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Thai Journal Obstetrics and Gynaecology (TJOG) is the official journal of The Royal Thai College of Obstetricians and Gynaecologists (RTCOG). This is a double-blind peer-reviewed journal aiming to promote academic knowledge and provide a forum for publication in Obstetrics and Gynecology. Manuscripts submitted to TJOG will be accepted on the understanding that the author must not have previously submitted the paper to another journal or have published the material elsewhere.

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Improvement of TJOG has been documented. TJOG was accepted for indexing by the Thai Journal Citation Index (TCI) in December 2010 and indexed in the ASEAN Citation Index (ACI) in September 2015. TJOG also received the National Journal Award from the 3rd TCI-Scopus-TRF Journal Awards on December 15, 2016.

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EDITORIAL

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RTCOG Annual Meeting 2020 will be held during 14-16 October 2020 at The Royal Jubilee Building, Bangkok, Thailand. The theme of this meeting is "**50 Years Golden Jubilee RTCOG: New Normal**". All RTCOG members are cordially invited to participate this scientific meeting.

Residents who would like to publish their researches in TJOG should submit their works before September 30, 2020. Our editorial team and constructive reviewers will let them know the results before December 31, 2020.

The quality of Thai Journal of Obstetrics and Gynaecology has been improved. Thai Journal of Obstetrics and Gynaecology has been indexed in many database: Scopus, TCI, ASEAN Citation Index, DOAJ, EuroPub, and Google Scholar.

Wish to see you at RTCOG Annual Meeting 2020 at Dusit Thani Pattaya Hotel, Chonburi, Thailand

Wish RCOG members and families safe from COVID-19.

Prof. Vorapong Phupong, M.D.
Editor in Chief

Thai Journal of Obstetrics and Gynaecology
July 2020, Vol. 28, No. 3, pp. 130-135

SPECIAL ARTICLE

Pre-implantation Genetic Testing for Aneuploidy (PGT-A)

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ABSTRACT

Preimplantation genetic diagnosis (PGD) or embryo selection was first performed in 1989 using PCR for gender selection to avoid X-linked recessive disorder. However, there was a misdiagnosis due to allele drop out (ADO). Therefore, fluorescent in situ hybridization (FISH) was recommended for gender selection and detection of chromosome abnormalities and PCR was for monogenic disorders. Since then, a number of advanced modern analysis methods for preimplantation genetic testing (PGT) of chromosome balance were developed. A more sophisticating comparative genomic hybridization microarray (aCGH) was introduced in 2011 providing detailed copy number variation (CNV) of 24 types of chromosomes (22 pairs, X and Y). A single aCGH protocol was used for PGT of aneuploidy (PGT-A) and PGT of segmental rearrangement (PGT-SR) for every chromosome in one go. Next generation sequencing (NGS) replaced aCGH in 2015 due to its better resolution and lower cost. Single nucleotide polymorphism microarray (aSNP) with karyomapping analysis for simultaneous PGT of monogenic disorders (PGT-M) and PGT-A is still more expensive. In this article, various embryo biopsy and chromosome analysis techniques are discussed. The pros and the cons of each techniques are also included.

Keywords: comparative genomic hybridization microarray (aCGH), embryo selection, next generation sequencing (NGS), pre-implantation genetic testing for aneuploidy (PGT-A), pre-implantation genetic screening (PGS).

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Pre-implantation Genetic Diagnosis (PGD) was first introduced by Alan Handyside in 1989[1]. Sex determination was performed on biopsied single cells from in-vitro fertilization (IVF) embryos at six to eight cells stage by deoxyribonucleic acid (DNA) amplification of Y chromosome-specific sequence in order to avoid hereditary X-linked mental retardation condition. This allows unaffected embryos to be identified and chosen to transfer to the uterus. Traditionally, prenatal diagnosis (PND) using chorionic villus sampling (CVS), amniocentesis or fetal blood sampling (FBS) followed by cytogenetic, biochemical or molecular analysis of cells recovered from the fetus can be performed for couples at risk of having babies with severe genetic condition in particular thalassemias and Down's syndrome[2]. However, in case of the fetus is affected,

abortion is offered as an option. Therefore, PGD is an alternative to PND for monogenic disorders and chromosome abnormalities i.e. preimplantation genetic testing for monogenic disorders (PGT-M), preimplantation genetic testing for aneuploidy (PGT-A) and preimplantation genetic testing for segmental rearrangement (PGT-SR). The article focuses on PGT-A and relevant embryo biopsy and modern analysis technology.

Preimplantation genetic testing for aneuploidy (PGT-A)

In IVF treatment, the choice of selecting best quality embryos for transfer depends on their morphology i.e. number of pronuclei, number and regularity of blastomeres and fragmentation. However, some good quality embryos on the morphology criteria failed to implant. Joyce Harper demonstrated that 46% of human embryos developed chromosomal abnormalities during preimplantation stage using 3-color fluorescent in situ hybridization (FISH)[3]. Using a more sophisticated single cell comparative genomic hybridization (CGH) techniques on 12 embryos, Dagan Wells and Joy Delhanty showed that 75% of preimplantation human embryos developed complicated chromosome abnormalities[4]. Possible reason may be because of the abnormal chromosome composition within the embryos. These may explain the low success rates of IVF and natural conception. For this reason, preimplantation genetic for aneuploidy screening (PGS) or preimplantation genetic testing for aneuploidy (PGT-A) was employed to identify chromosomally balanced or euploid embryos for transfer with the hope to improve pregnancy rates of IVF. Embryos with chromosomally balance are chosen for transfer with the hope that they will have more chance of developing into successful pregnancy with the principle of excluding embryos with abnormal chromosomes. Indications for PGT-A are advanced maternal age, repeated miscarriages with normal parental karyotype and repeated implantation failure.

Embryo biopsy techniques

- Polar bodies biopsy

During preconception period, polar bodies can be taken for analysis. They are unused maternal genetic material which will degenerate very soon. Two famous centers were keen to perform polar bodies biopsy are Yury Verlinsky[5] and Santiago Munne[6]. However, both first and second polar bodies are needed for comprehensive results which is labor intensive. Paternal genetic materials are not included in the analysis, therefore, in the recessive condition all oocytes with mutant allele will be discarded while half of them will be heterozygous if fertilized with sperm with normal allele. Dominant disorder inherited from the father cannot be diagnosed by polar bodies biopsy. Moreover, post-zygotic events cannot be revealed by this technique. Therefore, polar bodies biopsy is not popular elsewhere.

- Cleavage stage embryo biopsy

The very first clinical PGD reports employed cleavage stage embryo biopsy at day 3 when there are 6-8 cells[1]. One or two blastomeres are taken for diagnosis. It does not adversely affect the embryonic development[7]. Cleavage stage embryo biopsy had been the most popular technique during 1990-2010. However, only 1 or 2 cells can be obtained for the analysis which can sometimes be technical restriction. The biopsied single cells may be missing and mosaicism are common for FISH analysis leading to problematic diagnostic conclusion. Amplification failure (AF), allele drop out (ADO) and contamination are major obstacles for PCR analysis leading to misdiagnosis[8]. Moreover, most IVF centers require day 4 embryo transfer, therefore, only 24 hours or less is available for analysis. Cleavage stage embryo biopsy was superseded by blastocyst biopsy since early 2010s worldwide because of the improved embryo culture techniques and the need of more biopsied cells for CGH array analysis.

- Blastocyst biopsy

Until recently, with the improved knowledge of embryo culture that allow IVF laboratory to grow human embryos up to day 5 effectively. At blastocyst stage with about 150 cells, 5-10 trophectoderm cells can be taken for the analysis[9]. More biopsied cells help in facilitating the analysis techniques for both monogenic disorders and chromosome balance, including for microarray and

next generation sequencing (NGS) analyses. The chance of AF and ADO markedly reduced[8]. The number of surviving embryo to blastocyst stage is markedly reduced due to natural selection. This reduces workload and cost for the analysis. Since the endometrium at day 5 post-fertilization is not suitable for embryo transfer, all biopsied embryos are stored under liquid nitrogen waiting for transfer in the future. Therefore, there are more time for genetic testing. For this reason, blastocyst biopsy has become the most popular techniques worldwide[10].

- Blastocyst fluid biopsy

Future technique includes blastocyst fluid biopsy. Blastocyst fluid contains DNA from death cells from trophectoderm and inner cell mass (ICM)[11]. With the present advanced analysis techniques, the analysis of blastocyst fluid is possible. However, validation of accuracy is needed before clinical application.

Molecular analysis techniques

- Fluorescent in situ hybridization (FISH)

PCR was used in the very first cases of PGD for sexing[1]. However, misdiagnosis was encountered. This was because of the event called allele drop out (ADO) where one of two alleles in a heterozygous cell fails to amplify and leads to misdiagnosis[12]. This is a unique problem of single cell PCR. Since then FISH was recommended for chromosome abnormalities and sexing[13]. DNA sequences complimentary to particular chromosomes were used as probes for in situ hybridization. Fluorochromes with different colors were tagged in order to identify up to 5 chromosomes at a time. Original applications of PGT-A using FISH were for inherited chromosome abnormalities i.e. Robertsonian and reciprocal translocations. By identifying chromosomally balanced embryos for transfer, PGT-A helps the couples carrying translocations to avoid recurrent miscarriages and get pregnant with a healthy baby.

FISH is a sensitive, accurate and quick method to identify the particular chromosomes. It can be applied to polar bodies, blastomeres and trophectoderm. However, disadvantages of FISH include hybridization efficiency, split signals and overlapping signals. Original FISH was home grown with a few colors. The popular commercial FISH, Aneu Vysis, comprised 5 colors for chromosomes 21, 18, 13, X and Y. FISH was superseded by CGH array in 2011.

- Comparative genomic hybridization microarray (aCGH)

Comparative genomic hybridization (CGH) is a technique using the testing DNA as a probe labeled with green fluorescent dye to co-hybridize with the control DNA labeled with red fluorescent dye to cultured lymphocytes. Areas with orange signal are interpreted as balanced, green as additional and red as deletion. This reveals copy number variation (CNV) information of the 24 types of chromosomes[4]. However, manual CGH was labor intensive and time consuming. CGH became popular when the probes were transferred onto microarray. The hand on laboratory and analysis, even still quite sophisticated, have become more user friendly and reduce hand on time from 72 to 16 hours[14].

At the beginning of aCGH era, most IVF labs were still doing day 3 embryo biopsy. Soon after that the trend of embryo biopsy shifted to day 5 biopsy which provides more cells for the analysis per embryo and fewer embryos for testing. This reduces the cost of analysis. aCGH provides detailed CNV information of all 24 types of chromosomes in one go[15]. Therefore, aCGH replaced FISH in most PGT-A analysis very soon. The most popular aCGH was 24SURE from BlueGnome which was later taken by Illumina. However, main pitfalls of aCGH include the detection of triploidy, mosaicism and balanced translocation. PGT-A using aCGH was employed with the belief that transferring euploid embryos would improve pregnancy outcomes of IVF.

- Single nucleotide polymorphism microarray (aSNP)

Single nucleotide polymorphism (SNP) is the variations of single base pair without causing disease. SNP can be found every 1,000 bp through out human genome. SNP microarray (aSNP) includes probes for genotyping of SNPs throughout human genome. By comparing with control reference DNA, aSNP can provide CNV information, even though not as good as aCGH. However, aCGH gives the advantage of parental origins information of the unbalanced regions. Moreover,

balanced translocation is also possible to identify by aSNP. Employing SNPs information around the particular genes as haplotype blocks and comparing with references from the members of the family, it is possible to perform haplotyping analysis in the embryos, aka karyomapping[16]. Karyomapping using aSNP can be used as a universal linkage analysis protocol for PGT-M and PGT-A at the same time[17, 18]. This can reduce expenses and time for developing new protocol for each new disease. The only drawback of karyomapping at the moment is that its cost is far more expensive that PCR and aCGH or NGS.

- ***Next generation sequencing (NGS)***

It took 13 years and $3-billion for the Human Genome Project to complete human genome sequencing using Sanger sequencing techniques. At present it only takes 16 hours and $1,000 to do whole genome sequencing using next generation sequencing (NGS) or massive parallel sequencing (MPS). For PGT-A, the sequencing results are compared with the reference sequences. This allows CNV analysis in the embryos[19]. NGS is also used for non-invasive prenatal testing (NIPT) analyzing fetal free DNA maternal plasma[20]. Technically, NGS provides a better sensitivity than aCGH for chromosomal mosaicism detection[21]. However, with its more details results, NGS provides more chance of reporting variants of unknown significance (VUS). The cost for NGS-based aneuploidy testing for PGT-A is lower than aCGH. Therefore, NGS replaced aCGH in 2015 and has become the most popular platform for PGT-A.

Preimplantation genetic screening (PGS) for aneuploidy

A meta-analysis was carried out to assess the benefit of PGS[22]. Live birth rate per woman was the primary outcome. Randomized controlled trials comparing IVF/ intracytoplasmic Sperm Injection (ICSI) with PGS versus IVF/ICSI without PGS were included. Nine trials using 5-color, 7-color, 8-color and 9-color FISH were included. No trial using techniques other FISH met the inclusion criteria. In all studies, embryos with best morphology were transferred in the control group and embryos with chromosomally normal were transferred in the intervention group. In the IVF/ICSI with PGS group live birth rate per woman was significantly lower compared to the IVF/ICSI without PGS group in women of advanced maternal age and women with repeated IVF failure (OR=0.59, 95% CI=0.44-0.81 and OR=0.41, 95% CI=0.20-0.88, respectively). Women with a good prognosis exhibited similar trend, although without statistical support (OR=0.50, 95% CI=0.20-1.26). Both cleavage stage biopsy and blastocyst biopsy show similar results. However, other comprehensive chromosome analysis testing methods i.e. aCGH and NGS were not included in this meta-analysis.

This meta-analysis suggests that PGS using multicolor FISH reduces live birth rates in women of advanced maternal age and those with repeated IVF failure. This may be because of the discard of the embryos with abnormal chromosome testing results which leads to a reduced number of available embryos for transfer. There may be no embryo with normal chromosome results for transfer at all in some PGS cycles. Some biopsy blastomeres or trophectoderm cells with abnormal chromosome testing results are from embryos with chromosomal mosaicism, while the rest of the embryos are chromosomally normal and discarded. In addition, some embryos with abnormal chromosomes may undergo trisomic rescue event and turn out to be chromosomally balanced later on. These embryos, if have a chance to transfer, can produce successful normal pregnancy. Therefore, PGS should not be employed routinely.

Conclusion

Since late 2010s PGT-A, PGT-SR and PGS using day 5 blastocyst biopsy and cytogenetic analysis using NGS have become standard worldwide. With the benefit of optimal blastocyst culture technology and culture medium, blastocyst culture provides more biopsied trophectoderm cells for genetic analysis. Blastocyst freezing following the biopsy provides longer time for the analysis allowing possible laboratory cost efficiency management and cost saving. PGT for inherited conditions i.e. monogenic diseases and chromosomal translocations is for

avoiding the transfer of the affected embryos. PGT for aneuploidy screening aims to improve IVF efficiency by reducing time to pregnancy and the chance of implantation failure and recurrent pregnancy loss. However, there is still no prove for the benefit of PGS in increasing pregnancy rate in IVF. Additional technology that may help in evaluating the prognosis of the embryos includes time-lapse imaging, metabolomic study and mitochondrial DNA functions. It seems like karyomapping using aSNP is the best platform for simultaneously analysis of monogenic disorders and chromosome balance at present. However, long term safety of the procedures is still needed to be confirmed.

Potential conflicts of interest

The author declares no conflict of interest.

