SEX-RELATED DIFFERENCES IN THE LEVELS OF URINE 6-SULFATOXYMELATONIN IN VERY LOW BIRTH WEIGHT INFANTS

H. Kuzienkova

Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine

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ABSTRACT

Background. The sex-related differences of the urinary 6-sulfatoxymelatonin have not been studied in premature infants yet. The purpose of the work was to measure the daily urinary 6-sulfatoxymelatonin in premature infants with a very low birth weight.

Materials and Methods. Fifty premature infants (28 males and 22 females) with gestational age less than 33 weeks and body weight from 999 g to 1499 g were involved in the study. Urine 6-sulfatoxymelatonin was assessed using urine collection on the 1st day and on the 10th–14th days of life.

Results. The level of urine 6-sulfatoxymelatonin on the 1st day of life showed a significant increase in its excretion in females compared to males. The median values in males were 202.0 (95% CI 77.1–390.9) pg/ml and in females 437.0 (279.6–501.0) pg/ml, p=0.0103. Its level on the 10th–14th days of life significantly decreased both in males 57.0 (95 % CI 45,0–99.7) pg/ml, p=0.0028 and in females 90.0 (51.9–160.7) pg/ml, p=0.0021 without differences in sex-related distribution, p=0.3940.

Conclusions. The melatonin metabolite as urinary 6-sulfatoxymelatonin in premature infants with a very low birth weight demonstrates sex-related differences with significant increase in females compared to males on the 1st day of life and no sex-related difference on the 10th–14th days of life. The trend of reduced pineal function is a key point in understanding the neuroendocrine reactivity in male preterms. Future investigation of sex-related aspects of urinary 6-sulfatoxymelatonin excretion in children, especially premature infants, is required.

Key words: *urine, 6-sulfatoxymelatonin, premature infants, very low birth weight.*

INTRODUCTION

It is known that one of the main functions of melatonin in the organism is regulation of "sleep–wake". Melatonin is synthesized both by the pineal gland and other tissues. Its metabolism leads to formation of 6-sulfatoxymelatonin (aMT6s), which can be excreted in various fluids, mainly urine [1].

Due to the differences in the endocrine system in females and males, the role of gender differences in the functional activity of melatonin has been studied. The published findings are rather contradictory even in the adult population. One published study has found that females exhibited significantly greater levels of plasma melatonin than males and there are differences between males and females in its circadian rhythm [2]. Meanwhile, the effect of different light intensities on blood melatonin concentrations in females and males has been, and there were no gender differences in light sensitivity [3].

The recent studies of melatonin effects have shown not only its biological "sleep-wake" function. In the last decades, its role as a powerful antioxidant protection has been demonstrated in both adults and children [4–6]. Despite the fact that positive results have been obtained in the use of melatonin in newborns, sex-related differences in them, and especially in premature babies, have not been sufficiently studied [7].

Purpose, subjects and methods:

1. The purpose of the study was to measure the daily urinary aMT6s in premature infants with very low birth weight (VLBW) as well as evaluate its sex-related differences.

2. Subjects & Methods. *Design.* This simple, cohort, one-center, descriptive, retrospective study involved 50 premature infants with VLBW.

Corresponding Author:
Hanna Kuzienkova, MD, PhD student, Kharkiv Medical Academy of Postgraduate Education, Ukraine.
E-mail: annakuzenkova15@gmail.com

Inclusion criteria: premature infants with gestational age less than 33 weeks, body weight at birth from 999 g to 1499 g.

Exclusion criteria: gestational age more than 33 weeks and body weight ≥ 1500 g, degenerative and congenital diseases of the nervous system, chromosomal diseases; diseases with impaired renal function; orphan diseases.

Interventions. The data of a detailed case history and objective examinations, medical records, anthropometric measurements, and daily urine collection were studied. aMT6s in the 24-hour urine collection from premature infants was assessed by enzyme-linked immunosorbent assay using the BÜHLMANN 6-Sulfatoxymelatonin ELISA (BUHLMANN Diagnostics Corp., USA), offered by the manufacturer. The urine was collected on the 1st day and the 10th–14th days of life.

