamin D and 1,25(OH)2D.

 There has not been a widespread concensus on criteria for vitamin D status assessment (tab. 1.2). The cut-off-point of plasma 25(OH)D level < 75 nmol/L for of vitamin D insufficiency definition according to The Endocrine Society (ES) 2011 criteria is supported by the majority of experts and is based on studies of relationships between plasma 25(OH)D level and plasma parathomone, calcium absorption in guts and consequences of vitamin deficiency.

*Table 1.1.* **Criteria for vitamin D status assessment**

|  |  |
| --- | --- |
| **Author** | **Vitamin D status by plasma 25(OH)D (nmol/L)** |
| **Deficiency**  | **Insufficiency** | **Sufficiency** | **Toxicity** |
| Hollis 2005  | < 80 | 80 - 225 | > 225 |
| Holick 2007  | <50 | 50 - <75 | 75 – 225 | > 225 |
| IOM 2010 | < 30 | 30 - <50 | ≥ 50 |  |
| ES 2011 | < 50 | 50 - <75 | 75 – 250 | > 250 |

***1.1.3. Vitamin D deficiency***

 Vitamin D deficiency is very common in the world, especially in pregnant women. In Vietnam, it is also very common in women and its prevalence range from 52.0 to 60.0%.

***1.1.4. Recommendations on vitamin D supplementation and treatment of vitamin D deficiency***

 There has not been also a widespread concensus on vitamin D supplementation and treatment of vitamin D deficiency. Institute of Medicine (IOM) recommended dietary allowance of vitamin D is 600 IU (2010), meanwhile ES vitamin D daily requierment is 600 IU for pregnat women, 600 to 1000 IU and 1500 to 2000 IU daily for pregnant women at risk of vitamin deficiency at age of 14 to 18 and 19 to 50 year old, respectively (2011).

\* ***Tolerable upper intake level of vitamin D*** for the adult (including pregnant women) is 4,000 and 10,000 IU daily according to IOM (2010) and ES (2011), respectively.

**1.2. Gestational Diabetes Mellitus and insulin resistance**

***1.2.1. Definition and diagnostic criteria of GDM***

 GDM is defined by WHO as glucose intolerance of variable severity with onset or first recognition during pregnancy (1999). The International Association of Diabetes in Pregnancy Study Groups (ISDPSG) 2010 diagnosis criteria, applied by The American Diabetes Association (ADA) since 2011 and WHO since 2013, separates two forms of diabetes first time detected during pregnancy: 1) GDM and 2) "overt diabetes" that is diagnosed when blood glucose levels meet the diagnostic criteria for diabetes in the non-pregnant:

*Table 1.2.* **Classification and diagnostic criteria for hyperglycemia first time detected during pregnancy (ADA 2011 and WHO 2013)**

|  |  |  |
| --- | --- | --- |
| **Criterion** | **IADPSG/ADA 2011/WHOa** | **Overt DMa** |
| FPG b | 5.6 – 6.9 | ≥ 7.0 |
| 1-h PG b | ≥ 10.0 | - |
| 2-h PG b | 8.5 – 11.0 | ≥ 11.1 |
| Random PG c | - | ≥ 11.1 |

*Note:* PG unit is mmol/L, a: Diagnosed if there is one or more criteria; b: OGTT; c: + clinical symptoms of hyperglycemia.

***1.2.2. Insulin resistance in GDM***

 IR is condition in which normal blood insulin concentration produces lower biological actions on carbohydrate and lipid metabolism compared with normal people.

 IR in women with GDM comprises of physiological pregnancy-induced IR and that existing before conception, is higher compared with pregnant women without GDM, and starts to continuously rise from the second half of gestation until delivery.

***1.2.3. Homeostasis Model Assessment (HOMA) of IR***

HOMA was developed on basis of interaction between blood glucose and insulin in fasting steady state and non-linear equations derived in experiments. HOMA1 was introduced by Mathews in 1985, using simple approximate formula for calculation of IR. The computerized HOMA or HOMA2 developed by Oxford University (UK) has a number of advantages compared with HOMA1: IR is more accurately calculated by computer software, non-specific or specific blood insulin can be used, C-peptide can be used instead of insulin.

 The principal advantages of HOMA2: it is simple to carry out and yields results closely correlated to ones of reference method clamp technique (correlation coefficients range from 0.73 to 0.87).

***1.2.4. Studies on vitamin D and IR in GDM***

 Plasma 25(OH)D level is inversely correlated to IR in pregnant women with and without GDM even being adjusted by other IR related factors (Maghbooli 2008, Lacroix 2014). Vitamin D supplementation compared with placebo (2 studies by Asemi, 2013) or higher vitamin D dose compared with lower ones (Soheilykhah 2013) reduced IR in absolute or relative manor in pregnant women with and without GDM.