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OBSTETRICS

Accuracy of Hemoglobin E Screening Test Using Allelic Discrimination Assay

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ABSTRACT

Objectives: To determine the accuracy of allelic discrimination (AD) assay for hemoglobin E (Hb E) screening test in Chiang Mai Strategy for thalassemia prevention and control.

Materials and Methods: This study evaluated the AD assay compared with conventional Hb E screening tests used in Chiang Mai Strategy of Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. In this assay, two TaqMan probes were designed to discriminate heterozygous and homozygous by detecting normal and mutant nucleotides of Hb E gene.

Results: From 55 blinded DNA samples, the AD assay revealed the results with 100% sensitivity, specificity, positive predictive value, negative predictive value and efficiency when compared to the conventional Hb E screening tests of the Chiang Mai Strategy.

Conclusion: The AD assay is effective as an Hb E screening test in the thalassaemia prevention and control program. Moreover, AD assay can distinguish heterozygous from homozygous genotypes.

Keywords: allelic discrimination, E screening test, hemoglobin E, real-time PCR.

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ความถูกต้องของการทดสอบคัดกรองฮีโมโกลบินอีโดยวิธี Allelic Discrimination

สุปราณี อุปนันท์, สมเดช ศรีชัยรัตนกูล, ศิริวิภา ปิยะมงคล, วีรวิทย์ ปิยะมงคล

บทคัดย่อ

วัตถุประสงค์: *เพื่อหาความถูกต้องของวิธี allelic discrimination (AD) สำหรับการตรวจคัดกรองฮีโมโกลบินอี (Hb E) ใน Chiang Mai Strategy สำหรับการป้องกันและควบคุมธาลัสซีเมีย*

วัสดุและวิธีการ: *การศึกษานี้เป็นการประเมินวิธี AD เทียบกับการตรวจคัดกรอง Hb E แบบเดิมที่ใช้ใน Chiang Mai Strategy ของโรงพยาบาลมหาราชนครเชียงใหม่ จังหวัดเชียงใหม่ ในวิธีนี้ TaqMan probe จำนวน 2 เส้น ได้ถูกออกแบบเพื่อใช้แยกเฮเทอโรซัยกัส (heterozygous) และโฮโมซัยกัส (homozygous) โดยตรวจหานิวคลีโอไทด์ปกติและผิดปกติ (mutant) ของยีน Hb E*

ผลการศึกษา: *จากตัวอย่างดีเอ็นเอ จำนวน 55 ตัวอย่าง พบว่า วิธี AD แสดงผลการวิเคราะห์ด้วยร้อยละ 100 ของค่าความไว (sensitivity) ค่าความจำเพาะ (specificity) ค่าทำนายผลบวก (positive predictive value) ค่าทำนายผลลบ (negative predictive value) และประสิทธิภาพ (efficiency) เมื่อเปรียบเทียบกับการตรวจคัดกรอง Hb E แบบเดิมของ Chiang Mai Strategy*

สรุป: *วิธี AD มีประสิทธิภาพสำหรับการตรวจคัดกรอง Hb E ในโปรแกรมป้องกันและควบคุมธาลัสซีเมีย นอกจากนี้วิธี AD สามารถแยกแยะยีนชนิดเฮเทอโรซัยกัสจากโฮโมซัยกัสได้*

คำสำคัญ: *allelic discrimination, การตรวจคัดกรองอี, ฮีโมโกลบินอี, Real-time PCR*

Introduction

Thalassemia syndrome and hemoglobinopathy are the most common single gene disorder and costs significant health and economic burden worldwide. Hemoglobin E disease is the most common hemoglobinopathy in Thailand with the average prevalence of 13%. It is an inherited hemoglobin disorder with a point mutation within β-globin gene (codon 26, G→A). β-Thalassemia/Hb E is one of prime targets in the public health policy to prevent and control severe thalassemias[1-4]. Therefore, effective laboratory tests for Hb E screening to identify Hb E gene carriers are very important. Since the types of thalassemias are very diverse and complicated, a single laboratory test is not sufficient for every type of thalassemia diagnosis. This study thus aims to develop and determine the accuracy of allelic discrimination (AD) assay for Hb E screening in comparison to the conventional Hb E screening tests (i.e. hemoglobin typing (%A2/E) or E screen test) in Chiang Mai Strategy of Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand[1, 4-8].

Following the instruction in the allelic discrimination guide of Applied Biosystem company for ABI 7500™ real-time polymerase chain reaction (PCR) machine (Applied Biosystems, California), TaqMan allelic discrimination is high-throughput for genotyping of single nucleotide polymorphisms[9]. This assay is a multiplexed real-time PCR reaction with end-point detection. The reaction mix contains deoxynucleoside triphosphate (dNTP) substrates, Taq deoxyribonucleic acid (DNA) polymerase, a forward primer, a reverse primer and two TaqMan probes detecting normal (G) and mutant (A) nucleotide in one tube. Each TaqMan probe consists of different fluorescent reporter dye (e.g. VIC® and FAM®) at 5' end and nonfluorescent quencher (e.g. NQF) at 3' end offering lower background signal. The specific site of probes was between forward and reverse primer sites. To detect variants of a single nucleic acid sequence at the codon 26 (G→A), a green dye (VIC®) TaqMan probe was designed to specific guanine (G) for normal allele while a blue dye (FAM®) TaqMan probe was designed to specific to adenine (A) for mutant allele of Hb E mutation. The primers and probes would anneal with their own matching complementary sequences. Taq DNA polymerase added dNTPs to the 3' end of primer for polymerization. Subsequently, the hybridization probe was cleaved by the 5' nuclease activity of Taq DNA polymerase and released a fluorescent signal due to separation of the reporter dye from the quencher dye; only specific sequence was completely amplified. The AD assay combines PCR and mutation detection in a single step by measuring an increase in the fluorescence intensity of the reporter dye and performing allelic discrimination on the post-PCR product using SDS software presenting the data as graph plot of heterozygosity and homozygosity (Fig. 1) normal (VIC® dye fluorescent signal only), homozygous Hb E (FAM® dye fluorescent signal only) and heterozygous Hb E (both VIC® and FAM® dye fluorescent signals).

Indeed, in addition to wildtype allele the AD assay can distinguish between homozygous and heterozygous Hb E gene mutations. Therefore, it would be an alternative accurate, rapid and sensitive test for Hb E screening in the thalassemia prevention and control program.

Materials and Methods

Blinded DNA samples (N=55) from 3 normal individuals and 52 thalassemia patients: Hb E trait (N=9), Hb E trait/α1-trait (N=1), homozygous Hb E (N=2), β-major (N=2), β-trait (N=25), β-trait/α1-trait (N=2) and α1-trait (N=11) were obtained from Thalassemia Center, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. The study protocol was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University, Thailand (Study Code: OBG-2559-03835/Research ID: 3835). The patients were diagnosed Hb E disorder using conventional Hb E screening tests, including %A2/E or E screen test, according to Chiang Mai Strategy of Maharaj Nakorn Chiang Mai Hospital[1, 4-8]. The sample size was calculated using statistic descriptive studies.

Primer and probe sequences for real-time PCR were designed using Primer3 software and were synthesized by Applied Biosystems, California. The

forward and reverse primers are 5'-GCA AGG TGA ACG TGG ATG AAG T-3' and 5'- GTC TCC TTA AAC CTG TCT TGT AAC CT-3', respectively. TaqMan probes for normal and mutant alleles were designed as 5'-VIC®-AGG GCC T[C]A CCA CCA-NFQ-3' and 5'- FAM®-CAG GGC CT[T] ACC ACC A-NFQ-3', respectively. DNA samples (1 µl) were mixed with the reaction mixture (9 µl) containing 2x TaqMan® Mix (5 µl), 40x Primer-Probe Mix (0.25 µl) and nuclease-free water (3.75 µl). The real-time PCR profile (95°C for 10 min, 40 cycles of 95°C for 15 sec and 60°C for 1 min) and the AD assay were operated on the ABI 7,500™ real-time PCR machine (Applied Biosystems, California) according to the manufacturer's instructions. The validity of the AD assay was determined by diagnostic indices using a two-by-two table in comparison to the conventional Hb E screening tests (%A2/%E/E screen) of Chiang Mai Strategy in order to evaluate sensitivity, specificity, positive predictive value, negative predictive value and efficiency. Chi square was employed for correlation analysis.

Results

From a total of 55 blinded DNA samples from normal cases and various types of thalassemia patients (Hb E, β-Thalassemia and α-Thalassemia), the AD assay interpreting from the allelic discrimination plot could discriminate 43 normal, 10 heterozygous Hb E and 2 homozygous Hb E samples (Fig. 1). The results of the AD assay were positive in 21.8% (12/55) and negative in 78.2% (43/55) (Table 1). In addition, the validity of the AD assay was evaluated in comparison to the conventional Hb E screening tests (%A2/%E/E screen) in the Chiang Mai Strategy. The diagnostic indices from chi square analysis were shown in Table 1. Interestingly, the AD assay showed 100% sensitivity, specificity, positive predictive value, negative predictive value and efficiency. This implies that this test can identify all Hb E traits and homozygotes with no false-negative or false-positive result.

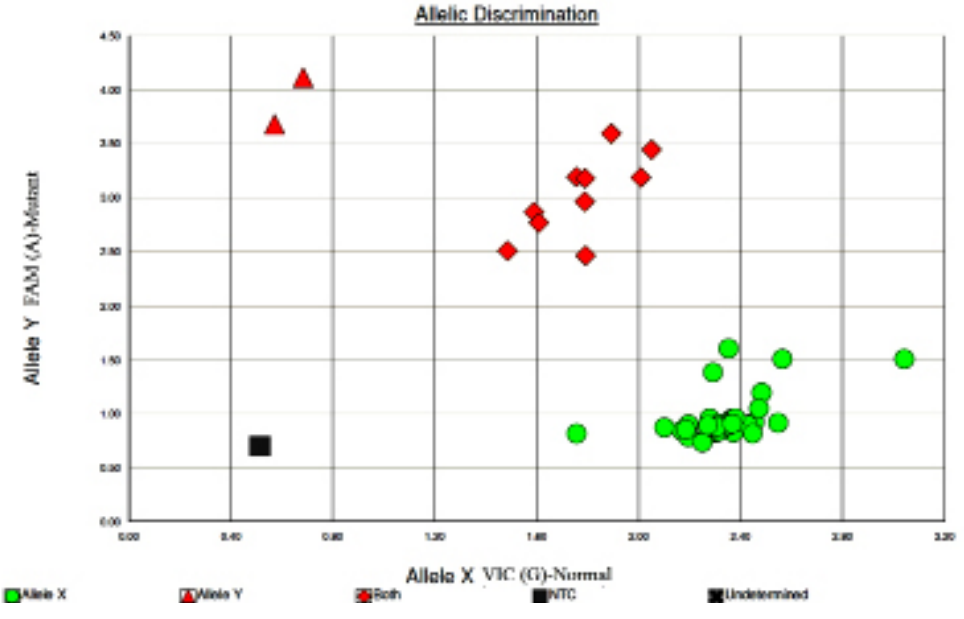

Fig. 1. Allelic discrimination plot of the AD assay of samples from subjects with normal, heterozygous and homozygous Hb E (β-globin gene codon 26, G→A) genotypes (N = 55). The x-axis is VIC (green) dye fluorescence signaling for normal allele with G nucleotide; the y-axis is FAM (red) dye fluorescence signaling for mutant allele with A nucleotide. Red triangles indicate homozygous Hb E. Orange diamond-shaped quadrangles indicate heterozygous Hb E. Green circles indicate normal allele. Grey square indicates no-template control (NTC).

Table 1. Two-by-two table showing the diagnostic indices of the allelic discrimination (AD assay results of DNA samples of normal, heterozygous and homozygous Hb E comparing with those using conventional Hb E screening methods (N = 55).

AD assay	Conventional Hb E screening tests (%A2/%E/Hb E screen)		
	Positive	**Negative**	**Total**
Positive	12	0	12
Negative	0	43	43
Total	**12**	**43**	**55**

Discussion

In order to prevent and control severe thalassemias, in particular β-thalassemia/Hb E disease according to public health strategies in Thailand, population screening for Hb E homozygotes or carriers is very crucial[1-4]. In the Chiang Mai Strategy, the assays from blood samples including Hb typing (%A2/E) or E screen were employed as the conventional Hb E screening tests for thalassemia diagnosis due to the cost of the analysis[4, 6, 10]. In the former, a single assay could not diagnose all types of thalassemias due to the limitations of the assays and the wide variety of different mutations. For instance, Hb typing using high performance liquid chromatography (HPLC) or electrophoresis is too expensive and complicated even though it can distinguish heterozygous and homozygous Hb E[5, 11]. Dichlorophenolindophenol (DCIP) test is cheap but possesses high false-positive results with blue color detection[1, 8, 12, 13]. DCIP and E screen cannot differentiate between heterozygous and homozygous Hb E genotypes[1, 7, 8, 12, 13]. Therefore, rapid and accurate molecular assays detecting the point mutation of Hb E gene with heterozygote and homozygote were developed. From only one reaction tube containing primers and probes specific for normal (G) and mutant (A) Hb E gene, the AD assay is one of such assays showing the allelic discrimination plot which can indicate the number of normal, heterozygous and homozygous Hb E samples. This demonstrated that the AD assay is not complicated for handle and analysis even though the ABI 7500 real-time PCR machine is really required and specific for this assay. Although Kho and colleagues had developed the AD assay for detection of Hb E gene mutation[9], the improved newly designed primers, probes and real-time PCR conditions in this study were developed and tested for its validity comparing with the conventional Hb E screening tests (i.e. hemoglobin typing (%A2/E) or E screen test) in the Chiang Mai Strategy. The AD assay in this study also showed 100% sensitivity and specificity without any false-negative and false-positive results. Therefore, it was demonstrated that the AD assay in this study could be used as an alternative test for Hb E screening as in the Chiang Mai Strategy. Obviously, it is a time-effective system using a reaction mixture and provides an easier result interpretation presenting heterozygous and homozygous genotype identification. Interestingly, the applications of this assay for dry blood spot and a non-invasive test such in DNA samples from buccal swabs is also possible.

Conclusion

In comparison to the conventional Hb E screening tests (i.e. hemoglobin typing (%A2/E) or E screen test) in Chiang Mai Strategy, the AD assay is a rapid and accurate assay with 100% sensitivity and specificity for Hb E screening. Additionally, it can discriminate Hb E genotypes (normal, heterozygous and homozygous Hb E) using one reaction tube and an easy interpretation plot. Therefore, the AD assay can be an alternative test for Hb E screening in the Chiang Mai Strategy for thalassemia prevention and control.

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Potential conflicts of interest

The authors declare no conflict of interest.

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OBSTETRICS

Association between Anemia in Pregnancy and Preterm Birth at Sunpasitthiprasong Hospital

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ABSTRACT

Objectives: To determine the association between anemia in pregnancy and preterm birth, maternal and neonatal complications.

Materials and Methods: A retrospective cohort study was conducted in pregnant women having a hemoglobin level at their first antenatal care and who delivered at Sunpasitthiprasong Hospital from January 2015 to December 2016. A total of 300 medical records of pregnant women were randomized from the database, of which 150 women were anemia (hemoglobin < 11 g/dL) and 150 women who were non-anemia (hemoglobin ≥ 11 g/dL). Maternal characteristics, gestation age of delivery, route of delivery, maternal and neonatal complications were recorded.

