Association between neonatal uterine bleeding and endometriosis-related symptoms later in life

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Article

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Abstract

Neonatal uterine bleeding (NUB) has been postulated to cause early-onset endometriosis, but so far there has been no information on whether young women with a history of NUB may manifest various symptoms related to endometriosis. In this retrospective case-controlled cohort study, we investigated the prevalence and risk factors of NUB and prospectively evaluated endometriosis-related symptoms by web-based questionnaire survey. Among 807 female babies born at our hospital between 2013 and 2017, NUB occurred in 25 cases with a prevalence of 3.1%. Multiple Logistic regression analysis indicated that younger age of the mother [odds ratio (OR) 0.92, 95% confidence interval (CI) 0.85–1.00, P = 0.048] and longer gestational age of 39 weeks (OR 3.04, 95% CI 1.43–6.45, P = 0.004) and ≥ 40 weeks (OR 4.54, 95% CI 2.20–9.39, P < 0.0001) of gestation were significantly associated with the occurrence of NUB. We confirmed the validity of the reported prevalence and risk factors of NUB. Young mothers and longer gestational age are the risk factors for NUB and newborn females with or without NUB similarly suffer from various endometriosis-related symptoms later in life during adulthood. Future prospective cross-sectional study is warranted to confirm the endometriosis-related symptoms in NUB + and NUB- cases by physical examination, ultrasound and/or MRI.

Introduction

Endometriosis is a multifactorial condition manifesting various problems on women health such as cyclic/acyclic menstrual pain, subfertility or infertility, recurrence of the disease after treatment and malignant transformation\(^1\). It is difficult to uniformly explain the development of endometriosis by a single factor. There is no consensus concerning the histologic origin of endometriosis. Although retrograde menstruation and/or coelomic metaplasia are the widely accepted theories of pathogenesis among others\(^2\), all cases of pre-menarcheal endometriosis cannot be explained by these theories. Endometriosis has been documented in young girls before menarche and in these cases it has been assumed that its pathogenesis and pathophysiology differ from adolescent and adult endometriosis. Recently, neonatal uterine bleeding (NUB) has been postulated to be the possible origin and progression of early-onset endometriosis\(^3–6\).

Neonatal uterine bleeding seems to occur in the first few days after birth. According to some reports, NUB may occur in female neonates on postnatal days 3–5 with a prevalence rate of 3–5% for overt NUB and 25–60% for occult NUB\(^3,5,7–10\). Similar to the adult endometrium, circulating maternal (placenta-derived) progesterone elicits decidual changes in the developing fetal endometrium and NUB is triggered by the rapid fall in circulating progesterone levels in the immediate post-natal period\(^3,8\). While this rapid decrease in steroid hormones occurs in all female neonates, occurrence of NUB is reported in only a small fraction of them. This could be due to the presence of a mucus plug in relatively long cervical canal of neonates and as a result NUB could be accompanied, conceivably, by a retrograde flux of endometrial cells, stromal fibroblasts, and/or endometrial stem/progenitor cells\(^7\). As such, the regurgitated endometrial cells resulting from the retrograde NUB may be deposited into the peritoneal cavity, sitting
dormant until thelarche or menarche when rising estrogen levels may activate these “sleeping beauty” cells, and resulting in the formation of endometriotic lesions. Thus, NUB may be the cause of early-onset endometriosis and, as such, the NUB hypothesis needs to be carefully evaluated.

Regarding risk factors of NUB, it has been reported that preeclampsia, low birth weight, post-maturity and/or dysmaturity, and fetomaternal blood incompatibility were significantly associated with the occurrence of NUB. However, due to the possible causal relationship between NUB and early-onset endometriosis, it may be timely to revisit the issues to strengthen the validity of the prevalence of NUB and its risk factors in association with NUB.