***1.2.5. Mechanisms of vitamin D actions on IR***

 Vitamin D reduces IR though: 1) Increasing insulin receptor expression; 2) Stimulating synthesis of PPARδ that is a transcription factor for proteins participating in lipid metabolism; 3) Regulating and maintaining intracellular calcium homeostasis; 4) Suppressing synthesis of pro-inflammatory cytokines causing IR and 5) Suppressing renin-angiotensin system

**Chapter 2. SUBJECTS AND METHODS**

**2.1. Study subjects**

 Subjects were pregnant women at 24 to 28 gestational weeks at The National Hospital of Gynecology & Obstetrics and National Hospital of Endocrinology comprising of two groups:

 - The group with GDM

 - The control group [without GDM, NGDM group)]

 GDM was diagnosed by the ADA 2011 criteria as following:

*Table 2.1.* **ADA 2011 criteria for diagnosis of GDM with 75g OGTT**

|  |  |
| --- | --- |
|  | **Plasma venous glucose level (mmol/L)** |
| Fasting | 5.1 - 6.9 |
| 1h | ≥ 10.0 |
| 2 h | 8.5 - 11.0 |

***- Selection criteria for GDM groups receiving vitamin D supplementation***

Pregnant women with GDM who have vitamin D insufficiency according to ES 2011 criteria with plasma 25(OH)D level < 75 nmol/L.

***- Exclusion criteria for GDM group:*** Subjects were excluded if having one or more of the following:

 Previously known diabetes or diabetes in pregnacy; past or present conditions affecting glucose metabolism; past or present use of drugs affecting glucose metabolism; present use of vitamin D containing drugs; present acute illnesses; present eclampsia; refusal of participation in the study.

***- Exclusion criteria for vitamin D supplementation groups***

 - Present use of vitamin D containing drugs.

 - Hypercalcemia: Total plasma calcium level > 2.5 mmol/L.

***- Exclusion criteria for the control group***

 1) DM family history; 2) Past GDM in previous pregnancies; 3) History of hypertension or dyslipidemia; 4) Preconception BMI≥ 23kg/m2; 5) Pour obstetrics history: still-birth, miscarriage, premature birth, gross-baby (with birth weight > 4000g).

**2.2. Place and time of study**

 The study was conducted in the National Hospital of Gynecology & Obstetrics and National Hospital of Endocrinology from April 2012 to April 2014.

**2.3. Study design:** Descriptive study to resolve objectives 1 and 2, and randomised control trial to resolve objective 3.

**2.4. Sample size:** The largest sample size among those for three objectives was 95 pregnant women with GDM. The real sample size was 104 women with GDM and 55 controls (NGDM).

**2.5. Study Implementation**

***2.5.1. Subject selection:*** Cumulative selection and block random assignment was carried out .

***2.5.2. Vitamin D supplementation intervention***

 The pregnant women with GDM having vitamin D insufficiency and giving consent to participate in the vitamin D supplementation trial were randomly allocated to one of the two groups taking daily 500 IU or 1500 IU of vitamin D3.

 Vitamin D3 drug: Aquadetrim manufactured by Medana Pharma (Poland): Solution with concentration of 15.000 UI/ml, 500 IU/drop , 10ml vial.

 Other vitamin D containing drugs were not used by the subjects during the supplementation trial.

 The visits following the baseline visit (visit 1) were: Visit 2 at gestational weeks 31 – 33, visit 3 at gestational weeks 36 – 38.

***2.5.3. Data collected at the visits***

*Table 2.3.* **Data collected at visits (marked with x)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Visit 1** | **Visit 2** | **Visit 3** |
| **NGDM** | **GDM** | **GDM** |
| Age | x | x |  |  |
| Familial and obstetrical history  | x | x |  |  |
| Gestational week | x | x | x | x |
| Preconception weight | x | x |  |  |
| Wight | x | x | x | x |
| Height | x | x |  |  |
| Plasma glucose | x | x | x | x |
| HbA1c |  | x | x | x |
| Fasting plasma Insulin | **x** | x |  | x |
| Fasting plasma C-peptide |  | **x** |  | x |
| Fasting plasma triglyceride |  | **x** |  |  |
| Fasting plasma HDL-C |  | **x** |  |  |
| Plasma total and ionized Calcium |   | x | x | x |
| Plasma 25(OH)D | x | x |  | x |

***2.5.4. Treatment of GDM***

 All the pregnant women with GDM were followed up at National Hospital of Endocrinology by diet and exercise. After 2 weeks, if blood glucose targets were not met, insulin was added.

**2.6. Data collection methods**

***2.6.1. Interview and anthropometric parameter measurement***

 - Demographic characteristics, personal, familial and obstetrical history, preconception weight and gestational week were collected.

 - Weight and height were measured

***2.6.2. Biochemical tests***

*- Oral glucose tolerance test* (OGTT) with 75g of glucose and 3 time points during 2 hours

- *Plasma insulin and C-peptide* were measured by electro-chemiluminescent immuno-assay; insulin plasma measurement unit is pmol/L and that of C-peptide nmol/L.

- *Plasma 25(OH)D* was measured by electro-chemiluminescent immuno-assay, the measurement unit is nmol/L.