Results: Preterm birth in the anemic group (n = 11, 7.3%) was higher than those in non-anemic group (n = 7, 4.7%) but there were no significant differences (p = 0.332). Maternal complications showed no significant differences between the groups (postpartum hemorrhage p = 0.442 and pregnancy induce hypertension p = 0.759). With respect to neonatal complications, there were no significant differences between the groups (low birth weight p = 0.821, birth asphyxia at 1 minute p = 0.315, neonatal unit admission p = 0.143 and respiratory distress syndrome p = 0.570). There were no birth asphyxia at 5 minutes, necrotizing enterocolitis and intraventricular hemorrhage in the relevant groups.

Conclusion: There was no significant difference of preterm births between the anemic group and the non-anemic group. Regarding the maternal and neonatal complications, there were no significant differences between the groups.

Keywords: maternal anemia, preterm birth, pregnancy outcomes.

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ความสัมพันธ์ระหว่างภาวะโลหิตจางในหญิงตั้งครรภ์กับการคลอดก่อนกำหนดที่โรงพยาบาลสรรพสิทธิประสงค์

สุธินี สลักเพชร, พงษ์สันต์ พันธะไชย

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาความสัมพันธ์ระหว่างภาวะโลหิตจางในหญิงตั้งครรภ์กับการคลอดก่อนกำหนด รวมถึงภาวะแทรกซ้อนของมารดาและทารก*

วัสดุและวิธีการ: *การศึกษานี้เป็นการศึกษาแบบย้อนหลังในผู้หญิงตั้งครรภ์เจาะฮีโมโกลบินเมื่อมาฝากครรภ์ครั้งแรก และคลอดที่โรงพยาบาลสรรพสิทธิประสงค์ ระหว่างเดือนมกราคม พ.ศ.2558 ถึงเดือนธันวาคม พ.ศ.2559 หญิงตั้งครรภ์จำนวนทั้งหมด 300 คน แบ่งเป็น 2 กลุ่ม โดยสุ่มจากฐานข้อมูลของโรงพยาบาลสรรพสิทธิประสงค์ คือ กลุ่มที่มีภาวะโลหิตจาง (ฮีโมโกลบิน < 11 g/dL) จำนวน 150 คน และกลุ่มที่ไม่มีภาวะโลหิตจาง (ฮีโมโกลบิน ≥ 11 g/dL) จำนวน 150 คน ข้อมูลที่เก็บประกอบไปด้วยข้อมูลพื้นฐานของมารดา, อายุครรภ์ที่คลอด, วิธีการคลอด, ภาวะแทรกซ้อนของมารดาและทารก*

ผลการศึกษา: *การคลอดก่อนกำหนดในกลุ่มที่มีภาวะโลหิตจาง (n = 11, 7.3%) พบมากกว่าในกลุ่มที่ไม่มีภาวะโลหิตจาง (n = 7, 4.7%) แต่ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (p = 0.332) ส่วนภาวะแทรกซ้อนของมารดาไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ทั้งภาวะตกเลือดหลังคลอด (p = 0.442) และภาวะความดันโลหิตสูงขณะตั้งครรภ์ (p = 0.759) ภาวะแทรกซ้อนของทารกไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ในเรื่องทารกแรกเกิดน้ำหนักน้อย (p = 0.821), ภาวะพร่องออกซิเจนในนาทีที่ 1 หลังคลอด (p = 0.315), ทารกนอนหอผู้ป่วยเด็ก (p = 0.143) และกลุ่มอาการหายใจลำบากในทารกแรกเกิด (p = 0.570) โดยทั้ง 2 กลุ่ม ไม่พบภาวะพร่องออกซิเจนในนาทีที่ 5 หลังคลอด, ภาวะลำไส้เน่าในทารกแรกเกิด และเลือดออกในโพรงสมองในทารกแรกเกิด*

สรุป: *ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ของการคลอดก่อนกำหนดระหว่างกลุ่มที่มีภาวะโลหิตจาง และกลุ่มที่ไม่มีภาวะโลหิตจาง ส่วนภาวะแทรกซ้อนของมารดาและทารกทั้ง 2 กลุ่ม ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ*

คำสำคัญ: *ภาวะโลหิตจางในหญิงตั้งครรภ์, การคลอดก่อนกำหนด, ผลลัพธ์ของการตั้งครรภ์*

Introduction

Anemia in pregnancy is a global health problem in developed and developing countries. It affects social and economic development. Anemia may result from several causes, with the most significant contributor being iron deficiency[1]. World Health Organization (WHO) defined anemia in pregnancy as hemoglobin (Hb) < 11 g/dL all trimesters[2]. The prevalence of anemia in pregnancy aged 15-49 years in 2011 was 17-63% (average 38.2%) worldwide[1]. In Thailand, the data from the Ministry of Public Health concluded that the rate of anemia during pregnancy was 10-20% between 1994 and 2010. Moreover, the trend of the prevalence increased to 39-45% between 2011 and 2013[3]. The record of first antenatal care (ANC) visits at Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand, the prevalence of anemia during pregnancy was 20-30%. The goal of the Ministry of Public Health is to reduce the rate of anemia in pregnancy to 10%.

Anemia in pregnancy increased the risk of low birth weight (LBW), preterm birth, small for gestational age (SGA) newborns, maternal and perinatal mortalities[4-9]. However, another study found no association between anemia in pregnancy and LBW & preterm birth[10]. Anemia resulting from iron deficiency adversely affects cognitive and motor development, causes fatigue and low productivity[1]. Children who were born to women classified as iron deficiency in the third trimester without iron supplementation had lower mental development at 12, 18, and 24 months of age, suggesting that prenatal iron deficiency is associated with mental development[4]. Anemia in the first trimester has been associated with a limited increased risk of preterm birth. However, third trimester anemia was associated with reduced risk for preterm birth[11].

The rate of preterm birth in 2010 ranged from 5% to 60% of babies born (11.1% of all live births worldwide)[12]. The preterm infant is susceptible to various serious medical complications during the newborn period as well as morbidities and mortalities. These complications, consequence of immature organs, are hypoglycemia, intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), hypotension especially respiratory distress syndrome (RDS). RDS results from immature lungs that are unable to sustain necessary oxygenation and resulting hypoxia is an underlying associated cause of neurological damage such as cerebral palsy. In addition, hyperoxia, a side effect of RDS treatment, causes bronchopulmonary dysplasia and ROP[4]. These morbidities are a significant cost to the health-care system, such as treatment of physical development, mental development and psychological care. Moreover, these problems affect physical and psychological health of caregivers.

We aimed to determine the association between anemia in pregnancy and preterm birth, maternal and neonatal complications.

Materials and Methods

A retrospective cohort study was conducted in pregnant women having Hb level at their first ANC and who delivered at Department of Obstetrics and Gynecology in Sunpasitthiprasong Hospital, Ubon Ratchatani, Thailand from January 2015 to December 2016. This study was approved by the Research Ethic Committee of Sunpasitthiprasong Hospital. Inclusion criteria were Hb level at first ANC visit, children born in Sunpasitthiprasong Hospital and singleton pregnancy. Those with fetal anomaly or fetal aneuploidy were excluded. Sample size was calculated based on a research question “whether anemia has impact on risk of preterm birth?”. According to a study by Okunade and colleagues (2014)[5], 21.0% of mothers with anemia had preterm births (p_1 = 0.210), while 9.4% of those without anemia had preterm births (p_2 = 0.094). At 80% power and 95% confidence level and with a ratio of exposed and non-exposed being 1:1 using n4Studies application, the sample size of 150 pregnant women were required for each group. 150 medical records of pregnant women were randomly selected from all pregnant women with and without anemia receiving

ANC and delivered at Sunpasitthiprasong Hospital during the study period.

Data on a main exposure, anemia and other risk factors at first ANC visit were obtained by careful medical record reviews. Anemia in pregnancy was classified according to the WHO as Hb less than 11 g/dL in all trimesters. WHO[2] defined severity of anemia as mild anemia is Hb 10.0-10.9 g/dL, moderate anemia is Hb 7.0-9.9 g/dL and severe anemia is Hb < 7.0 g/dL. If Hb level was < 11 g/dL, the woman received ferrous fumarate. If Hb level was < 8 g/dL, blood transfusions were given. Other risk factors included maternal age, ethnicity, body mass index (BMI) before pregnancy, occupation and education as well as gestational age (GA) at first ANC visit, parity, and Hb during pregnancy and interpregnancy interval (interval from one child's birth until the next pregnancy). Maternal and neonatal complications were obtained by medical record reviews. Neonatal complications were evaluated by pediatrician. GA was corrected based on last menstrual period and ultrasonography performed. Using logistic regression, risk factors of preterm birth applied from previous studies, Committee on Practice Bulletin No.130 (ACOG) : Prediction and prevention of preterm Birth, Williams Obstetrics 24th ed., interpregnancy interval in Scotland, risk for preterm delivery and severity of maternal anemia in Tanzania, maternal education and preterm birth in Michigan and characteristics and risk factors of preterm births in a tertiary center in Lagos, Nigeria[4, 13-17], including anemia (mild, moderate, severe anemia), teenage pregnancy, advance maternal age, interpregnancy interval < 18 months, BMI before pregnancy (normal 18.5-22.9 kg/m^2, underweight < 18.5 kg/m^2, overweight 23.0-24.9 kg/m^2, and obesity ≥ 25.0 kg/m^2)[18], below Bachelor degree and pregnancy induce hypertension (PIH).

Primary outcome was preterm birth (birth at GA 28-36^{+6} weeks). Secondary outcomes included maternal and neonatal complications. Maternal complications were postpartum hemorrhage (PPH, blood loss > 500 ml in vaginal delivery or > 1,000 ml in cesarean section) and PIH. Neonatal complications were LBW (birth weight < 2,500 grams), birth asphyxia at 1 and 5 minutes (Apgar score < 7), neonatal unit admission, RDS, IVH and NEC.

Statistics analyses were performed using IBM SPSS Statistics Version 22. Characteristics of the samples were described as number (%), mean (standard deviation, SD) and median (interquartile range, IQR) for categorical, normally and non-normally distributed continuous variables. Comparison of characteristics and outcomes between groups were done using chi-square test, independent sample t-test and Mann-Whitney U test for categorical, normally and non-normally distributed continuous variables, respectively. The association of anemia with preterm birth and secondary outcomes were examined using logistic regression with odds ratio (ORs) and 95% confidence interval (95% CI). A p value of < 0.05 was considered statistical significance.

Results

Table 1 shows maternal characteristics of 300 pregnant women participating in the present study, stratified by anemic status. Pregnant women with and without anemia were similar regarding age, ethnicity, occupation, parity and interpregnancy interval. In addition to lower Hb level at first ANC and lower last Hb level during pregnancy, those with anemia had lower BMI before pregnancy than those without anemia. Moreover, they were more likely to have more GA at first ANC than those without anemia and education below high school than those without anemia. Pregnant women in non-anemic group came to the hospital earlier for their first ANC than those women in the anemic group, so they received earlier folic acid or multivitamin supplementation than in the pregnant women with anemia. Then, these affected last Hb levels during pregnancy, pregnant women in non-anemic group had no anemia more than anemic group [median (IQR) 11.3 (10.6, 12.0)] and pregnant women in anemic group had anemia more than non-anemic group [median (IQR) 10.4 (9.7, 11.0)].

Table 1. Maternal characteristics.

Characteristics	Anemic group (150 cases)	Non-anemic group (150 cases)
Age* (years)	24.0 (19.0, 21.0)	26.0 (22.0, 31.0)
Ethnicity†		
Thai	149 (99.3%)	149 (99.3%)
Laos	1 (0.7%)	1 (0.7%)
Parity†		
0	67 (44.7%)	67 (44.7%)
1	51 (34.0%)	65 (43.3%)
≥ 2	32 (21.3%)	18 (12.0%)
Hemoglobin at first ANC* (g/dL)	10.2 (0.7)	12.3 (2.7)
Severity of anemia†		
# Mild anemia	109 (72.7%)	-
# Moderate anemia	40 (26.7%)	-
# Severe anemia	1 (0.6%)	-
Last hemoglobin during pregnancy‡ (g/dL)	10.4 (9.7, 11.0)	11.3 (10.6, 12.0)
Gestational age at first ANC‡ (weeks)	15.0 (11.0, 20.0)	10.0 (7.0, 12.0)
BMI before pregnancy‡ (kg/m^2)	20.4 (18.3, 20.9)	21.5 (19.2, 24.5)
Interpregnancy interval‡ (months)	43.0 (24.0, 74.3)	53.0 (36.0, 88.0)
Occupation†		
Housewife	72 (48.0%)	61 (40.7%)
Employee	39 (26.0%)	49 (32.7%)
Business owner	18 (12.0%)	15 (10.0%)
Government official	10 (6.7%)	14 (9.3%)
Others¶	11 (7.3%)	11 (7.3%)
Education†		
Primary school	16 (10.6%)	9 (6.0%)
High school	70 (46.7%)	58 (38.7%)
Vocational certification & high vocational certification	28 (18.7%)	30 (20.0%)
Bachelor & Master degree	36 (24.0%)	53 (35.3%)

BMI: Body mass index, * Mean (standard deviation) , † Number (%), ‡ Median (Interquartile range), ¶ Others were farmer, student and prisoner.

Table 2 shows causes of anemia in pregnancy at first ANC visit, iron deficiency anemia was the most significant cause of anemia (n = 111, 74.0%) in pregnant women in this study. In this study, thalassemia was Hb H disease. There were 2 pregnant women with Hb H received blood transfusion 1 year ago and were supplemented with folic acid, at that time. Other cause of anemia was normocytic anemia.

Table 2. Causes of anemia in pregnancy at first antenatal care visit.

Causes of anemia	Number (%)
Iron deficiency	111 (74.0%)
Thalassemia	2 (1.3%)
Other	37 (24.7%)

Comparisons of maternal outcomes, maternal and neonatal complications between pregnant women with and without anemia are shown in Table 3. Pregnant women with and without anemia had similar maternal outcomes and maternal complications, including GA at delivery (GA 38 weeks in anemia group vs 39 weeks in non-anemic group, p = 0.075), route of delivery (p = 0.071) as well as preterm birth [11 (7.3%) vs 7 (4.7%), p = 0.332], PPH (p = 0.442) and PIH (p = 0.759). Also, pregnant women with and without anemia had comparable rates of neonatal complications regarding LBW, birth asphyxia to neonatal unit admission. There were no birth asphyxia at 5 minutes, IVH and NEC in these two groups. Severe anemia did not associate with preterm birth because baby (n = 1) was born at term (GA 39 weeks). There was a still birth in the non-anemic group because the pregnant woman came to the hospital due to decreased fetal movement for 2 days and transabdominal ultrasound found no fetal heart movement. The cause of the stillbirth was unknown and the parents denied an autopsy.

Table 3. Maternal outcomes, maternal and neonatal complications.

