ANEMIA AND HYPERTENSION AS RISK FACTORS FOR PRETERM DELIVERY IN YEREVAN, ARMENIA

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Abstract

Objective: Anemia and hypertension are the most common disorders among women delivering at Yerevan maternities. Their role as potential risk factors for preterm delivery was assessed.

Design and Methods: A matched case-control study was conducted. Data were obtained from 5 maternities located at different sites, including the center of Yerevan. Approximately 2800 delivery records covering a six-month period (Sep 2002-Feb 2003) were reviewed. Women having a preterm delivery – less than 37 complete weeks of gestation at delivery – were defined as cases (n = 228). Women who delivered at term (37-41 weeks at delivery) and met the matching criteria were defined as controls (n =216). Age, parity, and first visit to antenatal care were used as matching criteria. In addition, both cases and controls were obtained from the same maternity to control for socioeconomic status (SES) and possible laboratory differences. They were matched also on season of delivery (autumn/winter).

Anemia was defined according to WHO standards as Hb less than 11 g/dL. Hypertension was defined by a systolic blood pressure greater or equal to 140 mm Hb and a diastolic blood pressure of greater or equal to 90 mm Hb or by a rise in blood pressure of systolic ≥ 30 mm Hg and diastolic ≥ 15 mm Hg. Data analysis was performed using the Stata statistical package (v 7.0). McNemar's test was used for odds ratio calculation and conditional logistic regression was used to control for other potential confounders, such as multiple pregnancy, prior premature labor, and placental abruption.

Results: The prevalence of anemia at the end of pregnancy was 42.5% in controls and 50% in cases; 15% of controls and 17.4% of cases had moderate anemia: Hb level 7.0-9.9 g/dl. There was only one case of severe anemia (Hb=6.8 g/dl), which was excluded from the analyses. There were no statistically significant differences in anemia prevalence at the end of pregnancy between cases and controls. The adjusted odds for preterm delivery in the presence of maternal anemia, defined as Hb less than or equal 9.0 g/dl, was three times higher as compared with the reference group of non-anemic women. However, the results were not statistically significant (95% CI = 0.9-10.6, p=0.067). The small number of observations with multiple pregnancy and prior preterm delivery precluded the inclusion of these variables in the final logistic regression model. After adjustment for potential confounders pregnancy induced hypertension (isolated hypertension, mild and severe preeclampsia) was positively associated with prematurity (adjusted odds ratio: 4.0; 95% CI = 1.9 to 8.7, p< 0.001). Chronic hypertension was also significantly different between cases and controls (adjusted odds ratio: 9.8, 95% CI= 1.2 to 80.5, p<0.05); however, the small number of observations with chronic hypertension resulted in a wide confidence interval. The adjusted variables were placenta abruption and premature rupture of membranes.

Conclusion: Maternal anemia at the end of the third trimester of pregnancy was not significantly associated with prematurity. However, anemia with Hb level less than 9.0 g/dl increases the risk of preterm labor threefold. Pregnancy induced hypertension (isolated hypertension, mild and severe preeclampsia), as well as chronic hypertension were significantly associated with preterm delivery. However, future studies with larger sample sizes are required in order to investigate these relationships in the presence of potential confounders.

Literature review

Preterm delivery

Premature or preterm delivery (delivery before 37 complete weeks of pregnancy) occurs in 7% to 12% of all deliveries, but accounts for over 85% of all perinatal morbidity and mortality (1). The incidence of preterm delivery is much higher in developing countries owing to socio-economic conditions (SES), low access to health care facilities, and poor antenatal care (2, 3). Preterm delivery is a public health problem in Armenia as it is the major cause of perinatal mortality and life-long disability (4). However, official data from the Ministry of Health of Armenia suggests that the rate of preterm delivery is relatively low in Armenia and it is comparable to those in the developed countries: 8.4% in 2000 (5). On the other hand, the prevalence of low birth weight babies, defined as the live births between 1kg and 2.5 kg, has been steadily increasing since 1990 (4). Although there is a clear distinction between these two conditions (preterm delivery and low birth weight babies), it might be assumed that the rate of preterm delivery has been increasing also, as the majority of babies with low birth weight, are born preterm (6).

There are many conditions and diseases that increase the risk of preterm delivery (7). Age, parity, maternal education, occupation and low SES are recognized risk factors for preterm delivery (8, 9). The preterm delivery rate is higher in women younger than 18 or older than 35 (9, 10). Grand-multipara women (> 8) have a higher preterm delivery rate than less parous women (10). Low maternal height (less than 145 centimeter) and maternal undernutrition, body/mass index (BMI) less than18.5, are also risk factors for preterm delivery (8, 10). Women who are engaged in the strenuous physical activities are also at risk for preterm delivery (9, 10).

Smoking more than 10 cigarettes per day decreases the overall length of gestation (8, 10). Pregnant women with preexisting medical illness (diabetes, chronic hypertension), previous poor pregnancy performance (prior premature delivery, fetal growth retardation, malformations, and placental accidents) are of more risk for preterm delivery (8, 9). History of prior preterm delivery strongly correlates with subsequent preterm labor (10).The risk of recurrent preterm delivery for those whose first delivery was preterm increased threefold compared to women whose first infant was mature (10).