**Pregnant women at GW**

**24 – 28**

**Vitamin D sufficient (n = 19)**

**GDM (n = 104)**

**NGDM (n = 55)**

**Vitamin D insufficient (n = 85)**

**Vitamin D3**

**500 IU/day (n = 30)**

**-Vitamin D insufficiency prevalence**

**-Insulin, C-peptide, HOMA2-IR**

**-Relationships between plasma 25(OH)D & HOMA2-IR**

**Comparison:**

**- Insulin, C-peptide**

**- HOMA2-IR**

***Figure 2.1.* Study design**

**Vitamin D3**

**1500 IU/day (n = 30)**

**Comparison at the baseline (Visit 1):**

**- Weight gain, BMI**

**- Plasma 25(OH)D**

**- Blood Glucose, HbA1c,**

**- Insulin, C-peptide, HOMA2-IR**

**Comparison at GW 36-38 (Visit 3):**

**- Weight gain, BMI**

**- Plasma 25(OH)D**

**- Blood Glucose, HbA1c**

**- Insulin, C-peptide, HOMA2-IR**

**Comparison at GW 31-33 (Visit 2):**

**- Weight gain, BMI**

**- Blood Glucose, HbA1c**

**2.7. Diagnosis and assessment criteria**

- GDM was diagnosed according to ADA 2011 criteria with 75g.

- Vitamin D status was assessed according ES 2011 criteria.

- Pregnant women pre-conception BMI was assessed according to the International Diabetes Federation criteria applied for the Asian:

 Increased: BMI ≥ 23,0; Not increased: BMI < 23,0 kg/m2

- Plasma insulin and C-peptide were assessed using the cut-off point of  ± 1SD of the control (NGDM) group.

- IR was calculated by HOMA2 calculator, version 2.2.3. 2013 produced by The Oxford University (UK).

 HOMA2-IR calculated with fasting glucose and insulin (HOMA2-IR-In) or C-peptide (HOMA2-IR-Cp).

- Increased IR was asserted when HOMA2-IR value was above the highest quartile of that of the control (NGDM) group (WHO 1999).

**2.8. Data analysis**

 Software SPSS13.0 was used to analyzing data.

 Vitamin D insufficiency prevalence was calculated as percents. The relationships between plasma 25(OH)D level and IR were examined by linear correlation and comparison of HOMA2-IR indices between vitamin D sufficiency and insufficiency groups. The effects of vitamin D supplementation on IR were examined by comparison of HOMA2-IR indices between two vitamin D supplementation groups after vitamin D supplementation.

**Chapter 3. STUDY RESULTS**

**3.1. Study subject characteristics**

 104 pregnant women with GDM and 55 ones without GDM (NGDM) determined at GW 24 - 28 participated in the study.

*Table 3.1.* **Distribution of study subjects according to age groups and mean age**

|  |  |  |  |
| --- | --- | --- | --- |
| **Age** | **NGDM(n = 55)** | **GDM (n = 104)** | **P value** |
| **n** | **Percentage** | **n** | **Percentage** |
| < 25 | 9 | 16.4 | 5 | 4.8 | ***< 0.05*** |
| 25 – 29 | 21 | 38.2 | 39 | 37.5 | *> 0.05* |
| 30 - 34 | 20 | 36.3 | 36 | 34.6 | *> 0.05* |
| ≥ 35 | 5 | 9.1 | 24 | 23.1 | ***< 0.05*** |
|  ± SD | 28.9 ± 4.3 | 30.8 ± 4.4 |  ***0.01*** |

***- Gestation week:*** There were no significant differences in distribution by GW and mean GW between GDM and NGDM groups (26.6 ± 1.3 and 26.9 ± 1.3 weeks, respectively, p > 0,05).

**3.2. Vitamin D status and its relationships**

Vitamin D insufficiencyprevalence in GDM group was 81.7%



*Chart 3.2.*  **Vitamin D status in GDM and NGDM groups**

*Note:* The values are  (SD)

 GDM group had significantly lower plasma 25(OH)D level and higher rate of vitamin D insufficiency, the GDM risk for vitamin D insufficiency increased by 2.18 times (95%CI 1.03 to 4.61) (chart 3.2)

*Table 3.2.* **Linear correlation between plasma 25(OH)D level and other factors in GDM group**

| **Factor** | **r** | **p value**  |
| --- | --- | --- |
| Age | 0.130 | *0.189* |
| Gestation week | 0.019 | *0.486* |
| Pre-conception BMI  | 0.006 | *0.951* |
| Weight gain from conception to visit 1 | -0.201 | ***0.041*** |
| BMI increment from conception to visit 1  | -0.230 | ***0.019*** |
| BMI at visit 1  | -0.122 | *0.219* |

*Table 3.3.* **Correlation between plasma 25(OH)D and blood glucose in OGTT**

| **Plasma glucose**(mmol/L) | **NGDM****(n = 55)** | **GDM****(n = 104)** | **All****(n = 159)** |
| --- | --- | --- | --- |
| **r** | **p** | **r** | **p** | **r** | **p** |
| 0 h  | -0,158 | *0,248* | -0,074 | *0,456* | -0,186 | ***0,019*** |
| 1 h | -0,206 | *0,132* | -0,033 | *0,740* | -0,232 | ***0,003*** |
| 2 h | -0,093 | *0,500* | 0,106 | *0,282* | -0,117 | 0,143 |

 When taking both groups together, plasma 25(OH)D level was significantly inversely correlated to plasma glucose levels at 0 and 1 h in the OGTT (tab. 3.3).

