KSÜ Tarım ve Doğa Derg 26 (3), 471-476, 2023
KSU J. Agric Nat 26 (3), 471-476,2023
https://doi.org/10.18016/ksutarimdoga.vi.1175469

Evaluation of Some Elements (Na^{+}, Cl^{-}, K^{+}, Fe^{+2}, Mg^{+2}, Ca^{+2}, Cu^{+2}, Zn^{+2}) Levels in Vernix Caseosa Substance

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ABSTRACT
The substance "Vernix Caseosa" (VC), which begins to be secreted in the third trimester of the human fetus, is a natural product that occurs spontaneously. This substance, which protects the baby against external factors, has a feature that facilitates birth by providing lubrication during normal birth. 52 infants were included in the study. A sterile soft-tipped device was used to collect the VC, and a swab was taken from the baby's skin immediately after birth. In addition, mothers under the age of 35 and over the age of 35 were grouped as multiparous and nulliparous and their VC was examined separately. In VC, the levels of the elements responsible for production and repair in the human body were investigated. Homogenized samples were measured by Atomic Absorption Spectrophotometer. Element results were given as mean and standard deviation; *Na+: 12.09 ± 0.58, *Cl-: 10.27 ± 1.08, *K+:1.84 ± 0.54, **Fe+2: 72.39 ± 4.84, **Mg+2: 67.07 ± 3.31, **Ca+2:864.51 ± 32.61, **Cu+2:12.98 ± 2.01, **Zn+2: 11.00 ± 1.55 (*:mg/g tissue, **:ug/g tissue). According to the results of literature review and to the best of knowledge, limited information on element levels of VC has been reported. The studied elements are found as cofactors in growth and development and in many biochemical mechanisms. Protein and lipid contents have been investigated in previous studies and it has been reported that VC has an important role in protecting the fetus from external factors and infections. In addition, the protection of amniotic fluid from maceration and the prevention of fluid and electrolyte losses of the skin are also mentioned. It is thought that this research will make an important contribution to the determination of the biochemical structure of VC, which is a natural and protective substance.

Biochemistry

Research Article

Article History
Received : 14.09.2022
Accepted : 15.11.2022

Keywords
Vernix caseosa
Elements
Newborn

Verniks Kazeoza Maddesinde Bulunan Bazı Element (Na^{+}, Cl^{-}, K^{+}, Fe^{+2}, Mg^{+2}, Ca^{+2}, Cu^{+2}, Zn^{+2}) Düzeylerinin Değerlendirilmesi

ÖZET
İnsan fetüsünun 3. trimestrinde salgılanmaya başlayan "Vernix Caseosa" (VC) maddesi kendiliğinden oluşan doğal bir üründür. Bebeği dış etkenlere karşı koruyan bu madde, normal doğum sırasında kayganlık sağlayarak doğumu kolaylaştıran bir özellik taşımaktadır. Çalışmaya 52 bebek dahil edilmiştir. VC toplanırken steril yumuşak uçlu aparat kullanılmıştır ve doğumdan hemen sonra bebeğin derisinden sürüntü şeklinde alınmıştır. Ayrıca 35 yaş altı ve 35 yaş üstü anneler, multipar ve nullipar olarak gruplandırılarak VC'si ayrı ayrı incelenmiştir. VC' da insan vücudunda bulunan, yapım ve onarımdan sorumlı elementlerin seviyeleri araştırılmıştır. Homojenize numuneler Atomik Absorbsiyon Spektrofotometre cihazı ölçülmüştür. Element sonuçları ortalama ve standart sapma olarak verildi; *Na+: 12.09 ± 0.58, *Cl-: 10.27 ± 1.08, *K+:1.84 ± 0.54, **Fe+2: 72.39 ± 4.84, **Mg+2: 67.07 ± 3.31, **Ca+2:864.51 ± 32.61, **Cu+2:12.98 ± 2.01, **Zn+2: 11.00 ± 1.55 (*:mg/g doku, **:ug/g doku). Literatür taramasının sonuçlarına göre ve bildindiği kadarıyla VC' nın element seviyeleri hakkında hiçbir bilgi rapor edilmemiştir. Çalışılan elementler, büyüme gelişmede ve birçok

Biyokimya

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 14.09.2022
Kabul Tarihi : 15.11.2022

Anahtar Kelimeler
Verniks kazeoza
Elementler
Yenidoğan

biyokimyasal mekanizmada kofaktör olarak bulunmaktadır. Daha önceki çalışmalarda protein ve lipid içerikleri araştırılmış ve VC'nin fetüsü dış etkenlerden ve enfeksiyonlardan korumada önemli rolü olduğu bildirilmiştir. Ek olarak amniyotik sıvının maserasyondan korunması ve cildin sıvı elektrolit kayıplarının önlenmesinden de bahsedilmiştir. Bu araştırmanın doğal ve koruyucu bir madde olan VC'nin biyokimyasal yapısının belirlenmesine önemli katkı sağlayacağı düşünülmektedir.