Despite evaluation of a panel of biomarkers, as of now, there is no clinically useful applicable marker for non-invasive diagnosis of endometriosis in general and of early-onset of endometriosis in particular. A sizeable proportion of adolescent girls suffer from constant moderate to severe cyclic/acyclic pain but many of them are reluctant to visit gynecologists, precluding for early diagnosis and management of endometriosis. As a result, a diagnostic delay of 4–14 years between onset of symptoms and final diagnosis is another big problem for these young women. Therefore, we need to search for new clinical marker to identify high-risk group of young women who are susceptible to develop early-onset endometriosis. The mechanistic basis whether NUB is causal for early-onset endometriosis is still unclear and is now under active investigation in our laboratory. To the best of our knowledge, whether there is a causal direct link between the occurrence of NUB and development of endometriosis-related symptoms later in life is unknown. Conceivably, it is logistically difficult and resource demanding to conduct decade-long prospective follow-up studies with cases until their onset of puberty in order to prove or disprove a link between NUB and endometriosis. Therefore, evaluation of web-based questionnaire survey to identify symptoms related to endometriosis in young women who were known to have a history of NUB at birth may be a suitable approach to establish a possible link between NUB and endometriosis.

The aims of this study were to investigate the followings: (1) To search retrospectively of recent (2013–2017) and older (1996–2000) medical records to reconfirm the prevalence of NUB and to identify risk factors associated with NUB using multivariate logistic regression analysis, (2) In an attempt to investigate the link between the history of NUB and various symptoms related to endometriosis in young women, we conducted a web-based questionnaire survey in order to identify endometriosis-related symptoms in these women who were born with and without NUB during the period of 1996–2000.

Results

Occurrence and prevalence of NUB. The annual year-dependent distribution of female newborns, NUB + cases and prevalence of NUB are shown in Table 1 (The prevalence of neonatal uterine bleeding (NUB) was detected by retrospective search of Obstetrical medical records during (A) 2013–2017 and (B) 1996–2000; *Data were not available).
Table 1

(A) Prevalence of NUB during 2013–2017

<table>
<thead>
<tr>
<th>Year</th>
<th>Female Newborns</th>
<th>NUB + cases</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>(n)</td>
<td>(%)</td>
</tr>
<tr>
<td>2017</td>
<td>153</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>2016</td>
<td>154</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>2015</td>
<td>157</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>2014</td>
<td>178</td>
<td>9</td>
<td>5.1</td>
</tr>
<tr>
<td>2013</td>
<td>165</td>
<td>4</td>
<td>2.4</td>
</tr>
<tr>
<td>2013–2017</td>
<td>807</td>
<td>25</td>
<td>3.1</td>
</tr>
</tbody>
</table>

(B) Prevalence of NUB during 1996-2000

<table>
<thead>
<tr>
<th>Year</th>
<th>Female Newborns</th>
<th>NUB + cases</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>(n)</td>
<td>(%)</td>
</tr>
<tr>
<td>2000</td>
<td>206</td>
<td>18</td>
<td>8.7</td>
</tr>
<tr>
<td>1999</td>
<td>215 (4*)</td>
<td>22</td>
<td>10.2</td>
</tr>
<tr>
<td>1998</td>
<td>227 (3*)</td>
<td>22</td>
<td>9.7</td>
</tr>
<tr>
<td>1997</td>
<td>221 (4*)</td>
<td>19</td>
<td>8.6</td>
</tr>
<tr>
<td>1996</td>
<td>224</td>
<td>24</td>
<td>10.7</td>
</tr>
<tr>
<td>1996–2000</td>
<td>1083 (11*)</td>
<td>105</td>
<td>9.7</td>
</tr>
</tbody>
</table>