**3.3. Insulin resistance and related factors**

*Table 3.4.* **HOMA2-IR-In in GDM and NGDM groups**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **KNGDM****(n = 55)** | **GDM****(n = 104)** | **p** |
| HOMA2-IR-In |  ± SD | 1.16 ± 0.44 | 1.44 ± 0.63 | ***0.001*** |
| *Mean difference* | *24.1%* |  |
| Upper cut-off-point | 1.42 |  |
| Increased (n, %) |  13 (23.6%) | 45 (43.3%) | ***<0.05*** |

GDM group had higher mean HOMA2-IR-In, by 24.1% and higher increased IR rate compared with NGDM one (tab. 3.4).

*Table 3.5.* **HOMA2-IR indices by preconception BMI status in GDM group**

|  |  |  |  |
| --- | --- | --- | --- |
| **Pre-conception BMI** | **n** | **HOMA2-IR-In** | **HOMA2-IR-Cp** |
| < 23 (kg/m2) | 86 | 1.39 ± 0.61 | 1.45 ± 0.59 |
| ≥ 23 (kg/m2) | 18 | 1.72 ± 0.67 | 1.82 ± 0.59 |
|  *p value* |  | ***< 0.05*** | ***< 0.05*** |

 Pregnant women with increased pre-conception BMI (≥ 23kg/m2) had significantly higher HOMA2-IR indices compared with the ones without increased pre-conception BMI (< 23 kg/m2) (tab. 3.5).

 HOMA2-IR indices were significantly correlated to pre-conception BMI, weight gain and BMI increment from conception to visit 1, BMI at visit 1 and fasting plasma triglyceride (tab. 3.6).

*Table 3.6.* **Linear correlation between HOMA2-IR indices and other factors in GDM group (n = 104)**

| **Factor** | **HOMA2-IR-In** | **HOMA2-IR-Cp** |
| --- | --- | --- |
|  **r** |  ***p*** |  **r** |  ***p*** |
| Age | -0,009 | *0,913* | -0,121 | *0,220* |
| Gestation week | 0,054 | *0,203* | 0,195 | ***0,047*** |
| Preconception BMI | 0,250 | ***0,001*** | 0,286 | ***0,003*** |
| Weight gain\*  | 0,354 | ***< 0,001*** | 0,274 | ***0,005*** |
| BMI at visit 1 | 0,387 | ***< 0,001*** | 0,371 | ***<0,001*** |
| BMI increment\*  | 0,356 | ***< 0,001*** | 0,277 | ***0,004*** |
| Fasting Triglyceride | 0,323 | ***0,001*** | 0,197 | ***0,045*** |
| Fasting HDL-C | -0,006 | *0,952* | 0,137 | *0,166* |

*Note:* from conception to visit 1

**3.4. Relationships between plasma 25(OH)D and IR**

***3.4.1. Linear correlation between plasma 25(OH)D and IR***



*Chart 3.3****.* Correlation between plasma 25(OH)D to HOMA2-IR indices**

 Plasma 25(OH)D level was significantly inversely correlated to fasting plasma insulin and C-peptide, and HOMA2-IR indices.

*Table 3.7.* **Correlation between plasma 25(OH)D and HOMA2-IR indices in multivariate linear regression model in GDM group**

|  |  |  |
| --- | --- | --- |
| **Factor** |  **HOMA2-IR-In** |  **HOMA2-IR-Cp** |
|  **r** |  ***p*** |  **r**  |  ***p*** |
| Model | 0,464 | ***<0,001*** | 0,496 | ***<0,001*** |
| Constant | 0,218 | *0,877* | -1,809 | *0,170* |
| Gestation week  | 0,040 | *0,591* | 0,190 | ***0,039*** |
| Preconception BMI | 0,158 | *0,102* | 0,278 | ***0,003*** |
| BMI increment | 0,158 | *0,129* | 0,121 | *0,215* |
| Plasma 25(OH)D | -0,225 | ***0,018*** | -0,283 | ***0,002*** |
| Fasting Triglyceride | 0,199 | ***0,041*** | 0,038 | *0,688* |

 Plasma 25(OH)D remained significantly inversely correlated to HOMA-2-IR values in the multivariate linear regression model.

***3.4.2. Relationships between vitamin D status and IR***

*Table 3.8.* **HOMA2-IR indices by vitamin D status in GDM group**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Vitamin D insufficient****(n = 85)**  | **Vitamin D sufficient****(n = 19)** | ***p*** |
| HOMA2-IR-In | 1,51 ± 0,64 | 1,15 ± 0,50 | ***<0,05*** |
| HOMA2-IR-Cp | 1,58 ± 0,61 | 1,20 ± 0,45 | ***<0,05*** |

 Vitamin D insufficient group had significantly higher HOMA2-IR indices (tab. 3.8).