Statistics. Statistical analysis was performed with MedCalc version 14.8 (©1993–2014) MedCalc Software bvba (Acacialaan 22 B-8400 Ostend, Belgium). Descriptive analysis such as median (Me), maximum (max), minimum (min), lower quartile (Lq), upper quartile (Uq), 95% Confidence Interval (CI) were used. Fisher's test to calculate the difference in two proportions, Mann-Whitney test (MW test) to compare of two independent samples, and Wilcoxon Rank-Sum Test (WRST) to compare of two dependent samples were used. The difference in parameters was considered statistically significant at $p<0.05$.

Results and discussion

The maternal pregnancy and delivery of infants with VLBW included the following conditions: premature rupture of membranes –19 (38.0%), abortion risk – 13 (26.0%), urogenital infections – 10 (20.0%), multiple pregnancy – 12 (24.0%), cesarean section – 29 (58.0%), fetal distress – 13 (26.0%). The demographic and clinical data of infants with VLBW are presented in *table 1*.

There was no significant difference between males and females among VLBW infants (p= =0.2330). Among them there was preference of moderate preterm: 32 weeks to <34 (p=0.0002) according to the WHO classification [8]. Table 1 presents the ranking of perinatal pathology in infants depending on the frequency of its prevalence. The presence of one or a combination of several pathological perinatal conditions was characterized by changes in the acid-base state of umbilical blood in infants with VLBW (*table 2*).

Table 1. Clinical and demographic data of the premature infants

Data	n (%)	95% CI
Males	28 (56.0)	41.2–70.0
Females	22 (44.0)	29.9–58.7
Gestation age		
≤29 weeks	8 (16.0)	7.0–29.0
30 weeks	12 (24.0)	13.0–38.1
31 weeks	7 (14.0)	5.8–26.7
32 weeks	8 (16.0)	7.0–29.0
33 weeks	15 (30.0)	17.8–44.6
Respiratory distress syndrome	50 (100)	92.8–100.0
Anemia of premature	29 (58.0)	43.2–71.8
Hypoxic-ischemic encephalopathy	26 (52.0)	37.4–66.3
Congenital pneumonia	19 (38.0)	24.6–52.8
Intraventricular hemorrhage	17 (34.0)	21.2–48.7
Sensorineural hearing loss	13 (26.0)	14.6–40.3
Neonatal sepsis	11 (22.0)	11.5–35.9
Severe asphyxia	9 (18.0)	8.5–31.4
Retinopathy of premature	7 (14.0)	5.8–26.7
Bronchopulmonary dysplasia	3 (6.0)	1.2–16.5
Necrotizing enterocolitis	3 (6.0)	1.2–16.5
Lethal outcome	2 (4.0)	0.4–13.7

Table 2. Acid-Base state in the umbilical blood in premature infants with VLBW

Parameter	pH, units	BEb, mEq/l
Median	7.1	–16
Lq; Uq	6.8; 7.3	–19; –11
min; max	7.0; 7.2	–22; –2

The combination of severe perinatal pathology led to the lethal outcome of 2 infants: hypoxic injury due to severe asphyxia and respiratory distress syndrome, second had moderate asphyxia and early neonatal sepsis.

The level of urine aMT6s on the 1st day of life showed a significant increase in its excretion in females compared to males (*Fig. 1*). The median values in males were 202.0 pg/ml (95% CI 77.1–390.9)

and in females 437.0 (279.6–501.0) pg/ml, MW test p=0.0103.

The level of urine aMT6s excretion on days 10^{th}–14^{th} of life significantly decreased both in males 57.0 (95% CI 45.0–99.7) pg/ml, WSRT p=0.0028 and in females 90.0 (51.9–160.7) pg/ml, WSRT p=0.0021. However, we did not find differences in sex-related distribution of urine aMT6s at the 10^{th}–14^{th} days of life, MW test p=0.3940 (*Fig. 2*).

Why did we study this particular question? In animals sex-related histological differences in the thymus after ectopic pineal gland was established [9].