Outcomes	Anemic group (150 cases)	Non-anemic group (150 cases)	p value
Maternal outcomes			
GA at delivery* (weeks)	38.0 (37.0, 39.0)	39.0 (38.0, 39.0)	0.075
Route of delivery†			0.071
Normal delivery	89 (59.4%)	73 (48.7%)	
Vacuum extraction	8 (5.3%)	8 (5.3%)	
Forceps extraction	0 (0.0%)	4 (2.7%)	
Cesarean section	53 (35.3%)	65 (43.3%)	
Preterm birth†	11 (7.3%)	7 (4.7%)	0.332
Mild anemia	7 (4.7%)	-	
Moderate anemia	4 (2.7%)	-	
Severe anemia	0 (0.0%)	-	
Maternal complications†			
Postpartum hemorrhage	17 (11.3%)	13 (8.7%)	0.442
Pregnancy induce hypertension	6 (4.0%)	5 (3.3%)	0.759
Neonatal complications			
Stillbirth	0 (0.0%)	1 (0.7%)	0.317
Low birth weight	11 (7.3%)	10 (6.7%)	0.821
Birth asphyxia at 1 minute	1 (0.7%)	2 (1.3%)	0.315
Birth asphyxia at 5 minutes	0 (0.0%)	0 (0.0%)	1.000
Neonatal unit admission	43 (28.7%)	32 (21.4%)	0.143
Newborn wards	40 (26.7%)	28 (18.7%)	0.081
NICU	3 (2.0%)	4 (2.7%)	0.703
Respiratory distress syndrome	17 (11.3%)	14 (9.3%)	0.570
Intraventricular hemorrhage	0 (0.0%)	0 (0.0%)	1.000
Necrotizing enterocolitis	0 (0.0%)	0 (0.0%)	1.000

GA: Gestational age, * Median (Interquartile range), † Number (%), NICU: neonatal intensive care unit

The associations of anemia and other factors with risk of preterm birth using logistic regression are shown in Table 4. In both the univariate and multivariate logistic regression models, either anemia or other risk factors did not associate with the risks of preterm birth in this cohort of pregnant women.

Table 4. Factors associated with preterm birth using logistic regression.

Factors	Crude ORs (95% CI)	Adjusted ORs (95% CI)
Anemia of first antenatal care visit		
Mild anemia	1.13 (0.42-2.99)	1.52 (0.50-4.67)
Moderate anemia	0.71 (0.24-2.10)	2.78 (0.73-10.64)
Severe anemia	0	0
Maternal age		
Teenage pregnancy	1.13 (0.36-3.56)	1.18 (0.32-4.39)
Advance maternal age	0.84 (0.18-3.75)	0.62 (0.13-3.09)
Interpregnancy interval < 18 months	1.04 (0.13-8.40)	0.74 (0.09-6.52)
BMI before pregnancy		
Normal	Reference	Reference
Underweight	0.97 (0.19-2.45)	0.73 (0.20-2.69)
Overweight	0.46 (0.13-1.64)	0.37 (0.10-1.40)
Obesity	0.77 (0.19-3.11)	0.67 (0.16-2.79)
Below Bachelor degree	1.61 (0.60-4.29)	0.49 (0.17-1.47)
PIH	1.21 (0.36-4.05)	2.00 (0.23-17.39)

ORs: Odd ratios, CI: confidence interval, BMI: Body mass index, PIH: Pregnancy induce hypertension
Odd ratios were adjusted for all factors in the table.

Discussion

Both groups found a few preterm births. The preterm birth in the anemic group was 7.3% (n = 11) and in non-anemic group was 4.7% (n = 7) similar to previous two studies. One study, perinatal outcomes among thalassaemia carriers in Hong Kong[7], preterm birth in anemic pregnant women was 3.4% and in the healthy group was 1.9%. 4.9% of thalassemia trait with anemia in pregnant women and 1.0% of thalassemia trait without anemia in pregnant women had preterm births. According to the study, anemia was defined as Hb level of < 11.0 g/dL in the first trimester and 10.5 g/dL in the second trimester which is in line with the current Centers for Disease Control guideline. In another study, disparities and relative risk ratio of preterm birth in six Central and Eastern European centers in 2007-2009[9], the study in the Czech Republic found 7.4% of preterm births in anemic group. In contrast, a Thai study, correlation of maternal anemia during pregnancy and low birth weight infant at Chonburi Hospital in 2004-2007[10], found approximately 15% of preterm births in pregnant women with and without anemia. According to the study, anemia was defined as hematocrit < 33% following the criteria of Department of Public Prosecution, Thailand. Similarly, studies of Okunade and colleagues (2014)[5], Bakhtiar and colleagues (2007)[6], Arora and colleagues (2015)[9], Kidanto and colleagues (2009)[15], Yuan and colleagues (2010)[19] and Rahman and colleagues (2016)[20], preterm birth in anemic group was 15-21% that they studied in developing (Nigeria, Pakistan, Romania, Ukraine and Tanzania) and

developed countries (Hungary, Slovakia and the United Kingdom). The definitions of anemia during pregnancy in those studies has shown anonymize. Okunade and colleagues[5]: anemia in pregnancy was defined as the pregnant women in which maternal packed cell volume fell below 30% because this definition of anemia is used in most parts of Africa. Bakhtiar and colleagues[6], Arora and colleagues[9] and Kidanto and colleagues[15]: anemia was classified according to the WHO standards as the Hb level < 11 g/dL which is as same as this study. Yuan and colleagues[19] used Hb < 10.5 g/dL as definition of anemia in pregnancy. Rahman and colleagues[20]: anemia was defined as the exposure variable with Hb concentrations < 11 g/dL or hematocrit < 33%.

Studies examining the impact of anemia on risk of preterm birth have shown inconsistent results. In this study, anemia did not significantly increase the risk of preterm birth as a few previous retrospective cohort studies. Yuan W and colleagues(19), 18.6% of maternal anemia had term births and 19.7% of maternal anemia had preterm births in Bristol, United Kingdom. The study at Chonburi Hospital[10] found no association between maternal anemic status and preterm birth (anemic group 15.1 % vs non-anemic group 15.2%, p = 0.223). In contrast to previous studies in Pakistan (Bakhtiar and colleagues[6]), Nigeria (Okunade and colleagues[5]) and systematic review and meta-analysis in South Asia, East-West Asia, African and South American regions (Rahman and colleagues[20]) which showed that the risk of preterm birth in anemic pregnant women was 1.5-3.0 times higher than in non-anemic pregnant women. In the perinatal outcomes among thalassaemia carriers in Hong Kong study[7], anemia in pregnancy was significantly associated with preterm deliveries (p = 0.020). A retrospective study in six Central and Eastern Europe[9] showed that in anemic women had preterm births 1.5-4.0 times higher than term birth in Czech Republic, Hungary, Slovakia, and Ukraine except in Romania. Anemic women had 18.5% of preterm birth and 18.8% of term birth (p = 0.820).

Because the anemic group in this study were living with the mild degree of anemia [n (%) = 109 (72.7%)], that it did not significantly increase the risk of preterm birth.

BMI before pregnancy in both groups was within the normal range so it did not increase the risk of preterm birth. Similar to ACOG Practice Bulletin No. 130: prediction and prevention of preterm Birth (2012)[13], Williams Obstetrics 24th ed.[4], Yuan and colleagues (2010)[19] and Di Renzo and colleagues (2011)[21], these studies found that underweight, overweight and obesity in pregnant women (BMI before pregnancy) increased the risk of preterm birth. In the anemic group, there had more pregnant women with below Bachelor degree education than in the non-anemic group [n (%) = 114 (76.0%) vs 97 (64.7%)]. Therefore, the preterm birth was affected by pregnant women with below Bachelor degree education. It is supported by a study of El-Sayed and Galea (2014)[16] which found that low maternal education (< 12 years) increased the risk of preterm birth.

Route of delivery was not significant difference between anemic group and non-anemic group, but this study found 4 forceps extraction in non-anemic group as indication of PIH. There was no forceps extraction in the anemic group. According to the study of Pitchaipraser and Siwadune (2009)[10], the forceps extraction rate in the anemic group was higher than control group and there were significantly differences (p < 0.001).

Both crude ORs and adjusted ORs (adjusted for all factors), all factors did not significantly increase the risk of preterm birth. The risk of preterm birth was 1 time in mild anemia and 2 times in moderate anemia as Kidanto and colleagues (2009)[15]. They found that the risks of preterm delivery increased in proportion to the severity of maternal anaemia. Teenage pregnancy had slightly increased the risk of preterm birth but there was not significant as a study of Kidanto and colleagues (2009)[15]. This study was similar to two studies of Butali and colleagues (2016)[17] and Di Renzo and colleagues (2011)[21] that advance maternal age did not significantly increase the risk of preterm birth. Smith and colleagues (2003)[14] found that there was a significantly increase the risk of preterm birth when interpregnancy interval was less than 6 months. Underweight, overweight or obesity before pregnancy increased the risk of

preterm birth but obesity had no significance[19-20]. Below Bachelor degree education increased the risk of preterm birth as a study of El-Sayed and Galea (2014)[16]. In this study found that PIH was 4%. In addition, a study of Rao and colleagues (2014)[22] found 21.4% of gestational hypertension and it increased significant risk of preterm birth. Similar to a study of Butali and colleagues (2016)[17] found that hypertension was significantly associated with all categories of preterm delivery (hypertension was defined as gestational or chronic hypertension including preeclampsia and eclampsia.).

Thus, all pregnant women should be recovered from anemia before pregnancy, the gap between pregnancy is 18 months or greater and the BMI before pregnancy must be within the normal range. Last, pregnant women must receive early ANC as well as regular follow up appointments to reduce the risk of preterm birth.

The strength of our study were randomly selected data and the first study of anemia in pregnancy and maternal and neonatal outcomes in Ubon Ratchatani. This study had some limitations. First, this study was a randomized study that the maternal characteristics were not similar between groups. So, those affected to increase or decrease the risk of preterm birth. Second, most pregnant women in this study had mild anemia, the preterm birth rate was slightly increased but there were no significant differences. And the lastly, samples were collected in a single hospital and represent only a limited number of patients.

Conclusion

There was no significant difference of preterm births between the anemic group and the non-anemic group. Maternal and neonatal complications showed no significant differences between the groups. All crude ORs and adjusted ORs of the factors which were associated with preterm birth were not statistically significant regarding the risk of the preterm birth.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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GYNECOLOGY

Can Video Enhance Confidence in Management of Vaginal Pessary: A randomized trial

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ABSTRACT

Objectives: To compare the confidence of women with pelvic organ prolapse (POP) in vaginal pessary management between those who had versus those who hadn't watched the teaching video.

Materials and Methods: This randomized clinical trial was conducted at two urogynecological clinics in Khon Kaen, Thailand, from September 2017 to May 2018. Women with POP that require for vaginal pessary were included and were randomized into two groups: 1) treatment group received brochure and watched teaching video, and 2) control group received only educational brochure. Each participant's knowledge was tested before and after receiving construction from a trained nurse. A retest, which examined participants' knowledge retention, self-confidence, and satisfaction in pessary use, was conducted at a two-week follow-up visit.

Results: A total of 50 subjects were enrolled: 25 in each video and non-video group. There was no statistically significant difference in median confidence scores between video (median; range = 10; 7-10) and non-video (median; range = 10; 5-10) groups, $p = 0.917$. There was statistically significant difference in mean pretest and posttest scores in both video and non-video group (3.72 [95%CI 2.98-4.46], $p < 0.001$ and 3.84 [95%CI 3.10-4.58], $p < 0.001$, respectively) but no significant difference between two groups (0.12 [-0.60-0.84], $p > 0.999$). However, the median time required to practice using pessary was significantly shorter in the video group (10 minutes [5, 30] and 15 minutes [7, 20], $p = 0.001$).

Conclusion: Additional teaching video didn't affect confidence in vaginal pessary management. However, this tool enhanced patients' learning.

Keywords: confidence, pelvic organ prolapse, teaching video, vaginal pessary.

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การใช้วีดีทัศน์ช่วยสอนสามารถเสริมความมั่นใจในการใช้อุปกรณ์พยุงช่องคลอดได้หรือไม่? : การทดลองแบบสุ่ม

ศิวรุจน์ บัวขม, ธีระยุทธ เต็มธนะกิจไพศาล, โฉมพิลาศ จงสมชัย, มาลีชาติ ศรีพิพัฒน์กุล, ประนอม บุพศิริ

บทคัดย่อ

วัตถุประสงค์: *เพื่อเปรียบเทียบความมั่นใจในการจัดการเกี่ยวกับห่วงพยุงช่องคลอดในสตรีที่มีภาวะการหย่อนของอวัยวะในอุ้งเชิงกรานในกลุ่มที่ได้ดูวีดีทัศน์เพื่อการสอนกับกลุ่มที่ไม่ได้ดู*

วัสดุและวิธีการ: *การทดลองแบบสุ่มนี้ดำเนินการที่คลินิกนรีเวชทางเดินปัสสาวะ 2 แห่ง ที่จังหวัดขอนแก่น ประเทศไทย ตั้งแต่เดือนกันยายน พ.ศ.2560 ถึงเดือนพฤษภาคม พ.ศ.2561 ในสตรีที่มีภาวะอวัยวะอุ้งเชิงกรานหย่อน และต้องการใช้ห่วงพยุงช่องคลอด โดยแบ่งเป็น 2 กลุ่ม กลุ่มรักษาได้รับเอกสารแผ่นพับความรู้และได้ดูวิดีทัศน์ ส่วนกลุ่มควบคุมได้รับเอกสารแผ่นพับความรู้อย่างเดียว อาสาสมัครทุกคนได้รับการประเมินความรู้ก่อนและหลังฟังคำอธิบายจากพยาบาล มีการติดตามประเมินการคงอยู่ของความรู้ ความมั่นใจของตนเองและความพึงพอใจในการใช้ห่วงพยุงช่องคลอดในอีก 2 สัปดาห์ถัดมา*

ผลการศึกษา: *อาสาสมัครจำนวน 50 คน แบ่งเป็น 25 คน ในแต่ละกลุ่ม พบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่ามัธยฐานความมั่นใจในการจัดการกับห่วงพยุงช่องคลอดของกลุ่มที่ดูวิดีทัศน์ (10; 7-10) และกลุ่มที่ไม่ได้ดูวิดีทัศน์ (10; 5-10), p = 0.917 พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่าเฉลี่ยของคะแนนด้านความรู้ก่อนและหลังการรับคำแนะนำในกลุ่มที่ดูวิดีทัศน์และกลุ่มที่ไม่ได้ดู (3.72 [95%CI 2.98-4.46], p < 0.001 and 3.84 [95%CI 3.10-4.58], p<0.001) แต่ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่าง 2 กลุ่ม อย่างไรก็ตามพบว่า ค่ามัธยฐานของระยะเวลาที่อาสาสมัครใช้ในการฝึกการใส่และถอดห่วงพยุงช่องคลอดสั้นกว่าอย่างมีนัยสำคัญทางสถิติในกลุ่มที่ได้ดูวิดีทัศน์ (10 [5, 30] และ 15 [7, 20], p = 0.001)*

สรุป: *การเสริมการสอนด้วยวิดีทัศน์ไม่ได้มีผลกระทบต่อความมั่นใจในการจัดการกับห่วงพยุงช่องคลอด แต่ช่วยเสริมการเรียนรู้*

คำสำคัญ: *ความมั่นใจ, อวัยวะในอุ้งเชิงกรานหย่อน, วีดิทัศน์การสอน, ห่วงพยุงช่องคลอด*

Introduction

Pelvic organ prolapse (POP) is a condition in which any of the pelvic organs, such as the uterus, vaginal wall, bladder, or rectum, descend from their normal position[1]. It can cause various symptoms such as pelvic pain, vaginal pain, urinary incontinence, constipation, and a feeling that something is falling out of the vagina. Although POP is not a life-threatening condition, it is closely associated with impaired quality of life. The prevalence of this condition is higher in the elderly[2]. Previous studies have found the prevalence of POP to be 30.8% in Swedish women age 20-59 years[3] and 43.3% in postmenopausal women in Thailand[4]. There are many options for POP treatment. Nonsurgical treatments are considered the first line of management and including pelvic floor muscle exercises and use of a vaginal pessary. Surgical treatments are offered when conservative management is ineffective[5]. Vaginal pessaries are widely used to support the pelvic organs. The advantages of pessaries are their ability to improve prolapse symptoms and that they can be used in cases in which the patient suffers from frailty or has a severe medical condition that precludes surgical intervention, or in which surgery has failed to remedy the condition[5]. It has been reported that a combination of vaginal pessary use and pelvic floor muscle training significantly improves the quality of life in women with POP to a greater degree than pelvic floor muscle training alone[6].