Among 807 female babies born at our hospital between 2013 and 2017, NUB occurred in 25 cases, yielding with a prevalence of 3.1% (Table 1, A). Among the 1083 female newborn babies born between 1996 and 2000, 105 cases had NUB, yielding with a prevalence of 9.7% (Table 1, B). Twenty-five NUB cases born between 2013 and 2017 showed overt bleeding 4.5 ± 1.8 days (median 4 days, range 1–8 days) after birth, and the bleeding lasted for 1.4 ± 0.8 days (median 1 day, range 1–4 days). In 105 cases born between 1996 and 2000, NUB was seen to occur 4.4 ± 1.2 days (median 4 days, range 0–7 days) after birth and continued for 1.9 ± 1.1 days (median 2 days, range 1–6 days). Five of 25 cases (20%) showed NUB in their diapers after the 7th day during the period 2013–2017 and 4 of 105 cases (3.8%) showed NUB on day 7 during the period 1996–2000. If any NUB continued until discharge from our hospital, the duration was the number of days until the day of discharge. Therefore, we could not collect any information on the possible occurrence of NUB after discharge from the hospital. Consequently
cases with occurrence of any NUB after discharge was not included in this study. The occurrence of any visible NUB until the time of discharge was observed by attending doctors or nurses and this information was routinely noted in medical records of our hospital. There was no significant difference in the presence or absence of NUB between babies, delivered by Cesarean section, staying in the hospital for 7 days or more and babies delivered vaginally staying in the hospital within 7 days.

The average gestational age at birth of NUB+ cases was 39.2 ± 1.3 weeks during the period of 2013–2017 and 39.6 ± 1.1 weeks in 1996–2000, and there was no statistically significant difference in gestational age between these two time periods. Maternal age of NUB+ cases was similar between mothers in 1996–2000 and in 2013–2017 (30.4 ± 4.4 vs. 31.2 ± 5.1 years, P = 0.58). On the other hand, gestational age at birth of all cases in 1996–2000 was statistically significantly longer than that of cases in 2013–2017 (37.9 ± 3.1 vs. 37.5 ± 2.6 weeks, P < 0.0001). Almost all NUB+ cases were term babies except for one case who was born at the gestational age of 36 weeks. The detail clinical characteristics of female babies born with and without NUB and their mothers during the period of 2013–2017 are shown in Tables 2 (Continuous variables were compared between groups using Wilcoxon rank sum test and categorical variables were compared using Fisher’s exact test. *These informations are not included in data analysis. **Associated fetal disease includes congenital fetal diseases, fetal growth restriction) and Table 3 (Continuous variables were compared between groups using Wilcoxon rank sum test and categorical variables were compared using Fisher’s exact test. *These information are not included in data analysis. **Coexisting diseases include maternal diseases (such as gestational diabetes, fibroid, ovarian tumor, cardiovascular disease and others), infection (hepatitis B, hepatitis C, chlamydia, HTLV-1 and others). ***Complications during pregnancy include preterm birth, threatened premature delivery, hypertensive disorder of pregnancy, preeclampsia, premature rupture of membranes, multiple pregnancy, placenta previa, blood type incompatible pregnancy, infection, thrombosis, placental abruption, low-lying placenta, hydramnion, oligoamnion, and other diseases).

Analysis of continuous variables between groups indicated that gestational age was longer, body height was taller, body weight was heavier, head/chest circumference was wider in NUB+ cases than in NUB- cases (Table 2). The occurrence of NUB in newborn girls was significantly higher in primipara than in multipara mothers without showing remarkable pregnancy related complications. The majority of NUB+ cases were born by vaginal delivery (Table 3).

Risk factors associated with NUB (2013–2017). To identify the risk factors for NUB, we selected several confounding factors such as gestational age at birth, maternal age, abnormalities at delivery, medical history of mother, pregnancy complications, and disease of the child. After a backward selection of variables with a p value of < 0.2 as analyzed by univariate analysis, we conducted multivariate logistic regression analysis. The risk of NUB was significantly increased in babies born at 39 weeks (odds ratio (OR) 3.87, 95% confidence interval (CI) 1.18–12.7, P = 0.026) or ≥ 40 weeks of gestational age (OR 10.2, 95% CI 3.58–28.9, P < 0.001) compared with the babies born at ≤ 38 weeks (Table 4). Younger age of the mother appeared to be significantly associated with the less occurrence of NUB (OR 0.92, 95% CI 0.85–1.00, P = 0.048) This finding Conversely indicates that as the maternal age increased by 1 year, the
frequency of NUB significantly decreased by 0.92 times. The presence of maternal intestinal disease, atopic dermatitis, non-reassuring fetal status (NRFS), and neonatal hydronephrosis were also the significant risk factors for NUB as shown in Table 4 (For multiple logistic regression analysis, we used one binary dependent variable (NUB) and six different independent variables. NUB, neonatal uterine bleeding; NRFS, non-reassuring fetal status; CI, confidence interval).