One study in the Netherlands on a population of 94 healthy children aged 2 to 18 years evaluated actigraphic data on 24-hour sleep-wake rhythm as a function of urinary aMT6s levels, sex differences, and body mass index. The methodology of the study included the children wearing of an actigraph device, collection of morning urine for 7

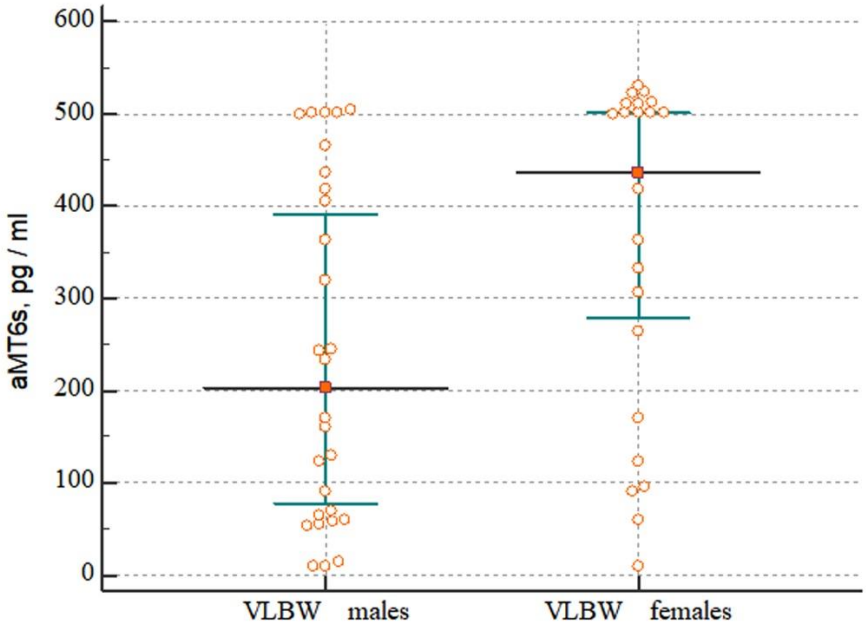

Fig. 1. The sex-related distribution of urine aMT6s in premature infants with VLBW on the 1^{st} day of life.

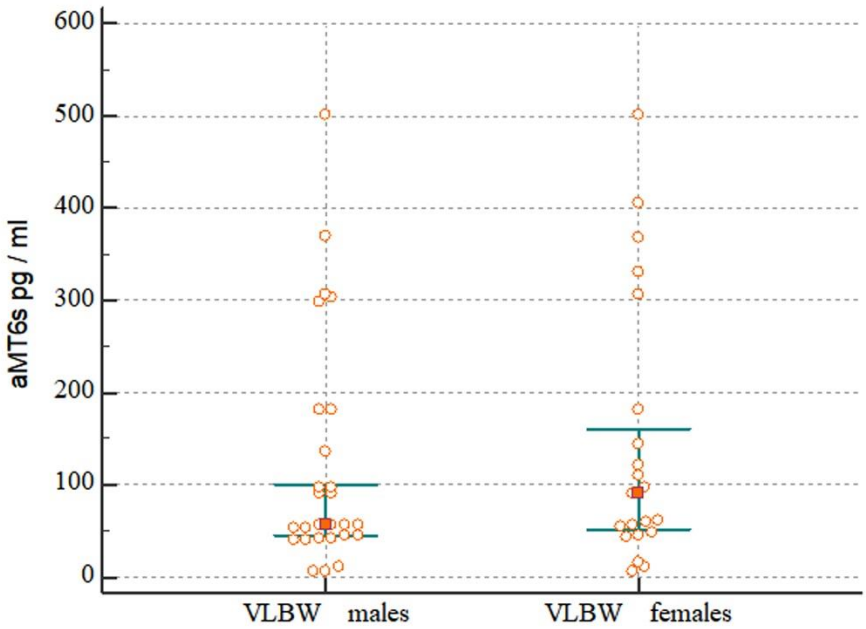

Fig. 2. The sex-related distribution of urine aMT6s in premature infants with VLBW on the 10^{th}–14^{th} days of life.

consecutive days, and a questionnaire. It was found that the levels of urine aMT6s decreased significantly as the child grew older. The authors also concluded that neither gender nor an increase in body mass index was associated with a difference in excretion of urine aMT6s [10].

Another study of the relationship between the excretion of the urine aMT6s, and prenatal, and intranatal, and postnatal changes and psychomotor development was carried out on a fairly large population of term infants (n=355). Poor child neurodevelopment has been associated with low levels of the urine aMT6s at 16 weeks, 3 and 6 months of age. The authors conclude that this association suggests a causal or prognostic relationship between melatonin and neurodevelopment in infants [11]. However, this study did not include premature infants, especially with LBW.