Atıf Şekli: Duyuran, R., Kılınç M.& Dağlı, H. (2023) Verniks Kazeoza Maddesinde Bulunan Bazı Element (Na+, Cl-, K+, Fe+2, Mg+2, Ca+2, Cu+2, Zn+2) Düzeylerinin Değerlendirilmesi . *KSÜ Tarım ve Doğa Derg 26* (3), 471-476. https://doi.org/10.18016/ksutarimdoga.vi. 1175469

To Cite : Duyuran, R., Kılınç M.& Dağlı, H. (2023). Evaluation of Some Elements (Na+, Cl-, K+, Fe+2, Mg+2, Ca+2, Cu+2, Zn+2) Levels in Vernix Caseosa Substance *KSU J. Agric Nat 26*(3), 471-476. https://doi.org/10.18016/ksutarimdoga.vi.1175469

INTRODUCTION

Vernix caseosa (VC) is a waxy or cheese-like white substance and is a layer formed by sebum, the sebaceous cells (Meng et al., 2021). The sebaceous cells formed in the skin of babies increase the secretion of oil by the effects of hormones that pass to them through their mothers. Its formation in the mother's womb begins in the last trimester (Visscher et al., 2015). It forms the vernix layer by combining it with the shredded epithelial cells from the skin of the baby (Nishijima et al., 2019). The periderm cells are continuously keratinized and poured into the amniotic fluid and get renewed from basal. The sloughed-off cells join to form VC, a protective layer that covers the fetal skin (Baker et al., 1995). VC along with sebum secretion of sebaceous glands protects the fetus in amniotic fluid throughout the fetal period (Paling & Koch, 2022). Sebaceous and sweat glands differentiate from the epidermis and develop into the dermis (Pickens et al., 2000). It is extremely rich in lipids but its composition has not yet been fully characterized despite many studies. The vernix is a biofilm that covers the fetus and is composed of about 80% water, 10% lipid, and 10% protein (Herman, 2022). This substance is formed by a human baby and is coated with a material that has endogenous cleaning ability before and after the birth of a baby. Such material is made up of lipids that can integrate easily with the skin surface and pores. The most abundant lipid classes found are wax esters, cholesterol esters, diol diesters, and triacylglycerols (Mihajlović et al., 2022). The high content of squalene and wax ester in VC is a significant portion of the lipid content and is strongly likely to be of sebaceous origin (Boiten et al., 2018). It is thought that several factors affect the vernix structure during the formation of VC such as the age of the pregnancy, the health of the mother and the baby, the drugs used during fetal development, or the chemical or biological elements exposed in the environment of the mother (Bamalan & Menezes, 2021). In general, this information can likely be used in present neonatology and for the benefit of some diseases in medical fields. VC shows features in the treatment of burn wounds and skin impairment after the loss of skin integrity as a result of dermatological diseases (Bosnar et al., 2022). It is emphasized that VC in the skin of newborns should be developed artificially for clinical therapeutic use. Peptide LL-37, one of the protective proteins in the dermal layer, acts as an antimicrobial barrier (Paling & Koch, 2022). Observing information from the past, VC is known to be used as a potential wound-healing ointment. (Setiawandari et al., 2022). The formation of synthetic creams as a prototype having a similar composition of VC has not yet been accomplished. Limited information is available about VC due to less number of studies on its chemical structure, its biological requirements, and its physical properties. As far as is known, no study has been done to find the elemental content of VC. Therefore, there is a need for work to be done in this regard. This study aims to reveal the values of some elements like Na+, Cl-, K+, Fe+2, Mg+2, Ca+2, Cu+2, and Zn+2 in VC content not known to much extent.

MATERIAL and METHOD

This study was designed in Kahramanmaraş Sütçü İmam University research and application Hospital, Department of Medical Biochemistry. After obtaining the necessary information and permissions about the study, the family signed an informed consent form. Approval was obtained from the Kahramanmaras Sutcu Imam University, Medical Faculty Ethical Committee before the study (doc.no:34/2017).