Providing instruction and demonstration of self-insertion and removal of the vaginal pessary is time-consuming. In our practice, we spent 20-25 minutes to teach in each patient. Therefore, instructional media such as pictures, brochures, and videos may play an important role in helping patients to understand and use vaginal pessaries with greater confidence and more effectively. A previous study found that half of the women with pelvic organ prolapse in the United States knew about vaginal pessary[7]. Another report found that women with pelvic organ prolapse who were taught how to use the pessary as well as being given the instructional media, such as brochures, had more confidence in their ability to use these devices[8]. In this study, we aimed to compare the confidence, knowledge and satisfaction of women with POP regarding vaginal pessary self-management between those who had versus those who had not watched a teaching video. We hypothesized that watching the video would make the patients more confident in pessary management.

Materials and Methods

This randomized clinical trial was approved by the Khon Kaen University Ethics Committee in Human Research (HE601252) and was conducted at two urogynecological clinics (Srinagarind hospital and Khon Kaen Regional hospital) from September 2017 to May 2018. The clinical trial registration number was TCTR20170906001. The inclusion criteria were that the patient had POP, required vaginal pessary treatment, and able to self-insert and remove pessary. After vaginal pessary fitting was performed by the physician and written informed consent was obtained, the participants had to complete a pretest questionnaire to test their knowledge of POP. They were then divided into two groups by using a block of 8 randomizations in sealed envelopes fashion by researcher nurse. Participants in the treatment group individually watched the educational video twice. The video consisted of basic POP knowledge, risk factors, treatment, and how to cope with common side effects (the same content covered in the educational brochure). In addition, it gave step-by-step visual instructions for pessary insertion, removal, and care. The control group did not watch this video. Next, participants in both groups were given basic knowledge regarding POP and trained in pessary insertion, removal, and care by trained nurse. They were then instructed to practice for themselves until they were familiar with the pessary. The duration of practice until each participant was able to use the pessary on their own was recorded. After this, the posttest was conducted. An educational brochure was given to all patients before leaving the clinic and an appointment was made for a two-week follow up. All patients were instructed to remove the pessary every night and reinsert it the morning. At a two-week follow-up, the same posttest was conducted, and patients were asked to rate their confidence, and overall satisfaction in vaginal pessary management and use.

Two educational brochures

The brochures were provided to each participant. The first one was a leaflet containing basic knowledge about POP, risk factors, treatment options, coping with common side effects, and POP prevention. The another one was a leaflet explaining the vaginal pessary and contained instructions in pessary use and management. The brochures were based on translated leaflets in Thai distributed by the Urogynecological Association (IUGA) [1] and the Thai Urogynecological Society (TUGS).

Preparation of the questionnaire

The questionnaire consisted of three parts. The first part consisted of questions about demographic data (age, parity, body mass index (BMI), educational level, and menopausal status). The second part consisted of 10 questions (10 scores) regarding the basic POP knowledge that was documented in the brochure (the definition of POP, the role of the pessary, use of lubricant gel, cleaning the pessary, and coping with vaginal or bloody discharge), each with four options (correct, incorrect, not sure, and unknown). The third part of the questionnaire evaluated self-confidence in pessary management using a 1-10 visual analog scale (VAS), with a higher score representing greater confidence. In addition, a five-level (1-5) questionnaire regarding overall satisfaction in pessary use was administered at the two-week follow-up. Answers were presented using five facial expressions, which represented very unhappy or very unsatisfied[1], unsatisfied, fair, satisfied, and very satisfied or very happy[5].

Preparation of the teaching video

The video was eight-minute long and covered basic POP knowledge, step-by-step instructions on pessary insertion and removal, pessary care, and coping with common side effects. The content of the video corresponded with the data in the brochure.

For sample size calculation, there was no data regarding to the effect of video on confidence in pessary management. Murray[8] reported that providing an education brochure was found to increase confidence in pessary management three point from ten compared to control group. We hypothesized that adding the video might increase confidence in pessary management at least equal to only educational brochure. We, thus, decided that 22 participants per group would be required to achieve 80% of the power of calculation.

Statistical analyses were performed using STATA version 10.0 (StataCorp, College Station, TX, USA). Confidence in pessary self-management, satisfaction in pessary use, and time required to practice pessary use were analyzed using a Mann-Whitney two-sample test. Events during pessary use were analyzed using a Fisher's exact test and chi-square test. Knowledge about pelvic organ prolapse and vaginal pessary were analyzed with a Bonferroni post-hoc test using Generalized Estimating Equations. A p value of less than 0.05 was considered statistically significant.

Results

A total of 50 subjects were enrolled in the study: 25 in the video group and 25 in the non-video group. There were four participants lost to follow-up at two weeks, (two in each group). There were 23 participants in the final analysis in each group, as shown in Fig. 1.

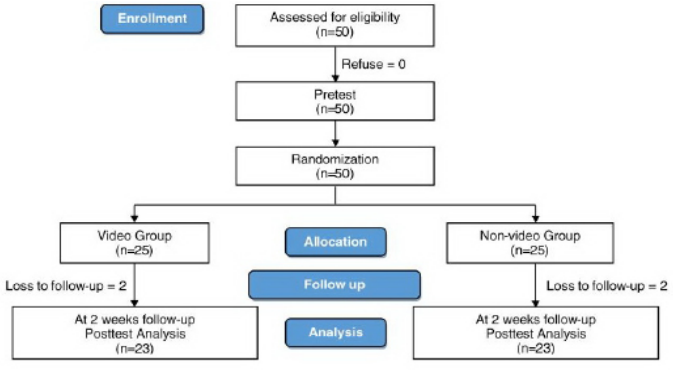

Fig. 1. Flowchart of the participants in the study.

Demographic data including age, BMI, occupation, education, and menopausal status, are shown in Table 1. The mean (SD) ages of participants in the video group and non-video group were 68.24 (9.42) and 67.28 (7.55) years, respectively. Most of the participants were the farmers, had a primary school education, had undergone vaginal delivery, were menopausal, and had POP stage III. The most common type of pessary use was ring pessary.

Table 1. Demographic data of patients in each group (N = 50).

Characteristics	Video group (n = 25)	Non-video group (n = 25)
Age, mean (SD), years	68.24 (9.42)	67.28 (7.55)
BMI, mean (SD), kg/m^2	23.14 (3.47)	24.32 (4.23)
Occupation, n (%)		
- Farmer	18 (72)	16 (64)
- Merchant	4 (16)	1 (4)
- Government officer	1 (4)	5 (20)
- None	2 (8)	3 (12)
Education level, n (%)		
- Primary	20 (80)	19 (76)
- Secondary	4 (16)	2 (8)
- Vocational/Technical	0(0)	1(4)
- Bachelor's degree or more	1 (4)	3 (12)
Parity, median (min, max)	3 (0,8)	3 (1,7)
Route of delivery, n (%)		
- Vagina	23 (92)	25 (100)
- Caesarean section	0 (0)	0 (0)
- Both	1 (4)	0 (0)
- None	1 (4)	0 (0)
Menopause, n (%)	24 (96)	24 (96)
Duration of POP, years, median (min, max)	2 (0.08, 20)	1 (0.08,10)
Stage of POP, n (%)		
I	0 (0)	0 (0)
II	6 (24)	9 (36)
III	13 (52)	12 (48)
IV	6 (24)	4 (16)
Type of pessary, n(%)		
Ring	18 (72)	17 (68)
Ring with support	7 (28)	7 (28)
Donut	0 (0)	1 (4)

SD: standard deviation, BMI: body mass index, POP: pelvic organ prolapse

Participants in both groups showed significant improvement in terms of their knowledge evaluation scores on the posttest compared to the pretest [5.40 (1.19) and 9.12 (1.01), p < 0.001 in the video group vs 5.16 (0.99) and 9.00 (1.12), p < 0.001 in the control group]. However, there was no statistically significant difference in the mean scores of the two groups (p > 0.999) (Table 2).

Table 2. Pretest and posttest knowledge scores.

	Video group (Total score 10)	N	Non-video group (Total score 10)	N	Mean difference (95%CI), p
Pretest score, mean (SD)	5.40 (1.19)	25	5.16 (0.99)	25	0.24 (-0.48-0.96), > 0.999
Posttest score, mean (SD)	9.12 (1.01)	25	9.00 (1.12)	25	0.12 (-0.60-0.84), > 0.999
2-week score, mean (SD)	9.00 (1.04)	23	8.96 (1.15)	23	0.04 (-0.71-0.79), > 0.999
Mean difference					
- Pre-Posttest (95%CI), p	3.72 (2.98-4.46), < 0.001		3.84 (3.10-4.58), < 0.001		
- Pretest-2 weeks (95%CI), p	3.60 (2.85-4.36), < 0.001		3.80 (3.05-4.55), < 0.001		

SD: standard deviation, CI: confidence interval

Participants' confidence in pessary self-management and the overall satisfaction regarding pessary use were high, and neither differed significantly between the two groups (Table 3). There was also no difference in terms of adverse events or side effects of pessary use between the two groups. However, the required practice time for vaginal pessary self-insertion and removal was significantly shorter in the video group [10 minutes (5, 30) vs 15 minutes (7, 20), p = 0.001] (Table 3).

Table 3. Confidence and satisfaction in vaginal pessary use at a two-week follow-up (N = 46).

Subject	Video group (n = 23)	Non-Video group (n = 23)	p value
Confidence in self-management of pessary			
- Median (min, max)	10 (7, 10)	10 (5, 10)	0.917
Satisfaction in using the pessary			
- Median (min, max)	4 (2, 5)	5 (3, 5)	0.209
Practice time required (minute)			
- Median (min, max)	10 (5, 30)	15 (7, 20)	0.001
Events during pessary use, n (%)			
- Pessary slippage	6 (26.09)	4 (17.39)	0.475
- Difficulty in pessary insertion and removal	5 (21.74)	2 (8.70)	0.414
- Failure to insert /discontinuing usage	0 (0)	0 (0)	
Side effect, n (%)			
- Pain, irritation	4 (17.39)	3 (13.04)	> 0.999
- Abnormal vaginal discharge	0 (0.00)	2 (8.70)	0.489
- Constipation	1 (4.35)	0 (0.00)	> 0.999

Discussion

Participants' confidence in vaginal pessary self-management was high in our study. This result was consistent with those of other studies, which found that teaching patients vaginal pessary management improved comfort and made the patients more likely to continue using the device[9, 10]. Palumbo[11] reported that patient education plays a vital role in successful pessary use. Studies have also shown that health literacy leads to patients being more aware of the importance of individual health maintenance[12,13]. Elderly patients are known to have difficulty in recalling information given to them by healthcare staff, particularly doctors. Additional media such as brochures, pictures/graphics, and videos, may enhance patients' understanding of their diseases and improve information retention and treatment plans[8-10].

In our study, we used a teaching video to enhance understanding of insertion and removal of a vaginal pessary. However, this did not affect patients' confidence in pessary self-management when compared with face-to-face consultation alone. The explanation for this might be that all patients were given slow verbal step-by-step instructions by a trained nurse, which helped them to become familiar with pessary use, which led to higher patient confidence in both groups. Thus, instruction performed by trained nurses and the provision of time for patients to practice pessary use and maintenance in the POP clinic are essential to pessary care[1-3, 14-16].

However, patients in the video group required significantly less time to practice self-insertion and removal of the pessary. This might be because the video provided patients with clearer picture as to how these devices should be used and managed. In addition, we showed participants the video prior to face-to-face consultation (verbal instruction), which served to enhanced patient's learning. Therefore, video presentation is useful and necessary to add in educating the POP patients that require pessary in both individual or group learning and it might reduce instruction time for health care personnel.

Patients' knowledge scores were significantly higher at post instruction. Retention at a follow-up visit at least two weeks later might also be an important indicator of awareness of the patients.

Time is needed to learn skill of self-insertion and removal of the vaginal pessary. If patients are skilled in this procedure, the continuation rate of pessary use will likely be high. Moreover, a two-step approach, in which information and advice regarding pessary use is first provided by the physician and then followed-up on by a trained nurse, enables prolapse patients to more effectively use the device and leads to a low rate of discontinuation[17]. However, Duenas[18] noted that most discontinuation occurs within the first week after device insertion. Brown[7] reported that lower levels of education tend to be correlated with declined pessary use. However, although most of our participants graduated from the primary school which contrast to Brown's reported a lower level of education trend to decline pessary use[7]. The Patients' self-confidence scores in vaginal pessary self-care were high in both groups. Overall satisfaction with pessary use was also high, which was consistent with results found in others reports[19, 20].

The strength of this study was the study design, the limitation of the study was a short time follow-up and the included participants were only the patients who were able to self-practice. Therefore, our data cannot apply in the dependent patients. Furthermore, a long time follow-up for retention rate evaluation of using the vaginal pessary, or develop new appropriate teaching media is required in the future research.

Conclusion

Additional teaching video did not affect confidence in self-management of the vaginal pessary. However, this tool enhanced understanding and led to a shorter time being required to practice pessary use.

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Potential conflicts of interest

The authors declare no conflict of interest.

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GYNECOLOGY

Parametrial Invasion in Early-Stage Cervical Cancer

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ABSTRACT

Objectives: To determine the rate of parametrial involvement and associated factors among patients who underwent radical hysterectomy and pelvic lymphadenectomy for early-stage cervical cancer.

Materials and Methods: Medical records of 165 patients who had complete data were reviewed. Early stage cervical cancer was defined as cervical cancer stage IA2 to IIA. We excluded patients who presented with neuroendocrine tumors or sarcoma. The lesion size was measured clinically during pelvic examination preceding surgery.

Results: The majority of patients (87.39%) were in stage IB1. One hundred twenty-five (75.8%) patients had tumors smaller than 2 cm in diameter. Pelvic lymph node metastases were noted in twelve (7.3%) patients. Parametrial invasion was noted in six (3.6%) patients. The rate of parametrial invasion was 10% among patients with tumors larger than 2 cm in diameter compared to 1.6% among those with smaller lesions. The rate of parametrial invasion was higher among patients who had deep stromal invasion (13.5% vs 0.9%) and pelvic node metastasis (41.7% vs 0.7%) compared to those without these two pathological factors.

Conclusion: The overall rate of parametrial invasion in patients with early stage cervical cancer in this study was 3.6%. The one significant preoperative predictor for parametrial invasion was tumor size. Significant pathological factors for predicting an increased risk of parametrial invasion included the presence of deep stromal invasion and pelvic lymph node metastasis.

Keywords: cervical cancer, parametrial invasion, radical hysterectomy.