One study showed results about maturation of fetal melatonin synthesis by measuring the urinary excretion of aMT6s in males aged 2–7 days and gestational age 26–42 weeks. A negative correlation was established between an increase in gestational age and the urine aMT6s excretion, which was also shown in our study. We demonstrated the same data but, on the 10^{th}–14^{th} days of life, and reduction of the urine aMT6s excretion. However, in contrast to the study above, we have demonstrated this phenomenon in both males and females [12].

Our study allowed to partially reject the hypothesis, that premature infants with VLBW have no sex-related differences in the profiles of urinary aMT6s in neonatal period. We showed a significant increase in urinary excretion of 6 in females compared to males on the1^{st} day of life, and the absence of sex-related differences of urinary aMT6s between males and females on days 10–14 of life.

Some publications suggest that melatonin plays a major antioxidant role in postnatal adaptation. It is possible that labor stress leads to the release of this hormone for antioxidant protection [4–6]. We consider another important issue for discussion, i.e. why we need to measure the level of the melatonin metabolite.

Low levels of metabolite in the urine in premature children provide for a subsidy of this hormone. But for today the melatonin supplementation for the prevention of hypoxic ischemic injury in newborns has been demonstrated at a high evidence level [13].

The effectiveness of melatonin supplementation as a neuroprotector in the first two weeks of extrauterine life in premature infants is just beginning to be studied. Our study showed that premature babies cannot produce enough melatonin, which is another contribution to research on the effectiveness of melatonin supplementation. Its antioxidant mechanism may be useful in preventing neurodevelopmental disorders. That is why the first study on the role of melatonin in preterm infants with very low birth weight was planned in 2020. It may serve as a basis for further research on melatonin as a neuroprotective strategy in this vulnerable population. [14]. Some publications showed the fact that premature infants have long-term neurodevelopmental disorders, cognitive and motor problems, and a low level of socialization and communication, it is extremely important to evaluate the role of melatonin as a neuroprotector during the first weeks of extrauterine life, to prevent disorders in the development of the nervous system [15–18]. We studied the relationship between the levels of the urine melatonin metabolite in the short-term formation of perinatal pathology in premature infants in the early and late neonatal period. We believe that the results of our study open up more prospects regarding the study of the effect of melatonin on the long-term aspects of the development of a child who was born prematurely. The limitation of our study was due to a small sample and time shortage.

Conclusions

The melatonin metabolite, urinary 6-sulfatoxymelatonin, in premature infants with a very low birth weight showed sex-related differences with significant increase in females compared to males on the 1^{st} day of life and no sex-related difference on the 10^{th}–14^{th} days of life. The trend of reduced pineal function is a key point to understanding the neuroendocrine reactivity in male preterms. Further studies of sex-related aspect of urinary 6-sulfatoxymelatonin excretion in children, especially premature infants are required.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure.

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Data Transparency

The data can be requested from the authors.