The following conditions and complications of pregnancy have been associated with increased risk of preterm delivery: multiple pregnancy, anemia, pregnancy induced hypertension, surgical interventions during pregnancy, urinary tract infection and sepsis (9, 11). However, in about the half of all cases, the cause of preterm delivery is unknown (8-10).

Anemia

Anemia, particularly iron deficiency anemia, is a major health problem in developing countries due to poor nutrition, inadequate birth spacing and multiparity (12). Anemia is more common in multiple pregnancies (13). Anemia in women is associated with parity: it is more frequent in multiparous women (12, 14). Women less than 20 years of age are at increased risk for developing anemia during pregnancy (14). Severe maternal anemia (Hemoglobin<7.0g/dl) is significantly associated with adverse pregnancy outcome, such as preterm birth, low birth weight, etc. (15, 16). Some authors set 9.0 g/dl as a critical value for Hb level; anemia with Hb level lower than 9 g/dl is associated with increased risk for preterm delivery (17). Some authors emphasize that only maternal anemia during early pregnancy (first trimester) is associated with an increased risk of preterm birth, while others believe that maternal anemia during the third trimester of pregnancy and at labor is associated with an increased risk of prematurity (15, 16).

However, there is still disagreement about whether anemia increases the risk of premature delivery (17).

Prior research has revealed that anemia is a public health problem in Armenia due to the low socio-economic conditions and poor diet (18, 19). The Strategic Program for Overcoming Poverty stated that 52.6% of population in Armenia was considered to be poor, from which 19.6% were very poor in 2001 (20). According to the report "The Health and Nutritional Status of Women and Children in Armenia", 15% of women of fertile age had either mild or moderate anemia in 1998 (18). Anemia was more common in pregnant women, 11-29%, with trends increasing as pregnancy advanced (18). According to the Demographic and Health Survey, Armenia 2000, the prevalence of anemia was 9.9% in urban areas, 16.5% in rural areas and 5.6% in Yerevan (19). According to a recent study by the Ministry of Health, the prevalence of anemia is as high as 50-60% among pregnant women in some regions of the country (4). In 1999, 121 women out of every 1,000 had anemia-related complications during delivery (21). Data from Health Department of Yerevan Municipality indicates that anemia is the most common condition encountered during pregnancy among women delivered at maternities in Yerevan (22).

Hypertension

Hypertensive disorders in pregnancy are a leading cause of maternal death worldwide, and even in developed countries increase perinatal mortality rates 5-fold (23, 24). Hypertension affects 10% of pregnancies in the United States and remains a leading cause of both maternal and fetal morbidity and mortality (23, 25). Hypertensive disorders (pregnancy induced hypertension and chronic hypertension) are more common in multiple pregnancies (26). Pregnancy induced hypertension (isolated hypertension, preeclampsia and eclampsia) is more common in nulliparous women (10). Chronic hypertension is more common in multiparas with advanced age (10). Maternal obesity adversely impacts pregnancy outcome primarily through increased rates of hypertensive disorders (27, 28).

According to UNDP, hypertension is one of the most frequent causes of maternal mortality in Armenia (29). Much of the neonatal loss attributed to hypertensive disorders is the consequence of preterm delivery, either from spontaneous labor or because of medically induced delivery (23, 28). Data from the Health Department of Yerevan Municipality indicates that hypertensive disorders are the second most common condition during pregnancy (22). Nevertheless, no information is available on the role of anemia and hypertension in developing preterm delivery in Armenia.

Methods

Research questions

The following research questions were addressed in the study:

1. What is the prevalence of preterm delivery in Yerevan, Armenia?
2. What is the prevalence of anemia at the time of delivery among women with preterm delivery? Is there a significant difference in proportions of anemia between women with preterm and term delivery (cases and controls)?
3. What proportion of women with preterm delivery had anemia during the first and second trimesters of pregnancy? Is there a significant difference in proportions of anemia during the first and second trimesters of pregnancy (before 28 weeks of pregnancy) between women with preterm and term delivery?

4. What proportion of women with preterm delivery had any form of pregnancy induced hypertension (PIH): mild/severe preeclampsia, isolated hypertension? Is there a significant difference in proportions of PIH between cases and controls?
5. Is there a significant difference in the proportions of chronic hypertension between cases and controls?
6. Is there any difference in the proportions of other risk/causal factors for preterm delivery between cases and controls: prior preterm delivery, body mass index (BMI), multiple pregnancy, and placenta abruption?

Study Design

Rationale for choice of study design

In order to study the association between premature delivery and variables of interest (anemia and hypertension) a matched case-control study was conducted with one control per case. This design is the most appropriate for answering the research question as preterm delivery is a relatively rare condition and information on exposure (anemia, hypertension) is available in medical records. In addition, the case-control design is not as expensive and time-consuming as the other designs, such as cohort, prospective, and experimental studies. A matched case-control study was chosen to control for potential confounders. The relationship of these variables and preterm delivery is well explored, and there was no interest in examining those variables in this study. Because these covariates are known to be true confounders, the statistical power should be increased by matching on them.