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การลุกลามเนื้อเยื่อข้างมดลูกในมะเร็งปากมดลูกระยะต้น

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บทคัดย่อ

วัตถุประสงค์: *เพื่อประเมินอัตราการลุกลามเนื้อเยื่อข้างมดลูกและปัจจัยที่เกี่ยวข้องในผู้ป่วยมะเร็งปากมดลูกระยะต้นที่ได้รับการผ่าตัดมดลูกแบบกว้างร่วมกับการเลาะต่อมน้ำเหลืองในอุ้งเชิงกราน*

วัสดุและวิธีการ: *ทำการเก็บรวบรวมข้อมูลประวัติการรักษาจากเวชระเบียนของผู้ป่วยมะเร็งปากมดลูกระยะต้น (มะเร็งปากมดลูกตั้งแต่ระยะ 1A2 ถึง 2A) จำนวน 165 ราย ยกเว้นผู้ป่วยมะเร็งปากมดลูกระยะต้นชนิด neuroendocrine หรือ sarcoma นอกจากนี้มีการวัดขนาดของรอยโรคก่อนผ่าตัดจากการตรวจภายในทางช่องคลอด*

ผลการศึกษา: *ผู้ป่วยส่วนใหญ่เป็นมะเร็งปากมดลูกระยะ 1B1 (ร้อยละ 87.39) และมีขนาดเส้นผ่าศูนย์กลางของรอยโรคน้อยกว่า 2 เซนติเมตร (ร้อยละ 75.8) มีการกระจายของมะเร็งไปต่อมน้ำเหลืองในอุ้งเชิงกรานจำนวน 12 ราย (ร้อยละ 7.3) มีการลุกลามไปเนื้อเยื่อข้างมดลูกจำนวน 6 ราย (ร้อยละ 3.6) โดยอัตราของการลุกลามเนื้อเยื่อข้างเคียงในกลุ่มที่มีขนาดเส้นผ่าศูนย์กลางของรอยโรคมากกว่า 2 เซนติเมตร (ร้อยละ10) มากกว่ากลุ่มที่มีขนาดรอยโรคน้อยกว่า 2 เซนติเมตร (ร้อยละ 1.6) นอกจากนี้พบว่า อัตราการลุกลามเนื้อเยื่อข้างมดลูกสัมพันธ์กับการลุกลามสโตรม่าชั้นลึก และการกระจายของมะเร็งไปต่อมน้ำเหลืองในอุ้งเชิงกราน โดยพบว่าอัตราการลุกลามเนื้อเยื่อข้างมดลูกในกลุ่มที่มีการลุกลามสโตรม่าชั้นลึก (ร้อยละ 13.5) จะสูงกว่ากลุ่มที่ไม่มีการลุกลามสโตรม่าชั้นลึก (ร้อยละ 0.9) และพบอัตราการลุกลามเนื้อเยื่อข้างมดลูกในกลุ่มที่มีการกระจายของมะเร็งไปต่อมน้ำเหลืองในอุ้งเชิงกราน (ร้อยละ 41.7) สูงกว่ากลุ่มที่ไม่มีการกระจายของมะเร็งไปต่อมน้ำเหลืองในอุ้งเชิงกราน (ร้อยละ 0.7)*

สรุป: *อัตราการลุกลามเนื้อเยื่อข้างมดลูกในผู้ป่วยมะเร็งปากมดลูกระยะต้นของการศึกษานี้เท่ากับร้อยละ 3.6 ปัจจัยสำคัญก่อนการผ่าตัดที่ช่วยทำนายการลุกลามเนื้อเยื่อข้างมดลูกคือขนาดของรอยโรคและปัจจัยสำคัญทางพยาธิวิทยาที่ช่วยทำนายการลุกลามเนื้อเยื่อข้างมดลูกคือการลุกลามสโตรม่าชั้นลึกและการกระจายของมะเร็งไปต่อมน้ำเหลืองในอุ้งเชิงกราน*

คำสำคัญ: *มะเร็งปากมดลูก, การลุกลามเนื้อเยื่อข้างปากมดลูก, การผ่าตัดมดลูกแบบกว้าง*

Introduction

Cervical cancer is a major health burden in less economically developed countries. Approximately 90% of cervical cancer-related deaths are in developing countries due to a lack of effective organized prevention programs[1]. Treatment of cervical cancer depends on the clinical stage of the disease. Clinical staging procedures in cervical cancer include detailed physical examination and diagnostic investigations to assess the size of the primary tumor and the extent to which it has spread to the surrounding tissues, retroperitoneal lymph nodes, and distant organs[2].

Radical hysterectomy is the surgical procedure performed to remove the uterus, cervix, upper vagina and parametria[3]. This procedure aims to ensure complete resection of the tumor[3]. Radical hysterectomy in conjunction with bilateral pelvic lymphadenectomy is the standard surgical treatment for stage IA2-IIA cervical cancer, when preservation of fertility is not necessary[4]. Radical hysterectomy results in treatment outcomes similar to those who receive pelvic radiation[4]. Radical hysterectomy, however, may be preferable to pelvic radiation among premenopausal women in order to preserve ovarian function and minimize sexual dysfunction[5].

One of the most distressing morbidities following radical hysterectomy is bladder dysfunction. Bladder dysfunction, caused by injury to the pelvic autonomic nerves during resection of the parametria (parametrectomy), may occur in up to 70% of women following radical hysterectomy[6, 7]. Other morbidities secondary to parametrectomy include ureteric damage, significant blood loss, fistula formation, and bowel denervation[8-10]. To prevent these potential complications, omitting parametrectomy during radical hysterectomy in women with an early stage cervical cancer might be reasonable if the risk of parametrial involvement is very low[2]. The purpose of this study was to determine the rate of parametrial involvement and associated factors among patients who underwent radical hysterectomy and pelvic lymphadenectomy for early stage cervical cancer.

Materials and Methods

After receiving approval from the Research Ethics Committee, we reviewed medical records of women with cervical cancer stages IA2-IIA who underwent radical hysterectomy from January 2005 to December 2016. In all cases, bilateral pelvic lymphadenectomy had been performed. Abstracted data included baseline characteristics, clinical stage, perioperative complications, and detailed pathological findings. Early stage cervical cancer was defined as cervical cancer stages IA2 to IIA. We excluded patients who received preoperative neoadjuvant chemotherapy or radiation therapy and those who presented with unusual histology such as neuroendocrine tumors or sarcoma.

The size of each tumor was clinically measured during pelvic examination preceding surgery. Parametrial invasion was defined as metastasis in either the parametrial tissue or parametrial lymph node. Deep stromal invasion was defined as a tumor invading cervical stroma to a depth of ≥ 10 mm. Lymphovascular space invasion (LVSI) was defined as the presence of neoplastic cells within vascular or lymphatic spaces lined by flattened endothelial cells.

Descriptive statistics were used to analyze patient characteristics. A 95% confidence interval (CI) of the rate of parametrial invasion was calculated to determine the precision of the data. A chi-square test or Fisher's exact test was used to univariately identify factors associated with parametrial invasion. A multivariate analysis using a logistic model was used to determine the independent effects of factors of interest. An adjusted odds ratio (aORs) with a 95%CI that did not include unity was considered statistically significant.

Results

This study reviewed medical records of 165 patients, all of whom had complete records. The mean age ± standard deviation (SD) of the patients was 45.7±7.9 years. Table 1. shows the patients' clinical and pathological characteristics. The majority

of patients (144 patients, 87.39%) were in stage IB1. Nine (5.5%) had tumors larger than 4 cm in diameter, and 125 (75.8%) had tumors smaller than 2 cm. Pelvic lymph node metastases were reported in 12 (7.3%) patients. Parametrial invasion was noted in six (3.6%) patients (95% CI 1.3-7.7%).

Table 1. Clinical and pathological characteristics of the patients.

Characteristics	Number (%)
Age	
< 40 years	47 (28.5)
≥ 40 years	118 (71.5)
Parity	
Nulliparous	10 (6.1)
Multiparous	155 (93.9)
Stage	
IA2	8 (4.8)
IB1	144 (87.3)
IB2	9 (5.5)
IIA1	4 (2.4)
Histology	
Squamous cell carcinoma	98 (59.4)
Adenocarcinoma	66 (40.0)
Adenosquamous carcinoma	1 (0.6)
Tumor size (largest diameter)	
≤ 2 cm	125 (75.8)
> 2 cm	40 (24.2)
Lymphovascular space invasion	
Presence	64 (38.8)
Absent	73 (44.2)
Not recorded	28 (17.0)
Deep stromal invasion	
Presence	37 (22.4)
Absent	113 (68.5)
Not recorded	15 (9.1)
Parametrial invasion	
Presence	6 (3.6)
Absent	159 (92.7)
Pelvic lymph node metastasis	
Presence	12 (7.3)
Absent	153 (92.7)

Table 2 displays the clinical and pathological factors for predicting parametrial invasion. Four clinical factors were analyzed; including the size of tumor, histological type, patient's age, and parity status, of which only tumor size was significantly associated with parametrial invasion. The rate of parametrial invasion was 10% among patients with tumors larger than 2 cm in diameter compared to 1.6% among those with smaller lesions. After adjusting by patient age and histological type, patients who had tumors larger than 2 cm in diameter were approximately seven times more likely to have parametrial invasion (aORs 7.24; 95%CI 1.24-42.17).

Three pathological factors were also examined; the presence of LVSI, deep stromal invasion (DSI), and pelvic lymph node metastasis, all of which were confirmed after pathological examination of surgical specimens. Patients found to have DSI or pelvic lymph node metastasis carried a higher risk of parametrial invasion (Table 2).

Table 2. Clinical and pathological factors predicting parametrial invasion.

Variable	Parametrial invasion		p value
	Presence	Absent	
Tumor size (n = 165)			
≤ 2 cm (n = 125)	2 (1.6)	123 (98.4)	0.031
> 2 cm (n = 40)	4 (10.0)	36 (90.0)	
Histology (n = 165)			
Squamous cell carcinoma (n = 98)	5 (5.1)	93 (94.9)	0.476
Others (n = 67)	1 (1.5)	65 (98.5)	
Age (n = 165)			
≥ 40 years (n = 118)	5 (4.2)	113 (95.7)	0.676
< 40 years (n = 47)	1 (2.1)	46 (97.9)	
Parity status (n = 165)			
Nulliparous (n = 10)	0 (0)	10 (100.0)	1.00
Multiparous (n = 155)	6 (3.9)	149 (96.1)	
Lymphovascular space invasion (n = 137)			
Presence (n = 64)	4 (6.3)	60 (93.7)	0.111
Absent (n = 73)	0 (0)	73 (100.0)	
Deep stromal invasion (n = 150)			
Presence (n = 37)	5 (13.5)	32 (86.5)	0.004
Absent (n = 113)	1 (0.9)	112 (99.1)	
Pelvic lymph node metastasis (n = 165)			
Presence (n = 12)	5 (41.7)	7 (58.3)	< 0.001
Absent (n = 153)	1 (0.7)	152 (99.3)	

Data presented as number (percentage)

Of the six patients with parametrial invasion, five (83.3%) had deep stromal invasion. The rate of parametrial invasion among patients without DSI was 0.9% (one of 113 patients). In addition, five of these

patients (83.3%) had pelvic lymph node metastasis. On the other hand, only one of the 153 patients without pelvic lymph node metastasis had parametrial invasion, a rate of 0.7%.

Discussion

The overall rate of parametrial invasion in early stage cervical cancer in this study was 3.6% (95%CI 1.3-7.7%). The one significant preoperative factor was the size of the cervical lesion. Significant pathological factors for predicting the risk of parametrial invasion included the presence of DSI and pelvic lymph node metastasis. Histological type and presence of LVSI were not significantly associated with the risk of parametrial invasion.

The rate of parametrial invasion in our study was in line with previously reported findings. In the present study, the rate of parametrial invasion was 3.6%. The rate of parametrial invasion in early stage cervical cancer found in previous reports varied from less than 1% to up to 10%[11-17].

The size of cervical lesions was the preoperative factor most strongly associated with the risk of parametrial invasion. Previous studies have found parametrial involvement to occur in less than 1% to 1.9% of women with cervical lesions 2 cm in diameter or smaller. In the present study, only 1.6% of patients whose largest tumor was smaller than 2 cm in diameter were found to have parametrial invasion, which significantly differed from the rate of 10% found among those with tumors larger than 2 cm (Table 2). After adjustment for patient age and histological tumor type, patients with tumors larger than 2 cm in diameter carried an approximately seven times higher risk of suffering from parametrial invasion (aORs 7.24; 95%CI 1.24-42.17). Tumor size therefore may be a potential indicator that can be used to tailor the radicality of a hysterectomy to minimize surgical morbidity associated with parametrectomy. However, effectiveness of simple hysterectomy for early stage cervical cancer needs to be critically evaluated in a randomized controlled trial before its relevance to patient care can be considered. In our analysis, we found status of the pelvic lymph node to be the pathological factor with the strongest association with parametrial invasion. The rate of parametrial invasion was approximately 42% in patients with pelvic lymph node metastasis compared to only 0.7% among patients without pelvic node metastasis. After adjustment for patient age and histological type, the presence of tumors larger than 2 cm in diameter was a significant independent factor associated with an increased risk of parametrial invasion (aORs 7.24; 95%CI 1.24-42.17). In addition, most patients who had parametrial invasion (83.3%) were also found to have pelvic lymph node metastasis (Table 2). These findings were similar to those of previous studies[12, 15, 16, 18], which have found the rates of parametrial involvement among patients with pelvic lymph node metastasis from 26.3% to 80% compared to 3.6% to 10% among those without pelvic lymph node metastasis[12, 15, 16, 18].

In this study, we defined lesions with DSI as tumors invading cervical stroma to a depth of ≥ 10 mm. Our study found a higher rate of parametrial invasion among patients who had DSI. The rates of parametrial invasion were 13.5% and 0.9% among patients with DSI and those without deep invasion, respectively (Table 2). Similarly, Vanichtantikul et al[18] reported rates of parametrial involvement among patients who had stromal invasion deeper than10 mm and those with more superficial invasions of 14.3% and 1.2%, respectively.

There are differences in the characteristics of patients with adenocarcinoma and those adenosquamous carcinoma, as well as differences in the natural course of disease between these two squamous cell carcinoma subtypes. This may raise questions regarding whether different histological subtypes of cervical cancer pose different levels of risk of parametrial invasion. In our study, histological type of cancer was not associated with risk of parametrial involvement. Our finding reaffirmed previously reported results which indicate a lack of significant impact of histological subtype on the risk of parametrial invasion in cases of early stage cervical cancer[12, 15-18].

Some limitations of this study are worthy of note. First, the lesion size measures in this study were solely based on physical examination or the so-called 'the clinical tumor size'. Different techniques in tumor size

measurement (i.e. pathological examination or radiologic imaging) may not yield a similar result. Second, a relative small sample size in this study resulted in a wide confidence interval of estimate measures.

Conclusion

In conclusion, the overall rate of parametrial invasion in stage IA2-IIA cervical cancer in this study was 3.6%. The one significant preoperative factor associated with the risk of parametrial invasion was clinical tumor size. Significant pathological factors for predicting the risk of parametrial invasion included the presence of DSI and pelvic lymph node metastasis.

Potential conflicts of interest

The authors declare no conflict of interest.

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OBSTETRICS

Prevalence of Maternal Hypovitaminosis D and Obstetric Outcomes at Chonprathan Hospital, Nonthaburi, Thailand

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ABSTRACT

Objectives: The primary aim of this study was to determine the prevalence of maternal hypovitaminosis D. The secondary aim was to determine the obstetric outcomes between a group of pregnant women with hypovitaminosis D and the normal control group at Chonprathan Hospital, Nonthaburi province of Thailand.

Materials and Methods: This study was a cross-sectional study. A total of 77 subjects were consecutively enrolled in the study. The inclusion criteria were pregnant women who had received antenatal care and delivered at Chonprathan Hospital, Nonthaburi, Thailand. The exclusion criteria included women who had a liver disease, kidney disease, gastrointestinal absorption disease, pulmonary tuberculosis, hyperthyroid disease, and pregnant women who used drugs that have an effect on vitamin D. Vitamin D deficiency was defined as 25-hydroxyvitamin D (25-OHD) < 20 ng/mL, insufficiency as 25-OHD 20–29.9 ng/mL, and sufficiency as 25-OHD ≥ 30 ng/mL. Hypovitaminosis D refer to vitamin D deficiency plus vitamin D insufficiency. A data interview was performed and the results recorded in a case record form by the research team. Venous blood samples were collected for 25-OHD, parathyroid hormone (PTH), calcium, phosphate, alkaline phosphatase (ALP), albumin, and magnesium on the day of labor.