Risk factors associated with NUB (1996–2000). In order to identify the risk factors associated with NUB of female babies born in our hospital between 1996 and 2000, we first performed univariate analysis with different confounding variables such as gestational age, maternal history, and neonatal biophysical conditions. A multivariate logistic regression analysis was performed on these and other 6 factors which were found to be significantly associated with NUB in the 2013–2017 analysis. We found that similar to the period of 2013–2017, NUB increased significantly in babies born at 39 weeks (OR 3.04, 95% CI 1.43–6.45, P = 0.004) and ≥ 40 weeks (OR 4.54, 95% CI 2.20–9.39, P < 0.0001) of gestation compared with ≤ 38 weeks of gestation (Table 5). Although maternal age did not show any risk association with NUB, increasing birth weight of newborns was modestly associated with the occurrence of NUB (OR 1.07, 95% CI 1.01–1.13, P = 0.025) during 1996–2000. Other confounding factors and their association with NUB are shown in Table 5 (OR: odds ratio; NUB, neonatal uterine bleeding; PROM, premature rupture of membranes; NRFS, non-reassuring fetal status; HBV, hepatitis B virus; HCV, hepatitis C virus).

Symptoms related to endometriosis based on questionnaire survey. In an attempt to find an association between the occurrence of NUB and symptoms related to endometriosis, we conducted a web-based questionnaire survey among young women who were born with and without NUB during the period of 1996–2000. Among 1083 female babies (11 cases with no available data and were thus excluded) born at our University Hospital, 105 cases showed occurrence of NUB. Among cases without NUB, 205 cases born at ≤ 36 weeks of gestation were excluded because the gestational ages of NUB+ cases at birth were all 37 weeks or later. We contacted 105 NUB+ cases and 771 NUB- cases by individual telephone call to the phone number listed in the medical records and we could approach to 55 cases with NUB (52.4%) and 149 cases without NUB (19.3%).

In the questionnaire, we properly explained the research purpose of our web-based questionnaire survey and the subjects gave us verbal informed consent. Finally, responses to the questionnaires survey were obtained from 31 NUB+ women and 52 NUB- women. A flow chart of cases who participated in the online questionnaire survey is shown in the Fig. 1. All participants declined to take a physical examination/ultrasound/MRI or laparoscopy to confirm the diagnosis of their symptoms, if any. The demographic profiles of each group of women who were born with and without NUB and responded to questionnaires are showed in Table 6 (Continuous variables were compared between groups using Wilcoxon rank sum test and categorical variables were compared using Fisher’s exact test. * Includes women who responded both mother and sister in the same family). Women with a history of NUB were significantly younger than those without NUB (median, 21 vs. 23 yrs. P = 0.039). Gestational age at delivery of NUB+ cases was significantly longer than that of NUB- cases (median, 40 vs. 39 weeks, P = 0.036).
In the questionnaire survey, participants responded to 10 different questions about the symptoms related to menstrual pain and use of medication. We compared the responses to the questionnaires between 31 NUB + cases and 52 NUB- cases. There was no significant difference in the complain of cyclic pain and/or severity of cyclic/acyclic pain (VAS score 7–10), number of painful days, disturbance of daily life activity during cyclic/acyclic pain, number of days missing school or work, taking hormonal or non-hormonal medication, duration and effect of medication (Table 7). (Continuous variables were compared between groups using Wilcoxon rank sum test and categorical variables were compared using Fisher’s exact test).