*Table 3.9****.* ANCOVA model with HOMA2-IR indices in GDM group**

|  |  |  |
| --- | --- | --- |
| **Factor** | **HOMA2-IR-In** | **HOMA2-IR-Cp** |
| ***p*** | **PES** | ***p*** | **PES** |
| Model  | ***<0,001*** | 0,211 | ***<0,001*** | 0,206 |
| Intercept | *0,755* | 0,001 | *0,383* | 0,008 |
| Vitamin D status | ***0,028*** | 0,048 | ***0,009*** | 0,067 |
| Increased preconception BMI | *0,082* | 0,030 | ***0,020*** | 0,054 |
| Gestation week at visit 1 | *0,729* | 0,001 | *0,071* | 0,033 |
| BMI increment | *0,067* | 0,034 | *0,102* | 0,027 |
| Fasting Triglyceride  | ***0,016*** | 0,057 | *0,344* | 0,009 |

*Note :* PES: Partial Eta Squared

 Vitamin D status was significantly associated with HOMA2-IR-In (p = 0,028) and HOMA2-IR-Cp (p = 0,009) in the analysis of covariance model (tab. 3.9).

**3.5. Effects of vitamin D supplementation on IR**

 60 pregnant with GDM at GW 24 – 28 and vitamin D insufficiency were randomly allocated to two groups: one receiving daily 500IU and the other daily 1500IU of vitamin D3.

***3.5.1. Characteristics of subjects before and after vitamin D supplementation***

 Before vitamin D supplementation, there were no differences between 2 supplementation groups in age, GW, pre-conception BMI, weight gain and BMI increment from conception to visit 1, BMI at visit 1, obstetrical and familial diabetes history, blood biochemical parameters and HOMA2-IR indices.

 There were no differences between the two groups in GWs at all the visits, vitamin D supplementation duration (9.7 ± 1.5 and 9.9 ± 1.7 weeks in 500 IU and 1500 IU groups, respectively, p > 0,05), weight gains and BMI increments from conception to all the visits and BMI at all the visits.

***3.5.2. Vitamin D change after vitamin D supplementation***

*Chart 3.4****.* Plasma 25(OH)D before and after vitamin D supplementation**

*Note:* Values are  (SD); p: comparison between 2 groups at each visit; visit 1 vs. visit 3 in each group: † p<0,01, ‡ p<0,001.

 After vitamin D supplementation 1500 IU group had higher plasma 25(OH)D (79.82 ± 10.11 vs. 67.41 ± 10.62 nmol/L, p < 0.001) and higher increment (16.91 ± 9.64 vs. 6.00 ± 10.48 nmol/L, p < 0.001 (chart 3.4).

*Chart 3.5.* **Vitamin D status**

**after vitamin D supplementation**

 After supplementation, 1500 IU group had significantly higher vitamin D sufficient rate than 500 IU one (chart 3.5).

***3.5.3. Treatment of GDM***

 The rate treatment with insulin and diet combination was 6.7% (n=2) and 10.0% (n = 3) in 500 IU and 1500 IU groups, respectively, the difference was not statistically significant.

***3.5.4. Changes of FPG and HbA1c after vitamin D supplementation***

*Chart 3.6.* **Fasting plasma glucose and HbA1c during the follow-up**

*Note:* Values are  (SD); p: comparison between 2 groups at each visit; visit 1 vs. visit 2 and 3 in each group: NS: non-significant, \*: p < 0.05; †: p < 0.01; ‡: p < 0.001.

 - 1500 IU group had lower FPG than 500 IU group at visit 3. FPG of 500 IU group did not change through the visits, meanwhile FPG of 1500 IU group significantly decreased at visit 2 and 3 compared with visit 1.

 - HbA1c of 500 IU group significantly increased at visit 2 and 3 compared with visit 1, meanwhile HbA1c of 1500 IU group did not significantly change through the visits.

***3.5.5. Changes of HOMA2-IR indices after vitamin D supplementation***

*Chart 3.7.* **HOMA2-IR indices from visit 1 to visit 3**

*Note:* Subjects treated with insulin were excluded; values are  (SD); p: comparison between 2 group at each visit; visit 1 vs. visit 2 and 3 in each group: NS: non-significant, \*: p < 0.05; †: p < 0.01; ‡: p < 0.001.

- At visit 3, 1500 IU group had significantly lower HOMA2-IR-In (1.64 ± 0.54 vs. 2.05 ± 0.54, *p < 0.01*) and lower HOMA2-IR-Cp (1.62 ± 0.50 vs.2.01 ± 0.50, *p < 0.01*).

- Changes of HOMA2-IR indices from visit 1(GW 24 – 28) to visit 3 (GW 36 – 38) after vitamin D supplementation: Both HOMA2-IR indices in 500 IU significantly increased, meanwhile only HOMA2-IR-In significantly increased but HOMA2-IR-Cp did not.

