VITAMIN D LEVELS IN UMBILICAL CORD AND ITS IMPACT ON INFANT AND CHILDREN'S HEALTH: A COHORT STUDY (VITADi STUDY)

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Protocol

Responsible Personnel

Principal Invesitagor Contact Information
Name: Sri Hardiyanti Putri
Title: MD
Institution: Department of Pediatrics, Faculty of Medicine, Hasanuddin University
Address: Jln. Perintis Kemerdekaan KM 11 Makassar, South Sulawesi, Indonesia, 90245
Telephone: +628114499339
Email: srihardiyantiputri@gmail.com

Other Contact
Name: Bahrul Fikri
Title: MD, PhD
Institution: Allergy and Immunology Division, Department of Pediatrics, Faculty of Medicine, Hasanuddin University
Address: Jln. Perintis Kemerdekaan KM 11 Makassar, South Sulawesi, Indonesia, 90245
Telephone: +62(411)584461
Email: bahrulfikri@unhas.ac.id

Summary:

This study is a cohort study that aims to assess the relationship between Vitamin D levels in infants and children, and the risk of anemia, atopic dermatitis, diarrhea and cardiovascular disorders in newborn. The subjects of this study are 100 women who met the inclusion criteria, which are in their third semester of pregnancy and agree to be included in this study with their babies. These subjects then will be examined for their weight, height and upper arm circumference using standardized CEBA digital scale, and blood sampling will also be done to these women to measure their vitamin D levels, calcium, parathyroid hormone, ferritin, Fe serum, IL-16, and IL-10. When subjects give birth, the babies will also be included in this study. Anthropometric examination will also be done on the babies, to measure their birth weight, birth length, and head circumference. Blood samples will also be taken from their umbilical cord to assess their complete blood count, ferritin, Fe serum, vitamin D levels, vitamin D receptors, IL-6 and IL-10. All subjects then will be monitored, and home visits will be done when the babies reach 3 months old and 6 months old. An assessment of the incidence of diarrhea, atopic dermatitis, and an assessment of cardiovascular disorders (checking pulse and blood pressure) will be carried out during the visit. At the end of the study, venous blood sampling will be taken to see levels of Vitamin D, calcium, parathyroid hormone, routine complete blood count, serum Fe, and ferritin, then data analysis is performed.

Introduction:

Vitamin D is essential for development, growth and the maintenance of the bone. (Huang et al., 2021) Although the main role of vitamin D is to maintain calcium homeostasis, vitamin D also has many roles in various organ system, including the immune system. The presence of vitamin D receptor (VDR) expression in immune system experimentally proves that vitamin D also plays an essential role in innate and adaptive immune system. Vitamin D is involved in immune system through intracellular VDR in monocyte/macrophage, T cells, B cells, natural killer cells (NK), and dendritic cells (DC). (Cyprian et al., 2019)

The presence of vitamin D receptors in the immunologic system, confers huge influence on the incidence of infectious diseases and allergies, such as diarrhea and atopic dermatitis. (Aluisio et al., 2013) (El Taieb, 2013, Kim JM, 2016) Epidemiological literature also explains that both vitamin D deficiency and insufficiency contribute to the incidence of cardiovascular disease and the incidence of metabolic syndrome, namely hypertension, obesity, insulin resistance and glucose intolerance. (Adams & Hewison, 2010) Several epidemiological studies and clinical trials also have found that vitamin D plays a role in iron homeostasis and erythropoiesis, hence the causal relationship with anemia. (Uwaezuoke, 2017)

The vitamin D levels of the intrauterine fetus is completely influenced by maternal vitamin D levels. 25-OH Vitamin D will go through the placental barrier and then into the baby's circulatory system with a half-life of about 2 months. (Wierzejska et al., 2017) There are several factors that influence vitamin D levels, such as maternal nutritional status, intake of calcium and phosphorus, maternal body mass index, and socioeconomic factors, meanwhile the main sources of vitamin D are sun exposure, food intake, supplementation, and fortified foods. (Holick, 2007)

Indonesia is a tropical country with high levels of sun exposure, but in fact vitamin D deficiency is still a problem that persists in the country. Data shows that children aged 2-12.9 years in Indonesia experience vitamin D insufficiency as much as 45.1%. (Koon Poh et al., 2016) The prevalence of pregnant women suffering from vitamin D deficiency is also quite high. (Adams & Hewison, 2010; Ayu Krisna Cahyaning Putri et al., nd; Cerami, 2017) This also increases the incidence of this micronutrient deficiency in newborns which can affect the growth and development of children later. Lack of vitamin D can also increase the likelihood of children suffering from several diseases later in life. By looking at the importance of vitamin D, researchers are interested in assessing the extent of the influence of maternal vitamin D levels on neonatal vitamin D levels, and how this vitamin D status affects the physical condition of the child later. This study also aims to see the effect of sun exposure on children's physical status.