Definition of disease and exposure

Premature delivery is defined as a delivery before 37 complete weeks of pregnancy, starting from the first day of the last menstruation period (11, 14). The lowest gestational age, considered as a preterm delivery is 23 weeks (14). Based on the WHO definition, Hb less than 11 g/dl is defined as anemia (18). Pregnant women with hypertension are usually divided into two groups: normotensive women who develop pregnancy-induced hypertension (isolated hypertension, preeclampsia, and eclampsia), which is uniquely related with pregnancy and regresses postpartum; and women with chronic hypertension, who become pregnant and are at increased risk for developing superimposed preeclampsia (14, 15). Pre-existing hypertension is defined as maximum diastolic pressure more than 80mm Hg in the first 20 weeks of pregnancy or in the presence of a confirmed history of chronic hypertension prior to pregnancy (11, 14). Antenatal care information available from the delivery records was used to confirm the history of chronic hypertension. Pregnancy induced hypertension was defined by a systolic blood pressure greater or equal to 140 mm Hg and a diastolic blood pressure of greater or equal to 90 mm Hg or by a rise in blood pressure of systolic $\geq$ 30 mm Hg and diastolic $\geq$ 15 mm Hg (11, 15, 16).

Definition of cases and controls, matching criteria

Study population are women, who delivered during Sep 2002-Feb 2003 in all maternities in Yerevan permitting data collection. Although it was originally planned to ask all 10 maternities to participate, only 5 was included in the study; the number of observations obtained from these 5 maternities was large enough to meet the desired sample size. Maternities were selected purposefully to cover the entire territory of Yerevan. If two maternities were located next to each other, only one was asked to participate.

Cases are defined as women with premature delivery during the specified period, who delivered in Yerevan. Controls are defined as women with term (at 37-41 weeks) delivery during the same period, living in Yerevan. Controls were matched on age (+/-2 years; for women under 20 matching criterion was stricter: +/-1 year, to assure equal number of young primigravida in cases and controls), parity, place of delivery, season of delivery, and on the trimester of pregnancy when first visit was made to antenatal care unit (first, second or third). The study definition of parity is number of all previous births, including stillbirths. Cases and controls were matched according to: no prior delivery, 1 or two previous deliveries, and 3+ deliveries. To control for seasonal differences in anemia development and other unknown confounders associated with time, cases and controls were matched on season of delivery: autumn/winter. The next consecutive delivery record, which fitted the matching criteria was chosen as a control.

Exclusion Criteria

Records with any unclear information on gestational age at delivery were excluded from the study. Records with incomplete information on matching variables, such as age, parity, were also excluded. Cases, who had no antenatal care visit during pregnancy were matched with controls without antenatal care visit. Records with incomplete information on several important variables at the same time were also excluded.

Potential confounders

Some of the known confounders were controlled initially by matching cases and controls by age, parity, SES (by matching on the place of delivery), and by the first visit to antenatal care unit (ACU). Both cases and controls were obtained from the same maternity to control for socioeconomic status (SES) and possible laboratory differences. Other potential confounders, which will be controlled while analyzing the data, are multiple pregnancy, prior preterm delivery, birth interval, and BMI.

Study Variables

The outcome variable was status at birth: preterm/term delivery. Independent variables were Hb level at the time of delivery (4 levels), Hb level before 28 weeks of pregnancy (4 levels) and hypertension (3 levels). Intervening variables were prior preterm delivery (2 levels), prior miscarriages (3 levels), prior abortions (3 levels), complications at delivery, including placenta abruption and premature rupture of membranes (2 levels), multiple pregnancy (2 levels), and BMI (3 levels).*

Sample Size Calculations

Sample size calculations were performed based on the formula for case-control designs (30). The preliminary estimates of proportions were obtained from the purposive sample of cases of preterm delivery, collected from 3 maternities in Yerevan and their matched controls.

$$1.96*(2*0.017*0.983/n)^{1/2} + 0.84*(0.017*0.983/n + 0.057*0.943/n)^{1/2} = 0.04$$

The calculated sample size for each group was equal to 211.

Data collection and preparation

Data were obtained from 5 maternities throughout Yerevan (Table 1). Approximately 2800 delivery records covering a six-month period (Sep 2002-Feb 2003) were reviewed. Information from both medical records and the antenatal care information sheet was collected. A record extraction form was developed for facilitating the data collection process. Information on 20 variables was gathered (including information on antenatal care).

*For detailed information on this variables see section on *Data Collection and Preparation*.

To control for the interval between births as a potential confounder, a spacing interval was calculated between last and current delivery. The date of current delivery was obtained from delivery record. However, there was a problem with the previous delivery date, as only the year of previous delivery was mentioned in the records. However, the assumption was made that births are evenly distributed throughout the year and the mid point (1 July) of a particular year was the best estimate of the prior delivery date. The interval between births was calculated by subtracting the estimated prior delivery date from the current one. Birth interval was categorized as 2 levels based on WHO guidelines for preferred spacing between births: less than 3 years and 3 years or more.

The Hb analysis that was taken at the closest time before delivery was considered the best estimate of anemia at delivery. The units of measurement of the Hb level were transformed into the international units (g/dl) by dividing them by 10 if units of g/l were used or by dividing them by 6 if mg% was used. Information regarding Hb level during first, second and third trimesters of current pregnancy was obtained from the antenatal care information sheet. Using the date of the Hb measurement, and the first day of the last menstrual period (LMP), gestational age was calculated and categorized into Hb level during the first (before 14 weeks of pregnancy), second (14-27) and third week of pregnancy (28 and above). Due to the small number of Hb measurements during the first and second trimesters of pregnancy, separate analyses of anemia during these trimesters was not appropriate. For that reason, another variables was generated by combining Hb measurements before week 28 of pregnancy. In the presence of multiple Hb measurements for a single study subject during the specified period the lowest Hb measurement was taken.