Study population: The VC samples were collected from 52 infants by swabbing with a sterile soft-tipped apparatus. There were several criteria taken into account for this study like the age of the mother, the gestation week, the number of gestations, the health of mother and baby, APGAR score for 1st and 5th minute, and gender of the babies. The babies were evaluated as a term (37-41 weeks+6 days), preterm (<37 weeks), and post-term (> 42 weeks). The age of the mother was divided into two categories: less than 35 years (<35) and more than 35 (>35) years.

It was placed in the tubes and kept at -20°C till the day of the experiment. For homogenization and solubilization, VC was added to 250 ml of PBS (pH 7.4)

containing 0.5% propylene glycol (Merck, Germany) and 2% sodium dodecyl sulfate (SDS). The solution was homogenized with a homogenizer at 4°C. The prepared homogenates were centrifuged at 4000 rpm for 10 min at 4°C. As a result, a solid layer of fat formed as the upper layer, and the liquid portion under the layer was carefully transferred to clean tubes and the samples were ready for analysis.

The levels of Na+, Cl-, K+, Fe+2, Mg+2, and Ca+2 elements in homogenized specimens were measured in a fully automatic autoanalyzer using a Siemens ADVIA 1800 Chemistry. The results of the test sample and the controls were calculated and reported based on the absorbance measurement during calibration. The levels of the other two elements Cu+2 and Zn+2 were analyzed on a flame photometer with Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 800, USA).

Samples and calibration standards were diluted with 10% glycerol in a ratio of 1/2 for copper measurement17 and it is by diluting with 5% glycerol in a ratio of 1/4 for zinc measurement18. Standards for the samples were prepared using standards of 1000 mg/L commercial Cu+2 and Zn+. The absorbance of unknown samples were calculated according to the standard curve.

Statistics: SPSS 16 program was used for the statistical analysis of results. The homogeneity of the groups was tested by Kolmogorov-Smirnov analysis and the Mann-Whitney U test method was chosen as the nonparametric test for comparison of the non-homogenous groups.

RESULTS

The average levels, standard deviation, and min-max values of elements Na+, Cl- K+, Fe+2, Mg+2, Ca+2, Cu+2, and Zn+ found in VC per gram in all cases are shown in Table 1. The VC element levels of male and female infants and their respective differences are shown in Table 2. According to this, in female infants, VC Ca+2 ($p < 0.016$) and Zn+2 ($p < 0.024$) levels were significantly higher than in male infants. The element levels of VC in infants (regardless of their gender) who were born from mothers of age groups above and below 35 are shown in Table 3. According to this, only the level of Cu+2 in VC of the infants of mothers over age 35 showed a significant decrease ($p < 0.03$). There was no significant difference between the element values of VC in infants of nulliparous and multiparous mothers ($p > 0.05$) as shown in Table 4.

In Table 2, only Zn and Ca elements show a significant difference ($p<0.05$) in element values between girls and boys. Zinc and calcium element amounts differ according to gender. It appears to be higher in female infants and lower in male infants. Na, Cl, K, Fe, Mg, Cu, Zn elements are similar in amount between the two genders.

There is a significant difference ($p<0.05$) only in Cu element between the elements of infants of mothers younger than 35 years old and above. Among the other analyzed elements, a remarkable difference appears in this element due to the age difference of the mothers. (Table 3).

Comparison according to the number of pregnancies was grouped as nulliparous mothers and multiparous mothers, and there was no significant difference ($p<0.05$) for any element value in Table 4.

Table 1. The average levels of Na^+, Cl^-, K^+, Fe^{+2}, Mg^{+2}, Ca^{+2}, Cu^{+2}, Zn^+ elements/ per gram of VC tissue.
Çizelge 1. Na+, Cl-, K+, Fe+2, Mg+2, Ca+2, Cu+2, Zn+ elementlerinin/ gram VC doku başına ortalama seviyeleri.

Elements							
*Na^+	*Cl^-	*K^+	**Fe^{+2}	**Mg^{+2}	**Ca^{+2}	**Cu^{+2}	**Zn^{+2}
12.01±58.40 (6.20-34.39) n: 50	10.46±1.08 (2.00-52.06) n: 47	1.84±0.54 (1.68-1.92) n: 52	78.19±5.48 (27.87-224.83) n: 48	67.07±3.31 (16.18-105.19) n:52	864.52±32.62 (119.88-332.00) n:52	14.69±2.14 (2.38-43.83) n:26	11.59±1.59 (2.33-47.62) n:34

*: mg/g tissue, **: ug/g tissue. All elements values were given as mean±standart error and min-max, n: subject number.