While acyclic pain was significantly higher in the group without NUB, this difference vanished in the multiple logistic regression analysis. Analgesics and hormonal drugs were useful in relieving pain in more than 90% of women who used them, but there was no difference between groups. Pregnancy was found in 3 women in the NUB- group only and therefore, could not be compared with NUB + group. A graphic representation showing responses to eight different questionnaires in women with and without NUB is shown in Suppl. Figure 1. Univariate and multivariate logistic regression analysis were performed to evaluate the associations between the questionnaire responses and each characteristic of participants (Suppl. Table 1A-F). (*Continuous variable: median (range), categorical variables: n (%), NA: no response).

Association between response to questionnaires and clinical profiles of participants. Six different outcomes (A1, A2, A4, A5, A6, A8 of Table 7) as binary dependent variable and 14 independent variables including NUB were considered for univariate and multivariate logistic regression analysis. Outcome A1: “do you feel pain during period (cyclic)?” NUB was not associated with the presence of cyclic pain but young women with their higher maternal age significantly more frequently experienced cyclic pain (OR 1.37, 95%CI 1.02–1.84, p = 0.039). There was a tendency that women with lower BMI (slender women) have cyclic pain (OR 0.74, 95%CI 0.54–1.01, p = 0.053) (Suppl. Table 1A).

Outcome A2: “do you feel pain beyond period (acyclic)?” As for acyclic pain, young women with a family history of acyclic pain significantly more frequently (6.6 times) complained of acyclic pain (OR 6.59, 95%CI 1.07–40.4, p = 0.042) but NUB was not associated with it (Suppl. Table 1B). Outcome A4: “degree of your cyclic pain (VAS score)?” Three independent variables were associated with this outcome. The cyclic pain with a VAS score of 7–10 (severe pain) was significantly associated with the longer gestational age at delivery than in women with mild to moderate levels of cyclic pain (VAS score 0–6) (OR 2.08, 95%CI 1.12–3.86, p = 0.020). Similarly, women complaining of the severe pain had significant (about 7 times) menstrual lag of ≥ 7 days (OR 6.96, 95%CI 1.46, 33.2, p = 0.015) but revealed no association with NUB. Moreover, young women with early onset of menarche tend to more frequently complain of severe cyclic pain (VAS score 7–10) than in women with mild to moderate degree of cyclic pain (VAS 0–6) (OR 0.66, 95%CI 0.43–1.02, p = 0.063) (Suppl. Table 1C).

Outcome A5: “degree of your acyclic pain (VAS score)?” The acyclic pain with a VAS score of 7–10 was not associated with the presence of NUB. Comparing to taller women (median, 160 cm), young women with shorter body height (median, 156 cm) had a tendency to more frequently complain of severe acyclic
pain than in women with mild to moderate degree of acyclic pain (OR 0.83, 95% CI 0.67–1.02, p = 0.074) (Suppl. Table 1D).

Outcome A6: “does your cyclic/acyclic pain disturb daily life activity?” Age was the only independent variable associated with this outcome. Younger women (median, 22 yrs.) complained that suffering from severe cyclic and acyclic pain significantly and more frequently reported disrupted daily life activity (OR 2.67, 95% CI 1.01–7.06, p = 0.047) (Suppl. Table 1E).

Outcome A8: “do you take any pain killer or hormonal therapy for your cyclic pain?” Among 14 independent variables, two variables were associated with this outcome such as age at menarche and normal menstrual cycle. Young women with early onset menarche (median, 12 yrs.) significantly more frequently needed to take pain-killer or hormonal therapy to relieve their cyclic pain (OR 0.61, 95%CI 0.38–0.97, p = 0.038). Women who had normal menstrual cycle significantly more frequently (> 6 times) needed to take pain killer or hormonal therapy to relieve their cyclic pain (OR 6.31, 95%CI 1.26–31.6, p = 0.025) (Suppl. Table 1F). NUB had no association with this outcome.