- The increments of HOMA2-IR indices of 1500 IU group were significantly lower than 500 IU group (0.18 ± 0.41 vs. 0.58 ± 0.57, *p < 0.01* and 0.10 ± 0.33 vs. 0.48 ± 0.47, *p = 0.001* for HOMA2-IR-In and HOMA2-IR-Cp increments, respectively).



*Chart 3.8.* **Changes of HOMA2-IR indices in percentage from visit 1 to visit 3**

*Note:* Values are  (SD); p: comparison between 2 groups

 - 1500 IU group had significantly higher percentage increases in HOMA2-IR indices than 500 IU group: HOMA2-IR-In increased by 18.6 ± 31.1% compared with 64.6 ± 74.7% (*p < 0.01*); HOMA2-IR-Cp increased by 9.4 ± 20.8% compared with 41.6 ± 38.6% (*p < 0.001*).

 - When taking HOMA-IR changes of 500 IU group as 100%, the increases in HOMA2-IR indices of 1500 IU group (relative differences) were lower by 71.2% and 77.4% in respect to HOMA2-IR-In and HOMA2-IR-Cp, respectively.

**Chapter 4. DISCUSSIONS**

**4.1. Study subjects**

 A total of 104 pregnant women with and 55 without GDM participated in the study, meeting the sample size requirements. The subjects were relatively homogenous in respect to GW (24 – 28 weeks) and blood glucose levels (subjects with “overt diabetes having higher blood glucose levels were excluded), which minimized confounding in exploring relationships between vitamin D and IR.

**4.2. Vitamin D status and related factors**

***4.2.1. Vitamin D insufficiency prevalence***

 The prevalence of vitamin D insufficiency, vitamin D deficiency and overall vitamin D insufficiency was 73.1%, 8.6% and 81.7%, respectively (chart 3.1). The vitamin D insufficiency prevalence is substantially higher than in other studies in pregnant and non-pregnant women in Vietnam: 58.6% and 52.0% in women of reproductive age in Hanoi and Hai Duong, respectively (V.T.T. Hien, 2007), 60% in pregnant women in a rural area of Ha Nam. Thus, vitamin D insufficiency is very common in Vietnam that although is located in the tropical zone and routine vitamin D supplementation for pregnant women, especially in the North part may be necessary.

***4.2.2. Plasma 25(OH)D level and related factors***

 Plasma 25(OH)D level was inversely correlated to weight gain and BMI increment from the conception to visit 1 (r = -0.201, *p = 0.041* and r = -0.231, *p = 0.019,* respectively) (tab. 3.2). The association between vitamin D deficiency and overweight or obesity could related to increased sequestration of vitamin D in adipose tissue in these conditions.

**4.3. Insulin resistance and related factors**

GDM group higher mean HOMA2-IR-In and higher rate of increased HOMA2-IR-In than NGDM one (tab. 3.4). Pregnant women with GDM have elevated IR compared with normal ones because besides physiologic pregnancy IR the former also have chronic IR existing before conception (Catalano 1997, Xiang 1999).

 HOMA2-IR indices of GDM group were significantly correlated to preconception BMI, weight gain and BMI increment from conception to visit 1, BMI at visit 1, fasting plasma triglyceride that are traits of IR present in metabolic syndrome, type 2 DM and GDM in which IR is the central factor.

**4.4. Relationships between vitamin D with insulin resistance, blood glucose and GDM**

***4.4.1. Relationships between vitamin D and insulin resistance***

*\* Linear correlation between plasma 25(OH)D and IR*

 Plasma 25(OH)D level was inversely correlated to HOMA2-IR indices in in univariate model (chart 3.3). In multivariate regression model with factors related to IR (GW, pre-conception BMI and present BMI at visit 1, BMI increment from conception to visit 1, plasma triglyceride), plasma 25(OH)D remained significantly inversely correlated to HOMA2-IR-In and HOMA2-IR-Cp with standardized β -0,225 (*p = 0,018*) and -0,283 (*p = 0,002*), respectively (table 3.7). *Thus, plasma 25(OH)D level was independently inversely correlated to IR in women with GDM.*

*\* Relationships between vitamin D status and IR*

 The vitamin D insufficient group had significantly higher and HOMA2-IR indices compared with the sufficient one (tab. 3.8). In the ANCOVA model in which HOMA2-IR was dependent variable and independent ones were vitamin D status and other related to IR including pre-conception BMI category (< 23 or ≥ 23 kg/m2), BMI at visit 1, BMI increment from conception to visit 1 and fasting plasma triglyceride, vitamin D insufficiency remained significantly associated to increased HOMA2-IR-In and HOMA2-IR-Cp (tab. 3.9). *Thus, vitamin D insufficiency was independently associated to increased insulin resistance in women with GDM.*

 Numerous studies worldwide had similar results: Maghbooli’s study (2008): plasma 25(OH)D was significantly inversely correlated to HOMA-IR (r = -0.20, *p = 0.002*)in pregnant women at GW 24 – 28 and the association remained significant when adjusted by BMI and parity number in multivariate analysis. Lacroix’s study (2014): plasma 25(OH)D at GW 6-13 was inversely correlated to HOMA-IR (r = -0.08, *p = 0.03*) and correlated to insulin sensitivity index Mastuda (r = 0.13, *p = 0.001*) at GW 24-28, and the associations remained significant when adjusted by the pregnant women waist circumference at GW 6-13 and plasma parathormone level.