Cutoff points for defining anemia were determined according to WHO guidelines and are as follows:

Hb >=11g/dl (no anemia),

Hb = 10-10.9g/dl (mild anemia),

Hb =7-9.9g/dl (moderate anemia) and

Hb<7g/dl(severe anemia).

However, considering that prior and current studies indicate the presence of no severe anemia in Armenia, the level representing severe anemia was excluded from analysis. Instead, taking into account the evidence from the literature about the strong relationship of preterm delivery and anemia with a cut off level at 9.0 g/dl, for the purpose of this study moderate anemia was classified as moderate anemia 1(Hb=9.1-9.9 g/dl) and moderate anemia 2 (Hb =7.0-9.0).

Hypertension was classified as pregnancy induced hypertension, chronic hypertension and no hypertension (for cut off levels see *Definition of disease and exposure* section). Small number of observations with isolated hypertension precluded separate analyses of relationship between preterm delivery and different forms of pregnancy induced hypertension (isolated hypertension, mild and severe preeclampsia).

Prepregnancy BMI was calculated by dividing the estimated prepregnacy weight in kilograms to the height in meters squared (kg/m^2). Estimates for prepregnancy weights were derived using a differential approach for cases and controls. Since average weight gain during pregnancy is 10kg (15 kg for twins), the best estimate for prepregnancy weight for controls was calculated by subtracting 10 (15 for twins) from the women's weight at the end of pregnancy. As for cases, the amount subtracted from the weight at the time of delivery was determined by the gestational age at delivery; for each 4 weeks (1 obstetrical month), one 1 kg was subtracted from the weight at the time of delivery. For example, if delivery occurred at anytime between 29 and 32 weeks of pregnancy 8 kg was subtracted from the weight at the time of delivery. BMI was categorized based on WHO guidelines:

BMI = 18.5-25 kg/^{2} (normal),

BMI<18.5 (underweight),

BMI>25 (overweight).

Maternal age was categorized onto three levels: below 20 years, 20-29, and 30 years and over.

Abortions were categorized according to the number of prior abortions: no abortion, 1 abortion and 2 or more abortions. Opportunity of exposure was considered, i. e. only women with more than one pregnancies had an opportunity of having prior abortions. Therefore, only multipara women were included in this variable. Prior miscarriages were categorized the same way.

The frequency of visiting the antenatal care unit (ACU) was defined as adequate (4 and more) and inadequate (less than 4) following to WHO guidelines.

Data entry and cleaning

Data were entered using Microsoft Access Forms. Separate forms were used for cases and controls as data on them were kept on separate tables. Data were checked for logical consistency for each of the variables. Range checks were used to identify extreme values for Hb (more then 145 and less than 60), number of pregnancies (more than 20 and less than 1), number of deliveries (more than 5), etc. Accuracy of matching was checked comparing cases and controls on age, parity and gestational age on first visit to ACU. There were more cases without antenatal care visits as compared to controls, for that reason some of cases remained unmatched.

Data analysis

Data were transferred to the Stata Statistical package (v. 7). The initial steps of the analysis consisted of summarizing all variables in order to assure accuracy of data. Unusual values were checked and corrected if necessary. Bivariate relationships were analyzed using cross-tabulations, paired t-test, McNemars test, and conditional logistic regression. Simple conditional logistic regression was used to assess the relationship between each of the independent variables and preterm delivery, and then multiple conditional logistic regression was used for introducing intervening variables. Intervening variables were investigated in a stepwise process, first looking at 1 variable at a time, combinations of 2 or more intervening variables were subsequently included in the model. Interactions were assessed via generation of new variables, which were included in the model and dropped when there was no statistically significant interaction ($p>0.05$). The best model was chosen with regards to the research question of interest as well as the statistical significance of variables. The result of the log-likelihood test was also taken into account when 2 models were similar with respect to the other important determinants.

Ethical considerations

Study proposal was reviewed and approved by the Student project IRB committee within AUA.

Results

Accuracy of matching

In total, data on 228 cases with preterm delivery and 216 controls with term delivery were obtained. 12 cases (5.3%) remained unmatched and were excluded from analyses, as no controls were found corresponding to the matching criteria. Distribution of study population across maternities is depicted in Table1.

In total, 17 records were omitted due to the incomplete information on matching variables or inaccurate information on gestational age at the time of delivery.

Cases and controls were similar with respect to age, parity, and gestational age at first visit to antenatal care: mean age of controls was 25.3 versus 25.4 in cases, 58.3 % controls and 56.6% of cases were nulliparous (Table 2).

Visits to ACU were not made by 11.6 % of controls and 12.3 % of cases (Table 2). For women making visits, the first visits to the ACU were normally distributed throughout pregnancy with mean/ mode gestational age of approximately 17 weeks at first visit (Table 2). One-third of women (34.3 %) first visited the ACU during the first trimester of pregnancy, 58 % during the second trimester and 7.7% during the third trimester (Table 2).

Prevalence of preterm delivery in Yerevan

There were 2754 deliveries during the period of Sep 2002-Feb 2003 (Table 3). The prevalence of preterm delivery ranged between 6.9-10.6 % across maternities: on average the prevalence was 8.2%. in these 5 maternities of Yerevan (Table 3).