**Discussion**

The hypothesis of the causal link between the occurrence of NUB and early-onset endometriosis has been proposed but never tested, and, as such, warrants investigation. Based on our retrospective search of medical records during two different time periods of 2013–2017 and 1996–2000, we further confirmed that visible uterine bleeding indeed occurs with a prevalence of 3.1% and 9.7%, respectively. Our five years retrospective finding during the period of 2013–2017 coincided with the previously published prevalence of 3–5%7, 8, 16. The three-fold difference in NUB prevalence during the period of 1996–2000 can be explained by the increasing distribution of younger maternal age and birth at longer gestational week. Different literatures reported various risk factors in the occurrence of NUB such as post-maturity, preeclampsia, low birth weight, and fetomaternal incompatibility8, 12. Multiple regression analysis with different confounding factors in our study identified that longer gestational age at birth and younger age of the mother were independent risk factors in the occurrence of NUB among others (2013–2017). The longer gestational age (post-maturity) at birth as a risk factor corresponds with the previous report3, 12, 17. In our separate analysis of confounding factors during the period of 1996–2000, we found that longer gestational age at birth remained the similar significant risk factor. Instead of maternal age, increasing birth weight of the baby (100 g) appeared as an additional risk factor after correcting for gestational age. This additional finding is in contradiction to the previously claimed risk factor (low birth weight) in the occurrence of NUB3, 12, 17.

The difference in the length of hospital stay between NUB + and NUB- cases could be a concerning factor in the occurrence of NUB. In fact, we did not observe any significant difference in the presence or absence of NUB between babies, delivered by Cesarean Section, staying in the hospital for 7 days or more and babies delivered vaginally staying in the hospital within 7 days. Therefore, it is expected that the length of hospital stay does not affect the occurrence of NUB. Our opinion can be corroborated by several previous
publications that NUB mostly occurs on the 3 to 5 days after birth\textsuperscript{5,7–10,12}. We presume that there might be some cases in which NUB could be observed after 7 days but those may be rare cases. We cannot ignore the possibility that there was NUB after discharge in the NUB- group. However, in our current and previous studies, the frequency of NUB itself was low and is therefore unlikely to refute our current findings.

In an attempt to find a possible association between NUB and endometriosis-related symptoms later in life, our web-based questionnaire survey confirmed that young women with a history of NUB at birth complained of severe cyclic/acyclic pain similarly to women without NUB. When we performed multiple logistic regression analysis with six different outcomes (binary dependent variable) and 14 independent variables including NUB, we found that NUB was not associated with any of these outcomes. Instead, longer gestational age at birth, early-onset menarche, slender women, and short body stature were significantly associated with the manifestation of severe cyclic and/or acyclic pain in these women. The majority of these endometriosis-related risk factors agreed with previous studies\textsuperscript{18,19}. In addition, younger women (median, 22 yrs.) with early-onset menarche and normal pattern of menstrual cycle mentioned that their daily life activity was disturbed and they frequently needed analgesics or hormonal therapy to relieve their debilitating symptoms. In fact, women with disturbance of daily life activity due to pain and resistance to medical treatment for alleviation of menstrual pain are among the high-risk groups of having endometriosis lesions\textsuperscript{20–22}. All these findings indicate that association between the occurrences of NUB and suffering from severe cyclic/acyclic menstrual pain/its consequences in these young women could be an incidental relationship rather than the cause of variable symptoms that might be related to the development of endometriosis.

Now two unclear issues remain to be resolved: (1) what role does the occurrence of NUB at birth exactly play in early puberty and young women? (2) how should we explain the cause of variable symptoms in relation to endometriosis in these women? These are issues at the current moment remain to be addressed. The retrograde flow of menstrual debris through the patent fallopian tube\textsuperscript{23}, metaplastic transformation of peritoneal mesothelium\textsuperscript{24,25}, theory of embryonic Müllerian rests in the pathogenesis of some cases of endometriosis in early puberty\textsuperscript{26–28} may explain some possibilities. Future study with imaging and laparoscopic intervention in these groups of women is necessary to confirm the cause-effect relationship between painful symptoms in NUB + and NUB- cases and endometriosis.