Objectives:

1. **General Objectives**
    a. To assess the relationship between neonatal vitamin D levels to the risk of anemia, atopic dermatitis, diarrhea, and cardiovascular disorders in newborns.
2. **Specific Objectives**
    a. To determine the third trimester pregnant women who do not have chronic infectious diseases and hypertension.

b. Measure the weight, height, and upper arm circumference of third trimester pregnant women.
c. Measure vitamin D levels, calcium, parathyroid hormone, ferritin, serum iron, routine blood, IL-16, and IL-10 in third trimester pregnant women.
d. Measure weight, body length, head circumference of newborns (whose mothers have been selected as research subjects)
e. Measure vitamin D levels, vitamin D receptors, IL-6, and IL-10 from umbilical cord blood of newborns (whose mothers have been selected as research subjects).
f. Monitor weight, body length, head circumference when the baby is 3 months old.
g. Monitor weight, body length, head circumference when the baby is 6 months old.
h. Measure levels of vitamin D, calcium, parathyroid hormone, ferritin, serum iron, routine blood, IL-6, and IL-10 when the baby is 6 months old.
i. Measure IgE levels when the baby is 6 months old.
j. Assess the incidence of diarrhea frequency in infants during monitoring.
k. Assess the incidence of atopic dermatitis in infants during monitoring.
l. Assess the incidence of hypertension during monitoring.
m. Check the levels of vitamin D, ferritin, hemoglobin, and reticulocytes when the baby is 6 months old.
n. Compare vitamin D levels in the umbilical cord blood of newborns with vitamin D levels at the age of 6 months.

Trial Design:
Observational study

Study Population

Women in third trimester of pregnancy in Makassar, Surabaya, Mataram who are selected during the screening test.

Number of Samples

100 samples.

Inclusion Criteria

a. Pregnant Women
- Women in third trimester of pregnancy
- Agree to take blood samples and are willing to include their babies as samples for the next study.

b. Infants
- Babies born from mothers who are willing to be included in the study
- Aterm
- Birth weight > 2500 grams

Exclusion Criteria

a. Pregnant women
- History of abortion in previous pregnancy
- Suffering from hypertension or preeclampsia

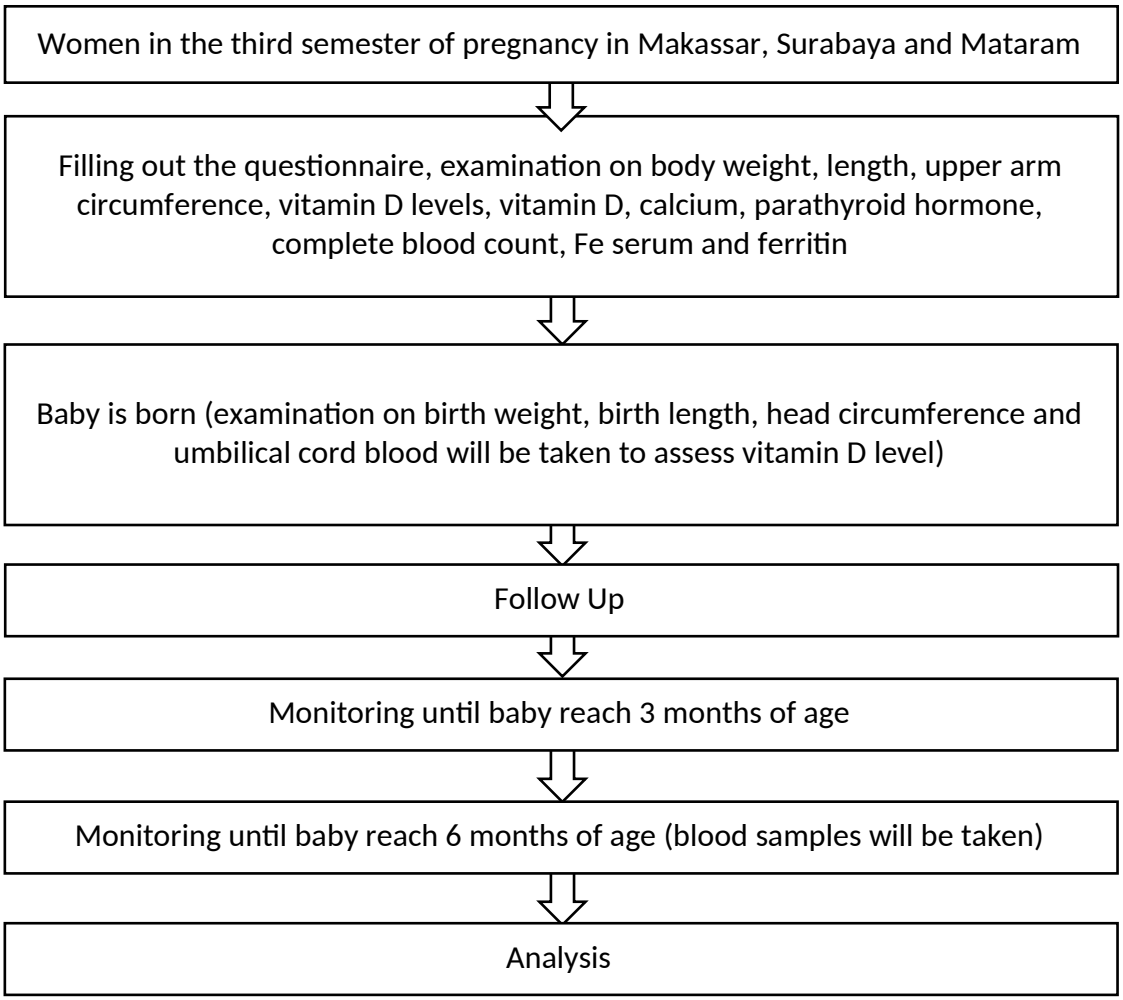
b. Infants
- Infants with congenital abnormalities at birth
- Infants with acute infection at birth