Prevalence of anemia at the end of pregnancy in cases and controls

Paired t-test was used to compare mean Hb levels at the end of pregnancy; it was significantly different between cases and controls ($p<0.05$) and was 11.1 g/dl in controls and 10.9 g/dl in cases. The prevalence of anemia at the end of pregnancy was 42.5% in controls and 50% in cases; 15% of controls and 17.4% of cases had moderate anemia (Table 4). There was only one case of severe anemia with Hb level of 6.8 g/dl, which was excluded from the analyses.

Simple conditional logistic regression revealed a weak association between anemia at the end of pregnancy classified according to the WHO and preterm delivery; the OR for delivering preterm was 1.5 times higher in case of mild anemia, and 1.4 times higher in case of moderate anemia compared with normal Hb values. However, the results were statistically insignificant ($p>0.05$).

When cutoff point of 9.0 g/dl for Hb was considered, moderate anemia was divided into 2 levels and included in the model (Table 5). The OR for delivering preterm in the presence of maternal moderate anemia with Hb level ≤ 9.0g/dl was on average 3 times higher compared with non-anemic women ($p<0.05$, 95% CI = 1.01 to 8.8) (Table 5).

Prevalence of anemia during first and second trimester of pregnancy

Information on Hb level before 28 weeks of pregnancy was not available for 19% of study subjects, as 12% of them had no antenatal care visits and 7% visited the ACU only during the third trimester of pregnancy (Table 2). Information on Hb measurements was missing in 98 cases (27.1%) of eligible subjects visiting the ACU before 28 weeks of pregnancy.

The prevalence of anemia before 28 weeks of pregnancy was 33.4% in controls and 31.3% in cases (Table 6). According to the results of simple logistic regression neither mild nor moderate anemia during the first and second trimester of pregnancy was significantly associated with preterm labor (Table 5).

Prevalence of pregnancy induced and chronic hypertension

The overall prevalence of all forms of hypertension among pregnant women was 12.2% (Table 7). The prevalence of both pregnancy induced hypertension and chronic hypertension was higher in cases as compared with controls (Table 7).

In simple logistic regression models both forms of hypertension are significantly associated with preterm labor: the OR for PIH was 3.3, 95% CI = 1.6 to 6.7, $p<0.05$, and OR in case of chronic hypertension was 8.0, 95% CI = 1.1 to 63.9, $p<0.05$ (Table 5).

Differences in other risk/causal factors for preterm delivery

The following variables are known/suspected confounders for preterm delivery and anemia and hypertension: short height, low BMI, prior abortions, prior miscarriages, prior preterm delivery, short birth interval (less than 3 years), inadequate visits to ACU(<4), multiple pregnancy, and placenta abruption.

Cases and controls were quite similar with respect to height. There was only one case with short stature - 140cm. Cases and controls were similar by weight as well: on average, 12% of women were underweight and 28% were overweight before pregnancy (Table 8). Simple logistic regression of prepregnacy BMI and preterm delivery revealed that there is no significant association between preterm delivery and underweight (BMI <18.5 kg/m^2) or overweight women (BMI>25 kg/m^2) as compared to normal women (BMI=18.5-25 kg/m^2) (Table 5). Being underweight was slightly protective for delivering preterm compared with normal weight, however, the results were not statistically significant (Table 5).

Overall, 36.6% of multiparous women had one or more abortions (Table 9). The number of abortions ranged from 1 to 15 with median of 2 abortions. Simple logistic regression, and McNemar's test revealed no significant association between prior abortions and preterm labor (Table 5).

Overall, 25.4% of multiparous women had one or more miscarriages (Table 9). There were no significant differences regarding the proportion with prior miscarriages between cases and controls (Table 5).

One-fourth of the multipara women had one or more prior preterm delivery. Logistic regression indicated that the OR for delivering preterm for women with prior preterm delivery was 10 times higher compared to the women without prior preterm delivery (95 CI=3.1-32.8, $p<0.001$) (Table 5).

Short birth spacing had 38.9% of controls and 44.4 % of cases. However, there was no significant association between birth spacing and preterm delivery; OR=1.6, 95 % CI =0.8- 3.2, $p>0.05$ (Table 5).

There was an inadequate number of antenatal visits(< 4) for 15.7 % of controls and 22.4 % of cases. The OR for developing preterm labor was 1.6 (95%CI =0.9-2.8) times higher for those women who had an inadequate number of antenatal visits, however, the results were insignificant (Table 5).

In total, there were 16 multiple pregnancies, including one triple pregnancy. There were no multiple pregnancies among controls; the prevalence of multiple pregnancy among cases was 7%. Multiple pregnancy was highly significantly associated with preterm labor ($p<0.001$).

Finally, 8% of cases and 1.5 % of controls developed placenta abruption during delivery. According to the results of simple logistic regression, there was statistically significant association between placenta abruption and preterm delivery; OR=6.0, 95% CI= 1.3-26.8, $p<0.05$ (Table 5).

Best Multivariable Model of the Odds of Preterm Delivery

The multivariable conditional logistic regression model was chosen based on the statistical significance, relevance to the research questions, and the results of log likelihood test. The variables included in the model were anemia at the end of pregnancy, hypertension (PIH and chronic hypertension), and complications at delivery, including placenta abruption and premature rupture of membranes (table 10).

Multiple pregnancy and prior preterm delivery(s) were also significantly associated with preterm delivery, however, the small number of observations precluded the inclusion of these variables in the final logistic regression model.