There are some limitations in our current study: (1) Sample sizes of NUB + and NUB- cases who participated in our web-based questionnaire survey are somewhat small. (2) We could not perform physical examination/ultrasound/MRI or diagnostic laparoscopy to confirm endometriosis who responded to our questionnaire survey, because none of participating women positively responded to our repeated requests to accept any of these investigation. (3) We did not include additional symptoms such as dysmenorrhea and abnormal uterine bleeding in our questionnaire survey that might be related to the occurrence of adenomyosis in these young women. (4) We could not include cases with NUB that may
occur after discharge from the hospital. Future studies are warranted with larger sample size to clarify all these unaddressed issues.

In conclusion, we reconfirmed that NUB indeed occurs in a proportion of newborn babies that is in agreement with previous studies. The longer gestational age, younger age of the mother, and overweight baby may be considered as high-risk factors for the occurrence of NUB. These findings may increase awareness among obstetrician, nurses and midwives who should keep these factors in mind and record them in clinical birth notes. In order to understand the true prevalence of NUB, parents should be encouraged to inform occurrence of any visible bleeding in the diapers of their infants after discharge from the hospital. In an attempt to find a link between NUB and endometriosis, our web-based survey results indicate that NUB + cases may manifest some symptoms related to endometriosis later in life during adulthood. Although it is premature to conclude an association between NUB and endometriosis in the presenting study, future prospective cross-sectional study is warranted to confirm the endometriosis-related symptoms in NUB + cases by physical examination/ultrasound/MRI. Although it is currently difficult to strongly emphasize that NUB could be associated in the development of early-onset endometriosis, our ongoing study on the mechanistic basis in the involvement of NUB in early-onset endometriosis may further illuminate this issue.

Materials And Methods

Study design. This is a combined retrospective and prospective case-controlled cohort study. The first part of this study consisted of retrospective observational cohort study during two different time periods (2013–2017 and 1996–2000) with an attempt to reconfirm the prevalence of NUB, to find the possible risk factors for NUB, and to examine any relevance or irrelevance in the findings between these two time frames. The search of medical records, with and without NUB, during the period of 1996–2000 was performed for the evaluation of questionnaire-based endometriosis-related symptoms later in life who are now adults, in addition to understand the differences in prevalence/risk factors of NUB between these two time periods. All available information such as the presence or absence of NUB, the period and timing of NUB, gestational week at birth, maternal age, coexisting diseases, abnormality in pregnancy, and neonatal profiles were obtained. The second part of this study consisted of a prospective cross-sectional web-based questionnaire survey among young women who had a history of NUB and no NUB during the period of 1996–2000 in order to identify the endometriosis-related symptoms among these women. We did not include symptoms (dysmenorrhea, abnormal uterine bleeding) related to adenomyosis in our questionnaires, because this was not the focus of our current study.

All retrospective searches of recent and old medical records and conduction of web-based questionnaire survey were carried out in accordance with the guidelines of the Declaration of Helsinki and with informed consent as necessary. This study was approved by the Ethics Committee of the Institutional Review Board (IRB) of Kyoto Prefectural University of Medicine (IRB approval No. ERB-C-1445-1).
Questionnaire survey. We retrospectively searched the medical records of female babies who were born at our department between 1996–2000. We noted all available contact information from the medical records and did telephone contact with NUB + cases and NUB- cases who are currently 20–26 years old. After clarifying the purpose of current study and obtaining informed consent, a prospective cross-sectional online questionnaire survey was conducted on the symptoms related to endometriosis. After approval by the Ethics Committee of the IRB of our University, this online questionnaire was developed using Google Forms (www.google.com/forms). All consented participants accessed the link shown on the homepage of our department (www.obgy-kpum.com) and answered the questionnaires. All questionnaire results were downloaded from the website for our study. As a token of gratitude and appreciation of their efforts, we distributed a Quo gift card equivalent to US$5.0 (equivalent to ¥700, Japanese yen) to each participant who responded to our questionnaires.