References

1. Batllori, M., Molero-Luis, M., Arrabal, L., Heras, J. L., Fernandez-Ramos, J. A., Gutiérrez-Solana, L. G., … & Artuch, R. (2017). Urinary sulphatoxymelatonin as a biomarker of serotonin status in biogenic amine-deficient patients. *Scientific reports*, *7*(1), 14675. https://doi.org/10.1038/s41598-017-15063-8
2. Gunn, P. J., Middleton, B., Davies, S. K., Revell, V. L., & Skene, D. J. (2016). Sex differences in the circadian profiles of melatonin and cortisol in plasma and urine matrices under constant routine conditions. *Chronobiology international*, *33*(1), 39–50. https://doi.org/10.3109/07420528.2015.1112396
3. Nathan, P. J., Wyndham, E. L., Burrows, G. D., & Norman, T. R. (2000). The effect of gender on the melatonin suppression by light: a dose response relationship. *Journal of neural transmission (Vienna, Austria: 1996)*, *107*(3), 271–279. https://doi.org/10.1007/s007020050022
4. Reiter, R. J., Mayo, J. C., Tan, D. X., Sainz, R. M., Alatorre-Jimenez, M., & Qin, L. (2016). Melatonin as an antioxidant: under promises but over delivers. *Journal of pineal research*, *61*(3), 253–278. https://doi.org/10.1111/jpi.12360
5. Bagci, S., Horoz, Ö. Ö., Yildizdas, D., Reinsberg, J., Bartmann, P., & Müller, A. (2012). Melatonin status in pediatric intensive care patients with sepsis. *Pediatric critical care medicine: a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*, *13*(2), e120–e123. https://doi.org/10.1097/PCC.0b013e3182191dc4
6. García, I. G., Rodriguez-Rubio, M., Mariblanca, A. R., de Soto, L. M., García, L. D., Villatoro, J. M., … & Borobia, A. M. (2020). A randomized multicenter clinical trial to evaluate the efficacy of melatonin in the prophylaxis of SARS-CoV-2 infection in high-risk contacts (MeCOVID Trial): A structured summary of a study protocol for a randomised controlled trial. *Trials*, *21*(1), 466. https://doi.org/10.1186/s13063-020-04436-6
7. Yıldız, E. P., Ekici, B., & Tatlı, B. (2017). Neonatal hypoxic ischemic encephalopathy: an update on disease pathogenesis and treatment. *Expert review of neurotherapeutics*, *17*(5), 449–459. https://doi.org/10.1080/14737175.2017.1259567
8. WHO and sub-categories of preterm birth, based on gestational age. Retrieved from https://www.who.int/news-room/fact-sheets/detail/preterm-birth
9. Susko, I., Alicelebic, S., Cosovic, E., Sahinovic, M., Kapic, D., Custovic, S., & Muzika, V. (2017). Gender-related Histological Changes in the Thymus Gland After Pinealectomy and Short-term Melatonin Treatment in Rats. *Medical archives (Sarajevo, Bosnia and Herzegovina)*, *71*(6), 385–390. https://doi.org/10.5455/medarh.2017.71.385-390
10. Rensen, N., Steur, L., Wijnen, N., van Someren, E., Kaspers, G., & van Litsenburg, R. (2020). Actigraphic estimates of sleep and the sleep-wake rhythm, and 6-sulfatoxymelatonin levels in healthy Dutch children. *Chronobiology international*, *37*(5), 660–672. https://doi.org/10.1080/07420528.2020.1727916
11. Tauman, R., Zisapel, N., Laudon, M., Nehama, H., & Sivan, Y. (2002). Melatonin production in infants. *Pediatric neurology*, *26*(5), 379–382. https://doi.org/10.1016/s0887-8994(01)00417-9
12. Commentz, J. C., Henke, A., Dammann, O., Hellwege, H. H., & Willig, R. P. (1996). Decreasing melatonin and 6-hydroxymelatonin sulfate excretion with advancing gestational age in preterm and term newborn male infants. *European journal of endocrinology*, *135*(2), 184–187. https://doi.org/10.1530/eje.0.1350184
13. Hurley, T., O'Dea, M., Aslam, S., Aly, H., Robertson, N., & Molloy, E. (2020). Melatonin treatment for newborns with hypoxic ischaemic encephalopathy. Cochrane database of systematic reviews. Retrieved from https://cochrane.altmetric.com/details/103680448 https://doi.org/10.1002/14651858.cd013754
14. Garofoli, F., Longo, S., Pisoni, C., Accorsi, P., Angelini, M., Aversa, S., … & Orcesi, S. (2021). Oral melatonin as a new tool for neuroprotection in preterm newborns: study protocol for a randomized controlled trial. *Trials*, *22*(1), 82. https://doi.org/10.1186/s13063-021-05034-w
15. Aita, M., De Clifford Faugère, G., Lavallée, A., Feeley, N., Stremler, R., Rioux, É., & Proulx, M. H. (2021). Effectiveness of interventions on early neurodevelopment of preterm infants: a systematic review and meta-analysis. *BMC pediatrics*, *21*(1), 210. https://doi.org/10.1186/s12887-021-02559-6
16. You, J., Shamsi, B. H., Hao, M. C., Cao, C. H., & Yang, W. Y. (2019). A study on the neurodevelopment outcomes of late preterm infants. *BMC neurology*, *19*(1), 108. https://doi.org/10.1186/s12883-019-1336-0
17. Park, J. (2020). Sleep Promotion for Preterm Infants in the NICU. *Nursing for women's health*, *24*(1), 24–35. https://doi.org/10.1016/j.nwh.2019.11.004
18. Cheong, J. L., Doyle, L. W., Burnett, A. C., Lee, K. J., Walsh, J. M., Potter, C. R., … & Spittle, A. J. (2017). Association Between Moderate and Late Preterm Birth and Neurodevelopment and Social-Emotional Development at Age 2 Years. *JAMA pediatrics*, *171*(4), e164805. https://doi.org/10.1001/jamapediatrics.2016.4805

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