Post-hoc power calculations

Power calculations were performed taking account actual sample size (228 and 216), observed proportions (2.3% in controls and 6.3% in cases) and level of significance (alpha=0.05). Power calculations for detecting significant difference between preterm delivery and anemia with Hb level ≤ 9.0mg/dl was 0.47.

Discussion

The overall prevalence of preterm labor in maternities of Yerevan was 8.2% on average, 21% higher than the official statistics of 6.8% in 2000. Comparison of the data from the maternities and the officially recorded data from Department of Health at Yerevan Municipality indicate that there is a difference of almost 50% in the prevalence of preterm delivery in some of the maternities. Possibly, this difference is connected with variations in defining preterm labor in different maternities: there were both cases of underreporting and overreporting of preterm labor. Some maternities rely on the weight of an infant (<2500) in defining preterm labor. Another explanation of recorded higher prevalence might be connected with unknown peculiarities of maternities included in this study. However, this is also consistent with the reports of prior studies pointing out that there were incomplete data on preterm delivery (6).

The prevalence of anemia at the end of pregnancy was uniformly high in all maternities, and was 42.5% in women with term deliveries and 50% in women with preterm deliveries. According to "The Health and Nutritional Status of Children and Women in Armenia" the overall prevalence of anemia in pregnant women was 15-30% on average, with rates increasing as pregnancy advanced. In urban areas, the prevalence of anemia was 17.6%. However, the total number of pregnant women in urban areas studied in that survey was only 51. According to the Demographic and Health Survey, anemia prevalence was 12% among pregnant women in 2000 (total number of pregnant women was 169). However, it is not clear yet during which trimester of pregnancy those women were studied, as there is a clear association between trimester of pregnancy and the risk of developing anemia. The results of the present study are more consistent with the 1994 data from the MOH, which pointed out that 50% of pregnant women had anemia at the time of delivery. Definitely, there are a number of reasons for such a high rate of anemia at the end of the pregnancy. Indeed, the poor quality of antenatal care is one of the major factors contributing to it.

Though anemia was not statistically significantly associated with preterm delivery, the OR of 3.1 with 95% CI from 0.9 to 10.6, indicates that there should be practical signifiacance in separating anemia with cutoff level of Hb at 9.0g/dl. The post-hoc power calculation indicate that sample size was not adequate for investigating this relationship.

The current study did not find a significant relationship between BMI and birth interval and preterm delivery, although these 2 variables are known confounders for the relationship between preterm delivery and anemia/hypertension. This non-finding might be due to the assumptions made in calculation of these variables (see section on *Data collection and preparation*).

The prevalence of hypertension also varied across maternities and ranged from 4-16.5%. On average it was 4.6% in controls and 15.4% in cases. All cases of chronic hypertension were from 2 maternities, there were no cases reported in the remaining 3, which was possibly due to the diagnostic discrepancies in defining chronic hypertension, and also lack of reliable data on blood pressure before pregnancy and during the first 20 weeks of pregnancy. Defining different forms of pregnancy induced hypertension was also a matter of discrepancies between maternities. While, some of the maternities rely on the contemporary definitions of pregnancy induced hypertension, the others used outdated classifications. Another problem was connected with the lack of reliable information on prior blood pressure, especially when women did not visit ACU.

All forms of hypertension were significantly associated with preterm delivery. However, the small number of observations with chronic hypertension resulted in a wide confidence interval. Another concern is that possibly not all forms of pregnancy induced hypertension are associated with preterm delivery. The prior studies indicate that mainly severe preeclampsia increases the risk of preterm delivery (8). Nevertheless, the small number of observations with isolated hypertension and mild preeclampsia precluded their investigation and separate analysis in the present study.

Study Limitations

There are study limitations connected with the design of the present study.Epidemiological studies, including case-control, have several threats to internal and external validity. Some of the limitations observed during this study are the following:

1) Diagnostic and instrument differences

Different diagnostic techniques were used for measuring Hb level in different maternities. While some of the maternities are well equipped and contemporary laboratory methods are used, the others still rely on outdated techniques. Possibly, this may contribute to the high anemia prevalence in some maternities and low prevalence in others (32-60%). The reliability of the anemia prevalence data, this fact may also limit the generalizability of findings to other maternities. However, it should have no impact on internal validity of other findings, as cases and controls were chosen from the same maternity. There might be similar discrepancies between diagnostic techniques used in ACUs and maternities.

There is a concern about the reliability of height and weight measurements between maternities, as well as between the different members of the personnel of the same maternity. Possibly, this may also account for the observed weak association between BMI and preterm delivery.

2) Selection bias

Not all maternities were included in the study. In case of differences between them and the maternities included in the study, the generalizability of the findings might be limited.

3) Small sample size

The sample size was adequate for answering some of the research questions, however, for answering other research questions, such as exploring the association of preterm delivery and anemia/hypertension after adjusting for all potential confounders, a larger sample size would be required. Unfortunately, the actual sample size did not allow to explore fully the relationship of anemia with cutoff level of Hb at 9.0 g/dl and preterm delivery, and different forms of pregnancy induced hypertension, such as isolated hypertension, mild and severe preeclampsia.