 *In summary, the study results demonstrated that plasma 25(OH)D was inversely related to IR in women with GDM and the association could be independent, that is vitamin D insufficiency could contribute to increased IR in those subjects. That could explain the association between decreased plasma 25(OH)D level with elevated blood glucose and increased risk of GDM as discussed above.*

***4.4.2. Relationships between vitamin D and blood glucose***

 When taking both GDM and NGDM groups, plasma 25(OH)D was significantly inversely correlated to plasma glucose levels at 0 and 1 hours in the OGTT (tab. 3.3). Similar studies showed that plasma 25(OH)D was inversely correlated to fasting plasma glucose (r = - 0.20; CI95% -0.31 − -0,08, Clifton-Bligh 2008) and fasting plasma glucose and OGTT 1-h plasma glucose (r = - 0.16, *p = 0.05* in both cases, Lau 2011).

***­4.4.3. Relationships between vitamin D and GDM***

 GDM group had significant higher plasma 25(OH)D level compared to NGDM one, and GDM risk increased by 2.18 times with vitamin D insufficiency (95% CI: 1.03 – 4.61) (chart 3.2). Meta-analysis studies showed that GDM risk due to vitamin D insufficiency increased by 1.609 times (95%CI: 1,19 – 2.17, Poel 2012) and by 1.49 times (95%CI: 1,18 – 1,89, Aghajafari 2013).

**4.5. Effects of vitamin D supplementation vitamin D on IR**

***4.5.1. Study design***

 IR in pregnant women starts to rise progressively from the beginning of pregnancy second half until its end, therefore study designs comparing two different vitamin D doses are appropriate to test a hypothesis that the higher dose results in less increase of IR during this period.

 The study chose two vitamin D3 doses, daily 500 IU, close to the Institute of Medicine daily recommended allowance (600 IU daily), and daily 1500 IU that is the lower limit of Endocrine Society recommended daily requirement range (1500 – 2000 IU daily) to compare their effects on IR.

***4.5.2. Characteristics of study subjects during vitamin D supplementation***

 There were no differences between two vitamin D supplementation groups in IR related characteristics and HOMA2-IR indices before the supplementation. There were not either differences between two groups in weight gains, BMI increments and gestation weeks at all the visits, and supplementation duration. That ensured that the comparison the vitamin D effects between two groups not confounded by these factors.

***4.5.3. Vitamin D supplementation duration***

 Mean vitamin D supplementation duration is similar between two groups ((9.7 ± 1.5 weeks and 9.9 ± 1.7 weeks, *p > 0,05*). Such vitamin D supplementation durations were long enough to detect its effects on IR. Two trials by Asemi demonstrated that vitamin D supplementation for 6 and 9 weeks reduced IR in pregnant women.

***4.5.4. Change of vitamin D after its supplementation***

 Vitamin D supplementation with 1500 IU/day was much more effective in respect to plasma 25(OH)D level (79.82 ± 10.11 vs. 67.41 ± 10.62 nmol/L, respectively, *p < 0,001*), its increment (16.91 ± 9.64 vs. 6.00±10.48 nmol/L, respectively, *p < 0,001*, chart 3.4), and vitamin D sufficiency rate (70.0% vs. 23.3%, respectively, *p < 0,001*, chart 3.5).

***4.5.5. GDM treatment methods***

 Two (6.7%) and three (10.0%) pregnant women in 500 IU and 1500 IU group, respectively had insulin therapy, the difference was not significant with *p > 0,05*. Those subjects were excluded from analysis involving insulin, C-peptide and HOMA2-IR indices. The similar insulin treatment rates of 2 groups minimized the subject exclusion influence on comparison of vitamin D supplementation between 2 groups.

***4.5.6. Changes in blood glucose, HbA1c and HOMA2-IR indices***

*4.6.6.1. Changes of blood glucose status*

 1500 IU group had better FPG evolution than 500 IU one: the former had lower FPG at visit 3 compared with the latter; FPG of the former significantly decreased at visit 2 and 3 compared with visit 1, meanwhile FPG of 500 IU group did not significantly change through all the visits (chart 3.6).

 1500 IU group had better HbA1c evolution than 500 IU one: HbA1c of the former did not significantly change through all the visits, meanwhile HbA1c of the latter at visit 2 and 3 were significantly increased compared with visit 1 (chart 3.6).

 Because both groups had similar blood glucose before vitamin D supplementation and were educated on diet and exercise in the similar manner, vitamin D supplementation with daily 1500 IU could have improving effects on blood glucose compared with daily 500 IU.