Results: The mean level of 25-OHD was 25.2% ± 7.9 ng/mL. The prevalence of vitamin D deficiency was 22.1%, vitamin D insufficiency was 44.1%, and vitamin D sufficiency was 33.8%. There was an association between vitamin D level and serum albumin but no association with the other blood parameters statuses (correct calcium,PTH, phosphate, ALP, magnesium,hematocrit), age, pre-pregnancy body mass index, or obstetric complications.

Conclusion: The prevalence of hypovitaminosis D was 66.2%, while vitamin D deficiency was 22.1%. There was no association between the vitamin D level and obstetric outcomes.

Keywords: vitamin D deficiency in pregnancy, vitamin D insufficiency in pregnancy, obstetric outcomes.

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ความชุกของภาวะการขาดวิตามินดีในสตรีตั้งครรภ์ และผลกระทบต่อการตั้งครรภ์ ณ โรงพยาบาลชลประทาน จังหวัดนนทบุรี ประเทศไทย

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บทคัดย่อ

วัตถุประสงค์: *วัตถุประสงค์หลักเพื่อศึกษาถึง ความชุกของภาวะการขาดวิตามินดีในสตรีตั้งครรภ์ วัตถุประสงค์รองคือ เพื่อศึกษาผลกระทบต่อการตั้งครรภ์ในสตรีกลุ่มที่มีภาวะขาดวิตามินดีเปรียบเทียบกับกลุ่มปกติ ณ โรงพยาบาลชลประทาน จังหวัดนนทบุรี ประเทศไทย*

วัสดุและวิธีการ: *การวิจัยนี้เป็นการศึกษาแบบตัดขวาง จำนวนกลุ่มตัวอย่างทั้งหมด 77 คน ถูกเลือกโดยการเรียงต่อกันตามลำดับ โดยมีเกณฑ์การคัดเข้าร่วมงานวิจัย คือ สตรีตั้งครรภ์ที่ได้รับการฝากครรภ์และคลอด ณ โรงพยาบาลชลประทาน มีเกณฑ์การคัดออก คือ สตรีตั้งครรภ์ที่มีโรคตับ, ไต, ทางเดินอาหาร, วัณโรคปอด, ไทรอยด์ และสตรีตั้งครรภ์ที่รับประทานยาที่มีผลต่อระดับวิตามินดี ภาวะพร่องวิตามินดี คือ ระดับวิตามินดีในเลือดน้อยกว่า 20 นาโนกรัมต่อมิลลิลิตร ภาวะวิตามินดีไม่เพียงพอ คือ วิตามินดีในเลือดอยู่ระหว่าง 20-29.9 นาโนกรัมต่อมิลลิลิตร และภาวะวิตามินดีปกติ คือ วิตามินดีในกระแสเลือดมากกว่าหรือเท่ากับ 30 นาโนกรัมต่อมิลลิลิตร นิยามภาวะขาดวิตามินดี คือ ภาวะพร่องวิตามินดีและ ภาวะวิตามินดีไม่เพียงพอ กลุ่มตัวอย่างจะถูกสมภาษณ์ข้อมูลเพื่อบันทึกในใบบันทึกข้อมูล และเจาะเลือดเพื่อส่งตรวจระดับวิตามินดี พาราไทรอยด์ฮอร์โมน แคลเซียม ฟอสเฟส อัลคาไลนฟอสฟาเตส อัลบูมิน และแมกนีเซียม ในวันที่มาทำการคลอด*

ผลการศึกษา: *พบว่าค่าเฉลี่ยของระดับวิตามินดีเท่ากับร้อยละ 25.2 ± 7.9 นาโนกรัมต่อมิลลิลิตร ความชุกของภาวะพร่องวิตามินดีเท่ากับ ร้อยละ 22.1 ภาวะวิตามินดีไม่เพียงพอ ร้อยละ 44.1 และภาวะวิตามินดีเพียงพอ ร้อยละ 33.8 พบความสัมพันธ์ระหว่างระดับวิตามินดีกับอัลบูมิน แต่ไม่พบความสัมพันธ์ระหว่างวิตามินดี กับ ค่าแคลเซียม พาราไทรอยด์ฮอร์โมน ฟอสเฟส อัลคาไลนฟอสฟาเตส แมกนีเซียม และฮีมาโตคริท รวมทั้งไม่พบความสัมพันธ์ระหว่างระดับวิตามินดีกับอายุ น้ำหนักก่อนตั้งครรภ์ และภาวะแทรกซ้อนระหว่างตั้งครรภ์ในสตรีกลุ่มตัวอย่าง*

สรุป: *พบความชุกของภาวะขาดวิตามินดี ร้อยละ 66.2 และภาวะพร่องวิตามินดี ร้อยละ 22.1 จากการศึกษาไม่พบความสัมพันธ์ระหว่างระดับวิตามินดีในกระแสเลือดและผลลัพธ์ทางสูติศาสตร์*

คำสำคัญ: *ภาวะพร่องวิตามินดีในสตรีตั้งครรภ์, ภาวะวิตามินดีไม่เพียงพอในสตรีตั้งครรภ์, ผลลัพธ์ทางสูติศาสตร์*

Introduction

There are many research studies in the literature showing that vitamin D is a beneficial nutrient for pregnant women and their babies. Studies have found the vitamin D receptor in various types of tissues in the human body, which suggest that vitamin D is not only useful for the bone-building system, but it also has benefits for the human body in other systems[1].

Vitamin D can be synthesized through human skin when skin is exposed to ultraviolet B-light or it can be absorbed from the intestines through the diet. Currently, the 25-hydroxyvitamin D (25-OHD) level measurement method is used to assess the vitamin D level in the human body[2].

Vitamin D in pregnant women plays an important role in both the mothers and babies. Vitamin D deficiency is often common in Northern Europe[1,3]. A recent study found that vitamin D deficiency is often common in pregnant women with certain risk factors, such as vegetarian groups, no or low sun-exposure groups, and especially women in dark-skin groups[4].

Some studies have found that vitamin D deficiency in pregnant women can have an effect on their pregnancy, such as preeclampsia[5], preterm labor[6], or gestational diabetes mellitus[7], and also have an effect on their infant, such as neonatal seizure due to hypocalcemia[8].

In Southeast Asian countries, especially Thailand, studies on vitamin D deficiency in pregnant women and its effects are limited. However, such studies are important as studying the prevalence of vitamin D deficiency will be guided to understanding the size of the problem and will inform guidelines for planning, prevention, and treatment in the future to overcome the problem.

The primary objective of this study was to determine the prevalence of maternal hypovitaminosis D. The secondary objective was to determine the obstetric outcomes between a group of pregnant women with hypovitaminosis D and the normal control group at Chonprathan Hospital, Nonthaburi province of Thailand.

Materials and Methods

This study was a cross-sectional study regarding the prevalence of vitamin D deficiency in pregnant women who had delivered at Chonprathan Hospital since 2016-2017. The study was approved by the Institutional Review Board of Chonprathan Hospital, Srinakharin Wirot University, Thailand. A total of 77 subjects were consecutively enrolled in the study. The inclusion criteria were pregnant women who had received antenatal care and delivered at Chonprathan Hospital. Informed consent was obtained from all subjects. The study was performed during the period October 2016-March 2017. The exclusion criteria included women who had a liver disease, kidney disease, gastrointestinal absorption disease, pulmonary tuberculosis, hyperthyroid disease, and pregnant women who used drugs that had an effect on vitamin D, such as anticonvulsant and steroid drugs. History taking and a physical examination were used to identify the exclusion criteria among participants. The volunteer group received a detailed description of the project. A data interview was performed and the results recorded in a case record form by the research team. The interview consisted of two parts: part 1- interview to record age, weight before pregnancy, height, underlying disease, regular medications, prenatal vitamins and minerals taken, pregnancy and previous pregnancy outcomes, duration of pregnancy, delivery methods and delivery complications of previous pregnancy, and pregnancy records for infants whose weight was above 4,000 grams or less than 2,500 grams; and part 2 – interview to record labor issues, including delivery methods, color of amniotic fluid, blood loss during delivery, and maternal and neonatal complications during pregnancy and labor (e.g., gestational hypertension (HT), preeclampsia, gestational diabetes mellitus (GDM), postpartum hemorrhage (PPH), preterm labor). Venous blood collection was performed for 25-OHD, parathyroid hormone (PTH), calcium, phosphate, alkaline phosphatase (ALP), albumin, and magnesium on the

day of labor. The time of interview was the same as the time of blood sampling. Gestational ages of participants were 36^{+1} to 41 weeks.

The researchers asked the interviewees about taking iron supplementation (e.g., natural, ferrous fumarate, and obimin AZ®) and confirmed with the out patient department card whether they took the supplementation reported in the interview. This was important as iron supplementation typically contains vitamin D; for instance, natural, ferrous fumarate, and obimin AZ® contain 400 IU vitamin D, so this can affect the vitamin D status of pregnant women.

Vitamin D deficiency was defined as 25-OHD < 20 ng/mL, insufficiency as 25-OHD 20-29.9 ng/mL, and sufficiency as 25-OHD ≥ 30 ng/m[9]. Hypovitaminosis D was defined as vitamin D deficiency plus vitamin D insufficiency.

Obstetric outcomes of this study included gestational HT, preeclampsia, GDM, PPH, fetal growth disorder, preterm birth, premature rupture of membranes, fetal distress, breech presentation, placenta previa, consumptive coagulopathy.

The sample size was calculated by using the formula:

$$n = \frac{Z^2_{\alpha/2} pq}{d^2} \quad \text{where} \quad Z_{\alpha/2} = 1.96$$

p = Prevalence of maternal hypovitaminosis D among pregnant women in the Thai population (75%), q = 1-p, d = The absolute error of the sample size (equal to 15%)

$$n = \frac{1.96^2 0.75 \times (1 - 0.75)}{(0.15 \times 0.75)^2} = 57$$

According to Pratumvinit, et al[10], the prevalence of maternal hypovitaminosis D among pregnant women in Bangkok, Thailand is 75.5%. The data collected for any missing data were 20%. Therefore, the sample size of this study was 69 people.

The plasma levels of calcium, phosphate, ALP, albumin, and magnesium were measured using an automated analyzer (Cobas C501, Roche Diagnosis, Germany). Intact PTH levels were measured using a chemiluminescent microparticle immunoassay (Abott Diagnostics, Wiesbaden, Germany). 25-OHD levels were analyzed using a chemiluminescent microparticle immunoassay (Abbott Diagnostics, Longford, Ireland). The laboratory investigation used 3 ml of blood for analysis in the laboratory at Panyananthphikkhu Chonprathan Medical Center. The inter-and intra-assay coefficient variant of 25-OHD test were 2.4% and 1.1%, respectively.

Statistic analysis was performed using SPSS version 17. Descriptive statistic (mean, percent, frequency and standard deviation) were used. Independent t test was used to compare mean of investigated factors between hypovitaminosis D group and control group. Fisher exact test were used to compare the percent of obstetric complications between hypovitaminosis D group and control group. A p value < 0.05 was considered statistically significant.

Results

In total, 77 pregnant women were included in the study. The mean age was 27.3 ± 6.1 years. Primiparity and multiparity were 42.9% and 57.1%, respectively. The mean weight and height were 53.6 ± 10.9 kg and 157.7 ± 6.2 cm, respectively. The pre-pregnancy body mass index (BMI) values in the range of underweight, normal weight, overweight, and obesity equivalents were 17.3%, 66.7%, 13.3%, and 2.7%, respectively. The mean gestational age was 38.6 ± 1.1 weeks. The women with blood group A, B, AB and O were 26%, 36.4%, 3.9%, and 33.8%, respectively. There was no Rhesus negative pregnant woman in this study.

Table 1 demonstrates blood chemistry status in this study. Mean level of 25-OHD, intact PTH, correct calcium, albumin, magnesium, phosphate, ALP were 25.2 ± 7.9 ng/mL, 40.8 ± 22.4 pg/mL, 8.9 ± 0.4 mg/dL, 3.58 ± 0.27 g/dL, 1.93 ± 0.18 mg/dL, 4.7 ± 2.8 mg/dL and 165.2 ± 51.5 unit/L, respectively.

The prevalence of vitamin D deficiency was 22.1%, vitamin D insufficiency was 44.1%, and vitamin D sufficiency was 33.8% (Table 2).

Table 1. Blood chemistry status of pregnant women in Chonprathan Hospital.

Blood chemistry	Normal range in pregnancy (3rd trimester)	Mean	Standard deviation
25-OHD (ng/mL)	10 - 18	25.2	7.9
Intact PTH (pg/mL)	9 - 26	40.8	22.4
Correct calcium (mg/dL)	8.8 - 10.3	8.9	0.4
Albumin (g/dL)	2.3 - 4.2	3.58	0.27
Magnesium (mg/dL)	1.1 - 2.2	1.93	0.18
Phosphate (mg/dL)	2.8 - 4.6	4.7	2.8
ALP (unit/L)	38 - 229	165.2	51.5

PTH: parathyroid hormone, ALP: alkaline phosphatase.

Table 2. Vitamin D status in pregnant women.

Vitamin D status in pregnancy	Number (total 77)	Prevalence (%)
Vitamin D sufficiency (25-OHD ≥ 30 ng/L)	26	33.8
Vitamin D insufficiency (25-OHD 20-29.9 ng/L)	34	44.1
Vitamin D deficiency (25-OHD < 20 ng/L)	17	22.1

OHD: hydroxyvitamin D

There was an association between the vitamin D level and albumin, but no association with the other blood parameters statuses (correct calcium,PTH, phosphate, ALP, albumin, magnesium, hematocrit), age, pre-pregnancy BMI, or obstetric complications (gestational HT, preeclampsia, GDM, PPH, fetal growth disorder, preterm birth, premature rupture of membranes, fetal distress, breech presentation, placenta previa, consumptive coagulopathy) (Table 3, 4).

Table 3. Compare mean of investigated factors associated with 25-hydroxyvitamin D.

Factors	n	Hypovitaminosis D (25-OHD < 30 ng/mL)	n	Vitamin D sufficiency (25-OHD ≥ 30 ng/mL)	p value
Age (years)	51	27.41 ± 6.35	26	27.04 ± 5.60	0.801
Gestational age (weeks)	51	38.77 ± 1.18	26	38.75 ± 0.09	0.953
Pre-pregnancy BMI (kg/m2)	51	21.92 ± 4.05	26	21.02 ± 3.77	0.351
Pregnant BMI (kg/m2)	51	27.62 ± 4.17	26	26.23 ± 3.92	0.163
PTH (mg/dL)	51	43.86 ± 23.08	26	34.89 ± 20.24	0.097
Correct calcium (mg/dL)	51	9.29 ± 0.43	26	9.25 ± 0.44	0.714
Phosphate (mg/dL)	51	4.48 ± 2.62	26	5.14 ± 3.08	0.332
ALP (unit/L)	51	169.2 ± 56.04	26	157.38 ± 41.15	0.345
Albumin (g/dL)	51	3.52 ± 0.26	26	3.69 ± 0.25	0.008
Magnesium (mEq/L)	51	1.91 ± 0.18	26	1.95 ± 0.19	0.391
Hematocrit (%)	51	35.23 ± 3.32	26	35.17 ± 2.28	0.935
Platelets (/mm3)	51	284,449.02 ± 65,567.39	26	257,076.92 ± 56,586.87	0.074
Blood loss (mL)	51	332.35 ± 210.43	26	338.46 ± 196.63	0.902

Data presented as mean ± standard deviation or n (%), OHD: hydroxyvitamin D, BMI: body mass index, ALP: alkaline phosphatase

Table 4. Comparison of the number (%) of obstetric complications between hypovitaminosis D group and a non-hypovitaminosis D group.