4) Accuracy of assumptions

Assumptions were made for BMI and birth interval calculations, which might be not true. However, as similar approach was used for both cases and controls, it is believed that the bias is conservative. Nevertheless, if other researchers implement different strategies for the calculation of BMI and birth interval, they may find different results

Recommendations

Area for further research

1. A prospective study for assessing the potential consequences of anemia during pregnancy and delivery, as well as postpartum, should be developed. Potential harm to fetuses and infants should be considered.
2. To estimate underreporting of preterm delivery in Armenia, and the medical records of women having late abortions might be used.

General Recommendations

1. The implementation of unique diagnostic standards for different diseases and disorders, particularly for preterm delivery, anemia and hypertension, is highly valuable. For that purpose, the written WHO guidelines might be used.

2. The range of moderate anemia is wide (7.0-9.9g/dl) and the potential harm to the fetus and complications for pregnant women may not be the same for those with lowest and highest Hb level based on the results of this study and supporting literature (17). It might be valuable to consider cut off level of 9.0g/dl for anemia in future studies of anemia in Armenia.
3. The quality of services provided by the ACU are not satisfactory, since this study indicates that almost half of all pregnant women had anemia at the end of the pregnancy. The quality of services should be improved via continuous education of medical staff of ACU.
4. The access to the antenatal care should be improved, as approximately 12% of all women had no antenatal care during pregnancy and one-fourth of women visited antenatal care units after 22 weeks of pregnancy.
5. Educational Programs for raising awareness of pregnant women on proper nutrition should be implemented.

Conclusion

Maternal anemia at the end of the of pregnancy was not significantly associated with prematurity. Nevertheless, anemia with Hb level less than 9.0 g/dl increases the risk of preterm labor fourfold. Although the results are not significant, the high OR indicates practical importance of this cutoff level. Pregnancy induced hypertension (isolated hypertension, mild and severe preeclampsia), as well as chronic hypertension were significantly associated with preterm delivery. However, future studies with larger sample sizes are required in order to investigate these relationships in the presence of potential confounders, as well as to explore the role of different forms of pregnancy induced hypertension in delivering preterm in Armenia.

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Tables

Table 1: The distribution of the study population by maternities

Maternity ID#	Frequency of Observations	Proportion (%)
1.	97	21.85
2.	69	15.54
3.	105	23.6
4.	123	27.7
5.	50	11.3
TOTAL	**444**	**100**

Table 2: The distribution of prenatal characteristics of cases and controls

Status / Variable		Control		Case		p-value
1. Age: mean (st. dev.)*		25.3	(5.5)	25.4	(5.5)	0.4958
2. Gestational Age at First visit to ACU in weeks: mean (st. dev.)*		17.2	(7.2)	16.7	(7.4)	0.5576
3. Parity**: [No. (%)]						
	nulliparous	126	(58.3)	129	(56.8)	
	1-2 prior delivery	83	(38.4)	92	(40.2)	
	3 > prior delivery	7	(3.1)	7	(3.2)	
4. Visited ACU**: [No. (%)]						
	Yes	191	(88.4)	200	(87.7)	
	No	25	(11.6)	28	(12.3)	
3. First Visit to ACU**: [No. (%)]						
	First trimester	66	(34.5)	68	(34.0)	
	Second trimester	112	(58.6)	115	(58.5)	
	Third trimester	13	(6.8)	17	(7.5)	

* Paired t-test

** Cross tabulation

Table 3: Prevalence of preterm delivery by maternities in Yerevan

Maternity ID#	Frequency of Preterm Delivery	Total # of Deliveries	Prevalence of Preterm Delivery (%)
1.	50	594	8.4
2.	35	470	7.5
3.	54	507	10.6
4.	63	805	7.8
5.	26	378	6.9
TOTAL	**228**	**2754**	**Average 8.24**

Table 4: The prevalence of anemia at the end of pregnancy in cases and controls, during Sep 2002- Feb 2003, in Yerevan**

Anemia (%) \ Status	Controls	Cases
No Anemia (Hb>=11.0 g/dl)	57.5	50.0
Mild Anemia (Hb10.0-10.9 g/dl)	27.6	32.6
Moderate Anemia (Hb7.0-9.9 g/dl)	14.9	17.4
TOTAL	**100**	**100**

** cross tabulation

Table 5: Simple conditional logistic regressions of odds of preterm delivery[1]

Variables	OR	95% CI	p-value
1. Hypertension			
Pregnancy Induced Hypertenison	3.5	1.7 -7.3	0.001
Chronic Hypertension	8.0	1.0- 63.9	0.05
3. Anemia at the end of pregnancy			
Mild (10.0-10.9)	1.5	0.9- 2.4	0.119
Moderate1 (9.1-9.9)	1.1	0.5- 2.2	0.814
Moderate2 (7.0-9.0)	3.0	1.1- 8.8	0.048
4. Complications at delivery			
Placenta abruption	6.0	1.6-36.9	0.019
Premature rupture of membranes	3.5	2.1-8.3	0.000
5. Abortions			
1 abortion	1.6	0.8-3.4	0.200
1> abortions	1.3	0.5-2.4	0.757
6. Miscarriages			
1 miscarriage	0.6	0.3-1.2	0.162
1>miscarriages	0.7	0.2- 2.3	0.566
7. Prior Preterm Delivery	10.0	3.1-32.8	0.000
8. Birth Interval	1.6	0.8- 3.2	0.174
9. BMI in kg/m^2			
<18.5	0.7	0.36-1.3	0.246
>25.0	1.2	0.74- 1.8	0.539
10. Anemia during first and second trimester of pregnany (before 28 weeks)			
Mild (10.0-10.9)	1.1	0.6-2.3	0.782
Moderate1 (9.1-9.9)	0.6	0.1-2.6	0.509
Moderate2 (7.0-9.0)	1.1	0.3-4.4	0.944
12. Frequency of visits to ACU	1.6	0.9-2.8	0.119