Table 2. The average levels of Na^+, Cl^-, K^+, Fe^{+2}, Mg^{+2}, Ca^{+2}, Cu^{+2}, Zn^{+2}elements/ per gram VC tissue in a girl and a boy baby.
Çizelge 2. Kız ve erkek bebekte VC dokusunun gram başına ortalama Na+, Cl-, K+, Fe+2, Mg+2, Ca+2, Cu+2, Zn+2 element seviyeleri.

	Newborn girls (n:23)	Newborn boys (n:29)	P values
***Na^+**	11.63±0.55	12.46±0.94	0.992
***Cl^-**	9.75±1.04	11.28±1.72	0.834
***K^+**	1.84±0,01	1.84±0.01	0.244
****Fe^{2+}**	67.87±7.51	77.54±4.88	0.126
****Mg^{2+}**	73.18±5.05	62.22±4.25	0.092
****Ca^{2+}**	961.94±40.83	824.46±39.56	**0.016**
****Cu^2**	18.75±3.85	13.12±2.30	0.310
****Zn^{2+}**	15.66±2.90	8.48±1.26	**0.024**

*: mg/g tissue, **: ug/g tissue. All elements values were given as mean±standart error and min-max, n: subject number.

Table 3. The element levels of VC in infants (regardless of their gender) taking birth from mothers of an age group above and below 35.

Çizelge 3. 35 yaş üstü ve altı annelerden doğan bebeklerde (cinsiyetinden bağımsız olarak) VK element düzeyleri

	Mothers age<35 years	Mothers age>35 years P values	Mothers age<35 years
*Na^+	11.31±0.37 (n:37)	12.64±1.04(n:13)	0.167
*Cl^-	9.12±0.74 (n:36)	11.68±1.30(n:11)	0.070
*K^+	1.83±0.01 (n:39)	1.85±0.01 (n:13)	0.127
**Fe^{2+}	76.70±4.95 (n:36)	61.83±8.30 (n:12)	0.159
**Mg^{2+}	68.68±3.21 (n:39)	62.24±9.27 (n:13)	0.596
**Ca^{2+}	890.02±31.30 (n:39)	870.92±76.00 (n:13)	0.874
**Cu^{2+}	16.89±2.49 (n:15)	9.95±2.21 (n:8)	**0.028**
**Zn^{2+}	10.67±1.27 (n:23)	8.70±2.00 (n:11)	0.467

*: mg/g tissue, **: ug/g tissue. All elements values were given as mean±standart error and min-max, n: subject number, #: p<0,05 significance.

Table 4. The element values of VC in infants of nulliparous and multiparous mothers.

Çizelge 4. Nullipar ve multipar annelerin bebeklerinde VC element değerleri

	Infants of nulliparous mothers	Infants of multiparous mothers	P values
*Na^+	11.75±0.93 (n:9)	12.15±0.68 (n:41)	0.990
*Cl^-	8.60±1.77 (n:9)	18.88±1.26 (n:38)	0.267
*K^+	1.83±0.01 (n:9)	1.84±0.01 (n:43)	0.510
**Fe^{2+}	80.83±11.17 (n:8)	74.00±3.56 (n:40)	0.599
**Mg^{2+}	58.44±8.72 (n:9)	68.87±3.56 (n:43)	0.280
**Ca^{2+}	848.04±78.86 (n:9)	893.06±32.8 (n:43)	0.594
**Zn^{2+}	6.66±1.30 (n:7)	12.51±1.89 (n:27)	0.088

*: mg/g tissue, **: ug/g tissue. All elements' values were given as mean ± standard error and min-max, n: subject number, #: p<0,05 significance.

DISCUSSION

Each produced material is used for a purpose in biological organisms. And for this reason, VC has a protective barrier effect for babies. It protects the deep surface of the skin from external factors and infections; moisturizes the skin and contributes to wound healing. It also facilitates childbirth by providing lubrication during normal labor (Visscher & Narendran, 2014). Moreover, it protects the amniotic fluid from maceration and prevents the loss of fluid and electrolytes. Looking at the content of VC, it is seen that it contains 80% water, 10% protein and 10% lipid in the rest. (Meng et al., 2021).

The publications so far have focused particularly on the analysis of its lipid content. The lipid composition of VC includes fatty acids in triglycerides form as well as it contains ceramides, cholesterol, sterol esters, wax esters, dihydroxy wax esters, squalene, diacylglycerol, monoacylglycerol and phospholipids (Boiten et al., 2018).