The questionnaires included variable questions such as cyclic/acyclic pain, severity of pain as measured by a 0–10 points of visual analogue scale (VAS) score (7–10 was considered as severe pain), disturbance of daily life activity caused by cyclic/acyclic pain, use of any pain-killer or hormone. All these manifestations are already validated and considered as symptoms related to endometriosis and as high-risk factors susceptible to develop laparoscopy-confirmed endometriosis. In addition to that, we also inquired about the demographic profiles of participants.

STATISTICAL ANALYSIS

Analysis of the risk factors for NUB (2013–2017). Univariate logistic regression analysis was performed to evaluate the associations between the prevalence of NUB and each newborn baby’s or mother’s characteristic for 218 variables. Candidate risk factors for inclusion in the multivariate analysis were selected on the basis of the results of univariate analyses (P < 0.2). Multivariate logistic regression analysis with backward variable selection (P < 0.05) was used to identify independent and significant risk factors for NUB and to estimate the respective odds ratios (ORs) and their 95% confidence intervals (CIs) of the selected factors. All reported P values were 2-sided.

Analysis of risk factors for NUB (1996–2000). Univariate logistic regression analysis was performed to evaluate the associations between the prevalence of NUB and each newborn baby’s or mother’s characteristic. Candidate risk factors for multivariate analysis were selected based on the results of univariate analysis (P < 0.2) and variables that were risk factors in analysis of the risk factors in 2013–2017 (gestational age, maternal age, maternal intestinal disease, non-reassuring fetal status (NRFS), neonatal hydronephrosis). Multivariate logistic regression analysis (P < 0.05) was used to identify independent significant risk factors for NUB and to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs) for each of the selected factors. All reported P values were 2-sided.

Analysis of the questionnaire survey. Univariate and multivariate logistic regression analysis was performed to evaluate the associations between the questionnaire responses and each characteristic (14 variables), and odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated. However,
because of missing data for "family history of cyclic/acyclic pain " and "normal menstrual cycle," if no association was found in univariate analysis (P > 0.2), they were not included in the multivariate analysis. Because body weight is highly correlated with BMI, it was not included in the multivariate analysis. All reported P values were 2-sided.

All results are expressed as mean ± SD and/or median. Continuous variables were compared between groups using Wilcoxon rank sum test and categorical variables were compared using Chi-squared test or Fisher's exact test. A value of p < 0.05 was considered statistically significant. All data analyses were conducted using SAS software version 9.4 (SAS Institute Inc. Cary, NC, USA).

Declarations

Ethical approval and consent to participate: All procedures were in accordance with the standards of the responsible committee of human study (institutional or national) and the Helsinki Declaration of 1964 and later versions. Informed consent was obtained from all participants in this study.

Data availability: The data underlying this article will be shared on reasonable request to the corresponding author.

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Author contributions: KO was involved in retrospective searching of medical records, web-based questionnaire survey, and writing manuscript draft; KNK contributed to original concept, study design, overall supervision, data collection/analysis/interpretation, and manuscript writing; HK, AK, AF, KI contributed to data interpretation; GH, ST contributed to data monitoring and statistical analysis; SWG, JK, TM were involved in stimulating discussion and editing of the article.

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References


**Table 2 To 7**

Table 2 To 7 is available in the Supplementary Files section.

**Figures**
Figure 1

A flow chart of cases who participated in the web-based questionnaire survey and were born with and without neonatal uterine bleeding during the period of 1996-2000.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suppl.Fig.1.docx
- SupplementaryInformation.pdf
- Table2To7.docx