1. Outcome variable is coded as term delivery=0, and preterm delivery=1

Table 6: The prevalence of anemia during first and second trimester of pregnancy (before 28 weeks of pregnancy)**

Anemia (%) \ Status	Controls	Cases
No Anemia (Hb>=11.0 g/dl)	66.7	68.7
Mild Anemia (Hb10.0-10.9mg/dl)	22.0	21.4
Moderate Anemia (Hb7.0-9.9mg/dl)	11.4	9.9
TOTAL	**100**	**100**

Table 7: Prevalence of pregnancy induced and chronic hypertension in cases and controls

Hypertension (%) \ Status	Controls	Cases
No Hypertension	94.9	81.1
Pregnancy induced hypertension (PIH)	4.6	15.4
Chronic hypertension	0.5	3.5
TOTAL	**100**	**100**

Table 8: Maternal Height, and Prepregnancy Weight and BMI in cases and controls

Variable \ Status	Controls	Cases	p-value
Maternal Height at the end of pregnancy in cm (mean/std.dev)*	160.5 (5.7)	161.1 (6.2)	0.349
Maternal Weight at the end of pregnancy in kg (mean/std.dev)*	69.7 (10.6)	68.8 (11.9)	0.467
Prepregnacy BMI, kg/m^2 (%)**			
<18.5	13.1	10.0	
18.5-25.0	60.6	60.4	
>25.0	26.3	29.6	

* Paired t-test

** Cross tabulations

Table 9: The prevalence of abortions and miscarriages in cases and controls

Variable \ Status	Cases	Controls
Abortions** (%)		
1 abortion	19.0	15.2
≥ 2 abortions	23.0	25.0
Miscarriages** (%)		
1 miscarriage	12.6	20
≥ 2 miscarriages	10.7	7.8

* Paired t-test
** Cross tabulations

Table 10: Unadjusted and Adjusted Results of Conditional Logistic Regression Models of the Odds of Preterm Delivery on Prenatal Characteristics*

	Unadjusted			Adjusted		
Variables	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Hypertension:						
Pregnancy Induced Hypertenison	3.5	1.7 -7.3	0.001	4.0	1.9-8.7	0.000
Chronic Hypertension	8.0	1.0- 63.9	0.05	9.8	1.2-80.5	0.034
Anemia at the end of pregnancy:						
Mild (10.0-10.9)	1.5	0.9- 2.4	0.119	1.5	0.9-2.6	0.147
Moderate1 (9.1-9.9)	1.1	0.5- 2.2	0.814	1.3	0.6-3.0	0.499
Moderate2 (7.0-9.0)	3.0	1.1- 8.8	0.048	3.1	0.9-10.6	0.067
Complications at delivery:						
Placenta abruption	6.0	1.3-26.8	0.019	7.8	1.6-36.9	0.010
Premature rupture of membranes	3.5	1.9-6.4	0.000	4.1	2.1-8.3	0.000

* Outcome variable is preterm delivery, coded as term=0, preterm=1

Appendix 1

A 1: The report of maternity services for the period of 1/Jan/2002-1/July/2002, Department of Health, Yerevan Municipality

	Maternity #1	Maternity #4	Maternity #8	Maternity "Saint Maria"	Malatia Center	Erebuni Center	Center for mother&child health protection
1.Preterm deliveries (%)	7.8	5.8	4.1	6.0	6.1	6.4	13.4
2. Cesarean Sections(%)	4.8	11.3	10.8	15.4	11.2	14.5	13.4
3. Number of preeclampsia/ eclampsia	15	2	17	7	31	17	43
4. Multiple pregnancies	2	2	4	7	3	7	12
5. Total # of Births	**270**	**452**	**435**	**537**	**295**	**613**	**605**

Appendix 2

Medical Record Extraction Form

ID: _ _ _ _ (1st digit: hospital number, 2nd -4th: record number)

Number of fetuses: ☐

Number of deliveries (all births/ life births): ☐

Number of pregnancies: ☐

Number of miscarriages/ abortions: ☐

Number of premature deliveries: ☐

Date of the last delivery: ☐

Dare of carrent delivery: ☐

Hemoglobin level at delivery: ☐

Weight: ☐

Height: ☐

Gestational age at delivery: ☐

Complications during and prior current delivery: (specify)

Complications during current pregnancy: (specify)

Delivery type: Vaginal: ☐ Cessarean: ☐

Delivery Induced for medical considerations: yes no

In-patient treatment during current pregnancy: ☐ specify:

Out-patient treatment during current pregnancy: ☐ specify:

Visited to antenatal care during current pregnancy: yes no

Gestational age at first visit to antenatal care unit: ☐

Diseases Identified at first visit to antenatal care unit: specify:________________________.

Weight at first visit: ☐

Total # of visits to antenatal care: ☐

Hg level during first trimester (before 13 weeks): ☐

Hg level during second trimester (14-27 weeks): ☐

Hg level during third trimester (more than 28 weeks): ☐