Míková et al. (2014) reported that the number of wax esters and triacylglycerol was higher in newborn girls than boys when they examined lipid composition compared to the sexes. This shows that some structural molecules will be different according to sex. A similar situation was also observed in Ca+2 and Zn+2 levels of VC in boy and girl infants in this work. Also, the same differences seen in Cu+2 elements of VC of the infants who were born from mothers of age groups above and below 35 were found statistically significant. In addition, it was reported that the hormonal level of the mother affects the chemical structure of the formation on the skin of the baby (Míková et al., 2014; Vavrušová et al., 2020).

In one of the studies, it was stated that estrogen has an accelerating effect and testosterone has a retarding effect on the formation of the skin barrierIts protein content is quite diverse and more than two hundred proteins are mentioned among which the important ones are enzyme modulators, hydrolyses, proteases, structural proteins, cytoskeleton-forming proteins, calcium-binding proteins, and other carrier proteins and proteins acting on immunity and other signal molecules (Hanley et al., 1996; Harazim et al., 2018). When the literature is examined, it is seen that there are not enough studies on element contents. Therefore, in this study, it is thought that it would be appropriate to consider the elemental contents of VC such as Na+, Cl-, K+, Fe+2, Mg+2, Ca+2, Cu+2, Zn+2. When the VC element levels in male and female infants were examined, Ca+2 and Zn+2 VC levels in male infants were lower than in female infants. In addition to this, it was observed that the Cu+2 level in VC of infants taking birth from mothers below 35 years of age was significantly higher than that of infants taking birth from mothers above 35 years of age. From the obtained values, it is seen that the level of some elements in VC varies according to the sex of the child and the age of the mother. Since there is not enough data to discuss VC in terms of element levels, further studies on the element content of VC are recommended as a result of this study. The increase in water percentage, lipids,

enzymes, and peptides (e.g., glutamine as a trophic agent) aids in a neonatal wound and burn to healing. Therefore, researchers are testing the possibility of applying similar methods to adult wounds and skin-grafting of burns with associate layers of a similar structure as the vernix; moreover, vernix-based topical creams might demonstrate sufficient potency in treating epidermal wounds and augmenting stratum corneum repair and maturation in infants (Bamalan & Menezes, 2021).

As mentioned in another study, The ceramide substance prevents the skin from drying out and protects it from the formation of microbial infections, and they are essential lipids for the skin barrier function. Together with free fatty acids and cholesterol, they form a membranous structure that superficially surrounds the corneocytes in the stratum corneum. The level of barrier-forming lipids is significantly lower than in the stratum corneum, and the predominant components of vernix caseosa are apolar lipids. (Schlessinger et al., 2021). This molecular structure composition prevents and protects the skin from direct contact with the amniotic fluid during the fetal period, and facilitates the maturation of the epidermis. (Harazim et al., 2018; Rissmann et al., 2006; Youssef et al., 2001)

There are many birth plan variations that a mother-to-be can choose regarding her pregnancy, it is known that leaving the vernix caseosa on the newborn skin immediately after birth and leaving this substance on the skin of the newborn has health and well-being benefits. It is known how important it is, especially when considering the thermal need of newborns and the risk that may occur if this need is not met (Kusari et al., 2019). Vernix caseosa, which is formed at 21 weeks of pregnancy, continues to act as a bacteria-fighting and antimicrobial barrier even after birth. It helps to prevent possible infections that may occur after the birth process is completed. (Buyuk et al., 2021; Qiao et al., 2019). Another important role of this layer is to maintain the hydration of the newborn. As it is known, newborn skin is very sensitive and when exposed to weather conditions without adaptation at first, cracking of the lips, drying of the skin, and crusting may occur. It is very important to moisturize the skin to prevent such situations. (Shamloul & Khachemoune, 2021). If the vernix does not clear the caseosa and is left on the baby, the baby's skin can maintain hydration longer. Improved mother/infant bonding is also observed, as moist skin better perceives contact earlier than when the baby is bathed immediately. Therefore, it can also aid sucking and breastfeeding abilities. On a closer look at these attributes, the health benefits and probabilities in risk and reward associated with vernix caseosa and delayed bathing may be significant.(Cui et al., 2016; Herman, 2022; Sampah & Hackam, 2020).

ACKNOWLEDGEMENTS

The study was supported by the individual research projects unit of Kahramanmaras Sutcu Imam University.

Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's Contributions

The contribution of the authors is equal.

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