Procedures:

1. Sampling will be preceded by giving explanations to the parents, then parents will fill and sign the informed consent paper if they agree to participate in this study.
2. All pregnant women who met the requirements as research subjects will be recorded by their name, age, parity, birth history, and history of illness.
3. Anthropometric measurements will be performed on pregnant women who met the criteria, including body weight using standardized digital CEBA scales, height, and upper arm circumference.
4. The pre-analytic in this study is the preparation of tools and materials to take 3 cc of blood serum samples (2 tubes for 1 patient), purple capped tubes (with EDTA) for routine blood count tests, and red tubes for ferritin, Fe serum, vitamin D levels, calcium, parathyroid hormone, IL-6, and IL-10.
5. After the mother gives birth, the baby who are qualified based on the inclusion criteria will also be included.
6. Birth weight, birth length and head circumference will be measured.
7. The pre-analytic in this study is the preparation of tools and materials for blood and serum sampling, collection of infants blood samples from the umbilical cord and children from venous blood (2 tubes for 1 patient), purple capped tubes (with EDTA) for routine blood count tests, and red tubes for ferritin, Fe serum, vitamin D levels, vitamin D receptors, IL-6, and IL-10. The amount of blood samples taken for the purple closed tube is 1.5 ml and for the red tube is 1.5 ml (which is the minimum value for each tube for examination).
8. Monitoring will be carried out until the baby is 6 months old. Visits will be made when the baby reach 3 months and 6 months old. Anthropometric status measurements will be done on infants to measure their weight, length, and head circumference, then we will determine the nutritional status by plotting to the WHO curve. There will be interviews with the parents/caregivers regarding complaints of diarrhea and signs of allergies such red rash and an assessment based on Hanifin Rajka's criteria will be made.
9. At the age of 6 months, a blood sample will be taken again. The pre-analytic in this study is the preparation of tools and materials for blood and serum sampling, collection of blood samples for infants from the umbilical cord and children from venous blood (2 tubes for 1 patient), purple capped tubes (with EDTA) for routine blood tests, and red tubes for examination of ferritin, serum Fe, levels of vitamin D, IL-6, and IL-10. The number of blood samples taken for the purple closed tube is 1.5 ml and for the red tube it is 1.5 ml (which is the minimum value for each tube for examination).

10. Unpleasant sensations like pain during blood sampling and fear of being stabbed with a needle will be minimalized by using experts in this field, such as laboratory assistant and a doctor as a companion.

Workflow



Data Analysis

The collected data are grouped based on the purpose and type of data, then the suitable statistical methods are used, namely:

a. Univariate analysis
   Used to describe the characteristics of the basic data, in the form of frequency, mean value, standard deviation, and range.
b. Bivariate analysis
   1) Wilcoxon test: Used to analyse data with independent variables with an ordinal/numeric scale, the data are not normally distributed and have different variances.

2) Mann Whitney test: Non-parametric test to compare ordinal or numeric scale variables whose data are not normally distributed and have different variances between two unpaired groups.

The results of the hypothesis test are determined as follows:

1) Not meaningful if p-value > 0.05
2) Significant if $p \leq 0.05$
3) Very meaningful if $p < 0.01$

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