*4.5.6.2. Changes in HOMA2-IR indices*

 After vitamin D supplementation, 1500 IU had clearly better IR status than 500 IU one:

- The former had a significant increase in only HOMA2-IR but not in HOMA2-IR-Cp, meanwhile the latter had significantly increased both HOMA2-IR indices (chart 3.7).

- At visit 3, the former had significant lower HOMA2-IR-In (chart 3.7).

- The former had significant lower increments of HOMA2-IR (chart 3.7).

- The former had significant lower percentage increments in HOMA2-IR-In (chart 3.8).

- When taking the increments in HOMA2-IR indices of 500 IU group as 100%, the increments of 1500 IU group were reduced by 71.2% and 77.4% by HOMA2-IR-In and HOMA2-IR-Cp, respectively (chart 3.8).

 IR progressively increases from the beginning of pregnancy second half until delivery. *Thus, vitamin D supplementation with 1500 IU/day could not absolutely reduce IR, but could substantially reduce IR increase for period from GWs 24 – 28 to GWs 36 – 38.*

 Other abroad studies also demonstrated that vitamin D supplementation versus placebo or vitamin D supplementation with higher doses versus lower doses reduced IR in pregnant women with and without GDM.

 - In a study by Asemi and co-workers vitamin D supplementation with daily 400 IU in pregnant women for 9 weeks, starting from GW 25, lowered FPG more than placebo (-0.65 vs. -0.12 mg/dL, *p = 0.01*) and decreased HOMA-IR, meanwhile the placebo increased this index (-0,34 vs. +0,60, p= 0,06).

 - In another study by Asemi and co-workers vitamin D supplementation with two doses of 50,000 IU two weeks apart with placebo in pregnant women at GWs 24 - 28 for 6 weeks decreased FPG (-17.12 ± 14.84mg/dl, *p < 0.001*) and HOMA-IR (-1.21 ± 1.41, *p = 0.001*), meanwhile, the placebo did not significantly change these indices.

 - A study by Soheilykhah and co-workers compared different vitamin D doses supplemented for pregnant women from GW 12 until the delivery. After the supplementation, the group with vitamin D supplementation of 50,000 IU every two weeks had lower increases in fasting plasma insulin (3.58 ± 4.16 vs. 6.9 ± 7 IU/ml, *p = 0.01*) and lower increases in HOMA1-IR (0.7 ± 1.04 vs. 1.46 ± 1.69, *p = 0.02*) compared with the group receiving daily 200 IU.

 In pregnant women, particularly in the ones with GDM, IR progressively increases from the beginning second half until the end of pregnancy. The present study compared effects of two vitamin D doses. The daily dose of 1500 IU resulted in better blood glucose and, although did not absolutely decreased IR, did reduced IR increase for that period compared with daily dose of 500 IU. The results were similar with those of Soheilykhah’s study in which higher doses of vitamin D (daily 2000 IU and daily 4000 IU) could only reduce IR increase from the pregnancy beginning to its end but could not absolutely decrease IR for this period.

 Vitamin D decreases IR by a number of mechanisms demonstrated in experiments, including the following: Increasing insulin receptor expression; Stimulating of synthesis of PPARδ, a transcription factor of proteins participating in lipid metabolism; Maintaining intracellular calcium ion level; Suppressing pro-inflammatory cytokines such as TNF-α, Interleukin-1β, Interleukin-6; Suppressing renin – angiotensin system.

**CONCLUSIONS**

 The study on 104 pregnant with GDM at The National Hospital of Gynecology & Obstetrics and The National Hospital of Endocrinology had the following conclusions:

**1. Vitamin D insufficiency prevalence in women with GDM** in gestation weeks 24 to 28 was 81.7%.

**2. Plasma 25(OH) D concentration was inversely associated to insulin resistance in pregnant women with GDM in gestational weeks 24 to 28.**

 Plasma 25(OH)D concentration was inversely correlated to insulin resistance with r = -0.298, p = 0.002 and r = -0.314, p = 0,001 for HOMA2-IR indices assessed by fasting plasma insulin and C-peptide, respectively. The association remained significant when adjusted by gestation week, preconception BMI, present BMI, BMI increment from conception and fasting plasma triglyceride.

 Vitamin D insufficiency was associated to increased insulin resistance. The association remained significant when adjusted by preconception BMI increase, BMI increment from conception, present BMI and fasting plasma triglyceride.

**3. Vitamin D supplementation with daily 1500 IU for period from gestation weeks 24-28 until 36-38 in pregnant women with GDM reduced insulin resistance compared with daily 500 IU.**

 After vitamin D supplementation, 1500 IU group reduced insulin resistance increase by 71.2% and 77.4% by HOMA2-IR indices assessed by fasting plasma insulin and C-peptide, respectively, compared with 500 IU one.

**RECOMMENDATIONS**

1. Because vitamin D insufficency is very common in women with GDM, this condition should be routinely detected in this group and vitamin D be supplemented for those with this condition.

2. It is necessary to further study effects of vitamin D supplementation as adjuvant therapy of GDM in women with vitamin D insufficiency, especially vitamin D deficiency, and effects on GDM prevention in early pregnancy, particularly in women at high risk for both conditions.