Obstetrics complications	n	Hypovitaminosis D (25-OHD < 30 ng/mL) n (%)	n	Vitamin D sufficiency (25-OHD > 30 ng/mL) n (%)	p value
Gestational HT	51	0 (0%)	26	0 (0%)	NA
Preeclampsia	51	0 (0%)	26	0 (0%)	NA
GDM A1	51	0 (0%)	26	1 (3.8%)	0.338
GDM A2	51	0 (0%)	26	0 (0%)	NA
PPH	51	2 (3.9%)	26	0 (0%)	0.547
IUGR	51	0 (0%)	26	0 (0%)	NA
Preterm birth	51	1 (2.0%)	26	0 (0%)	1
Premature rupture of membranes	51	3 (5.9%)	26	0 (0%)	0.547
Fetal distress	51	1 (2.0%)	26	0 (0%)	1
Breech presentation	51	5 (9.8%)	26	2 (7.7%)	1
Placenta previa	51	1 (2%)	26	0 (0%)	1
Coagulopathy	51	0 (0%)	26	0 (0%)	NA

OHD: hydroxyvitamin D, HT: gestational hypertension, GDM: gestational diabetes mellitus, PPH: postpartum hemorrhage, IUGR: intrauterine growth restriction

Discussion

Thailand is a country in Southeast Asia, and near the equator, so it experiences relatively strong sunlight throughout the year. Despite this, World Health Organization found the incidence of vitamin D deficiency in Thailand is relatively high. The present study discovered that the prevalence of vitamin D insufficiency (25-OHD 20-29 ng/mL) and vitamin D deficiency (25-OHD < 20 ng/mL) were 44.1% and 22.1%, respectively. The prevalence of hypovitaminosis D in this study was 66.2%, which is slightly different from one study in Bangkok showed that the prevalence of hypovitaminosis D (25-OHD < 30 ng/mL) was 75.5%[10]. However, the study from Thammasart university, Pathumthani province, Thailand found the prevalence of vitamin inadequacy (25-OHD < 75 nmol/L, which approximately < 24 ng/mL) was only 27.4% in third trimester pregnancy[11].

Our study found that serum albumin was the factor that associated with hypovitaminosis D. However mean serum albumin level in hypovitaminosis D group was slightly lower than vitamin D sufficiency. Although, there was no association between hypovitaminosis D and intact PTH but if we analyzed only vitamin D deficiency group (25OHD < 20 ng/mL), we found the association between vitamin D deficiency group and intact PTH. The vitamin D deficient group had intact PTH level concentrations higher than in the vitamin D sufficient group, which was in accordance with the findings in other studies[10]. However, the level of intact PTH is regulated by serum ionized calcium not by 25-OHD[12]. This phenomenon may have been because the intact PTH response to a low level of 25-OHD was reduced by the higher levels of calcium in women without secondary hyperparathyroidism[13]. The present study found no relationship between serum correct calcium level and vitamin D status.

Our study found no relationship between BMI before pregnancy and vitamin D status, in contrast with one study that showed that BMI had a significant effect

on vitamin D status. They explained that a person with a high BMI usually has a high content of body fat, which acts as a reservoir for lipid-soluble vitamin D. At the same time, lipid-soluble vitamin D release from fat is extremely slow. Excess body fat results in its increased sequestration and low availability and, as a consequence, low serum 25-OHD level[14]. The reason that there was no relationship found with BMI before pregnancy in our study might due to the limited sample size, as the obesity group represented only 2.7% of our sample set.

In terms of the maternal outcome, in our study, we were unable to find an association between vitamin D deficiency and meconium status, fetal distress, mode of delivery, preterm birth, or premature rupture of the membranes. One systematic review and meta-analysis result was opposite that found in our study concerning preterm birth. They found that vitamin D deficiency was associated with preterm birth, with serum 25-OHD levels < 75 nmol/l associated with an 83% (95%CI 1.23, 2.74) and 13% (95%CI 0.94, 1.36) increased risk of preterm birth measured at < 32 - 34 weeks and < 35 - 37 weeks, respectively[15]. However, from study by Bhupornvivat and Phupong found that the serum 25-OHD concentrations and the prevalence of vitamin D deficiency and insufficiency were not different between the preterm labor and the term labor groups[16]. Therefore, study about vitamin D deficiency and preterm birth is needed in this area in the future.

The results from many studies have shown no association between a premature rupture of the membranes and vitamin D deficiency, which is consistent with our study[17].

A study concluded that vitamin D levels were significant lower in a mother delivering by cesarean section due to suspected fetal distress and birth asphyxia[18], which is contrast to the results from our study. However, our study had a limited number of fetal distress cases, indeed only one case from our 77-sample set.

Although we concluded that vitamin D deficiency has a high prevalence, there was no relation between vitamin D deficiency and obstetric complications. Due to the limited evidence currently available to directly assess the benefits and harms of the use of vitamin D supplementation alone in pregnancy for improving maternal and infant health outcomes, the use of this intervention during pregnancy as part of routine antenatal care is not recommended. This study related to the suggestion from WHO 2016 that vitamin D supplementation is not recommended for pregnant women, and to improve maternal and perinatal outcome, pregnant women should instead be encouraged to receive adequate nutrition, which is best achieved through the composition of a healthy and balanced diet. For pregnant women with documented vitamin D deficiency, vitamin D supplements may be given at the current recommended nutrient intake of 200 IU (5 micrograms) per day[19,20].

The strength of this study was the first study about the prevalence of hypovitaminosis D in Nonthaburi province, urban area in Thailand. This study confirmed that there had high prevalence of hypovitaminosis D in this area. However, the limitation of this study was a few of participants had the level of 25-OHD less than 20 ng/mL which may deter us from finding the correlation between vitamin D deficiency and adverse obstetric outcome.

Conclusion

There is a high prevalence of vitamin D deficiency in pregnant women at Chonprathan Hospital, Nonthaburi, Thailand. However, there was no relation between hypovitaminosis D and adverse obstetric outcomes. Therefore, it should not recommend prescribing vitamin D supplementation for pregnant women attending Chonprathan Hospital for antenatal care.

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Potential conflicts of interest

The authors declare no conflict of interest.

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OBSTETRICS

The Outcomes of Ampicillin plus Azithromycin to Prolong Latency Period in Preterm Premature Rupture of Membranes between 24 and 33^{+6} Weeks of Gestation at King Chulalongkorn Memorial Hospital

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ABSTRACT

Objectives: To determine the success rate of ampicillin plus azithromycin to prolong the latency period in cases with preterm premature rupture of membranes (PPROM).

Materials and Methods: A retrospective descriptive study was conducted. Medical records of singleton pregnancies between 24 and 33^{+6} weeks of gestation who complicated with PPROM and received ampicillin and oral azithromycin to prolong the latency period at King Chulalongkorn Memorial Hospital between January 2010 and December 2016 were reviewed. Prolonged latency period more than 48 hours was defined as success. Descriptive statistics were used for data analysis.

Results: Eighty eight pregnancies were included in the study with mean ± standard deviation age of 30.4 ± 5.6 years and mean gestational age of 31.3 ± 2.5 weeks. The median of latency period was 96 hours (interquartile range 60-192 hours) and 76 cases (86.4%) reached more than 48 hours of latency period. Seven women (8.0%) complicated by chorioamnionitis with 1 case of maternal sepsis. Regarding neonatal outcomes, respiratory distress syndrome (RDS) complicated in 29 neonates (33.0%) and 12 cases (13.6%) needed ventilator. Forty eight cases (54.5%) found neonatal sepsis along with 3 (3.4%) neonatal deaths.

Conclusion: Antibiotic regimen including ampicillin and azithromycin was effective to prolong latency period in most women presented with PPROM. However, one-third of the neonates complicated with RDS and neonatal sepsis was found in more than half of the cases. Further study is needed to identify regimens that may improve neonatal outcomes.

Keywords: antibiotic, azithromycin, premature rupture of membrane, prolong latency period.

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ผลการรักษาของแอมพิซิลลินร่วมกับอะซิโทรมัยซินเพื่อยืดระยะเวลาก่อนคลอดในภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์ช่วงอายุครรภ์ระหว่าง 24 และ 33^{+6} สัปดาห์ ในโรงพยาบาลจุฬาลงกรณ์

นันท์นภัส สินรัตน์, สุรสิทธิ์ ชัยทองวงศ์วัฒนา

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาร้อยละของความสำเร็จในการยืดระยะเวลาก่อนคลอดในผู้ป่วยภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์ช่วงอายุครรภ์ก่อนครบกำหนด*

วัสดุและวิธีการ: *การศึกษาเชิงพรรณนาแบบย้อนหลัง โดยทบทวนเวชระเบียนของหญิงตั้งครรภ์เดี่ยวที่เข้ารับการรักษาตัวในโรงพยาบาลจุฬาลงกรณ์ เนื่องจากมีภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์ช่วงอายุครรภ์ระหว่าง 24 และ 33^{+6} สัปดาห์ และได้รับยาแอมพิซิลลินร่วมกับอะซิโทรมัยซิน เพื่อยืดระยะเวลาก่อนคลอด ระหว่างเดือนมกราคม พ.ศ. 2553 ถึงเดือนธันวาคม พ.ศ. 2559 ความสำเร็จของการยืดระยะเวลาก่อนคลอด นับเมื่อมีระยะเวลาก่อนคลอดนานกว่า 48 ชั่วโมง และวิเคราะห์ข้อมูลโดยใช้สถิติเชิงพรรณนา*

ผลการศึกษา: *การศึกษาข้อมูลหญิงตั้งครรภ์รวม 88 ราย มีอายุเฉลี่ย ± ส่วนเบี่ยงเบนมาตรฐาน เท่ากับ 30.4 ± 5.6 ปี และอายุครรภ์เฉลี่ย 31.3 ± 2.5 สัปดาห์ ค่ามัธยฐานของระยะเวลาก่อนคลอดเท่ากับ 96 ชั่วโมง (ค่าพิสัยระหว่างควอไทล์ 60-192 ชั่วโมง) โดยมี 76 ราย (ร้อยละ 86.4) ที่สามารถยืดระยะเวลาก่อนคลอดได้นานกว่า 48 ชั่วโมง ผู้ป่วย 7 ราย (ร้อยละ 8.0) เกิดการอักเสบของถุงน้ำคร่ำ และพบการติดเชื้อในกระแสเลือด 1 ราย ผลในทารกแรกเกิดพบภาวะกลุ่มอาการหายใจลำบากรวม 29 ราย (ร้อยละ 33.0) และ 12 ราย (ร้อยละ 13.6) ต้องใช้เครื่องช่วยหายใจ และพบทารกติดเชื้อในกระแสเลือดจำนวน 48 ราย (ร้อยละ 54.5) และมีทารกเสียชีวิต 3 ราย (ร้อยละ 3.4)*

สรุป: *สูตรยาปฏิชีวนะประกอบด้วยแอมพิซิลลินและอะซิโทรมัยซินมีประสิทธิภาพในการยืดระยะก่อนคลอดในหญิงตั้งครรภ์ส่วนใหญ่ที่เกิดภาวะถุงน้ำคร่ำแตกก่อนการเจ็บครรภ์ช่วงอายุครรภ์ก่อนครบกำหนดอย่างไรก็ตาม หนึ่งในสามของทารกแรกเกิดพบภาวะกลุ่มอาการหายใจลำบาก และพบทารกติดเชื้อในกระแสเลือดมากกว่าครึ่งหนึ่งของทารกทั้งหมด จำเป็นต้องมีการศึกษาต่อไปเพื่อหาสูตรยาปฏิชีวนะที่อาจช่วยให้ผลลัพธ์ในทารกแรกเกิดดีขึ้น*

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Introduction

Preterm birth, of which the global incidence approximately 15 million each year[1,2], is the leading cause of neonatal morbidity and mortality. In 2010, the estimated preterm birth rates worldwide were ranging from 5% to 18% of all livebirths[2]. Similarly, about 14% of all neonates born at King Chulalongkorn Memorial Hospital (KCMH) during the years 2008-2017 were preterm babies. Preterm premature rupture of membranes (PPROM) is accounted for one-third of spontaneous preterm births and frequently associated with intra-amniotic infection and inflammation[3,4]. PPROM could lead to maternal infectious complications including chorioamnionitis, sepsis and postpartum endometritis, while babies born after PPROM commonly complicated with respiratory distress syndrome (RDS), neonatal sepsis, pneumonia and death[5]. Surviving neonates may pose long-term neurodevelopmental consequences including cerebral palsy[6] that would be a burden to their parents.

The benefits of antibiotic therapy after PPROM were confirmed to prolong latency periods and reduce chorioamnionitis and short-term neonatal morbidities[7]. As a result, several national guidelines[8-10] recommend the use of prophylactic antibiotics in cases of PPROM. For management of PPROM between 24 and 33^{+6} weeks of gestation, the American College of Obstetricians and Gynecologists (ACOG) suggests a 7-day course of therapy with a combination of intravenous ampicillin and erythromycin followed by oral amoxicillin and erythromycin to prolong latency together with a single-course corticosteroids to promote neonatal lung maturity[10].

Erythromycin is endorsed by the ACOG and other national organizations for the first line antibiotic regimen for PPROM[8-10] because it is one of the well-studied regimens[7]. However, clinical use of erythromycin faces problems of poor compliance due to its gastrointestinal side effects and number of dosages per day. In addition, the intravenous erythromycin formulation is not available at KCMH. Azithromycin is a newer macrolide derivatives that has a longer half-life allowing for once a day regimen, excellent tissue distribution, minimal drug interaction and similar pharmacokinetics of intravenous and oral formulations[11-13]. Antibiotic regimens used for prolongation of latency period in cases with PPROM at KCMH includes intravenous ampicillin (2 g every 6 hours) for 48 hours following by 5 days of oral amoxicillin (250 mg every 8 hours) and oral erythromycin (250 mg every 6 hours) for 7 days. Due to easier administration, replacement of erythromycin with oral azithromycin (500 mg on the first day following by 250 mg every 24 hours for 6 days) for PPROM at KCMH is increasing. Because of limited information of azithromycin-included regimen, this study was conducted to determine the effectiveness of the antibiotic regimen including ampicillin plus azithromycin to prolong the latency period in cases with PPROM in term of success rate, maternal and neonatal morbidities.

Materials and Methods

This retrospective descriptive study was approved by The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Medical records of pregnant women who diagnosed as PPROM at gestational age between 24 and 33^{+6} weeks and admitted at King Chulalongkorn Memorial Hospital between January 2010 and December 2016 were reviewed. Singleton pregnancies between 24 and 33^{+6} weeks of gestation with clinical presentation of PROM without labor pain and received ampicillin plus azithromycin to prolong latency period were included. Cases having fetal lethal anomaly, history of fetal surgery or genetic amniocentesis in the current pregnancy were excluded. The perinatal outcomes of PPROM after genetic amniocentesis are significantly better than spontaneous PPROM at a similar gestational age[14] while outcomes of fetal surgery depend on the nature of diseases. Rupture of membranes was primarily diagnosed by combination of history of fluid leakage and visualization of amniotic fluid pooling in the vagina during sterile speculum examination and/or positive arborization test. The patients included in this study received antibiotic regimen using ampicillin plus azithromycin to prolong latency period. The regimen included ampicillin 2 g intravenously every 6 hours for 48 hours then amoxicillin 500 mg orally three times a day and azithromycin 500 mg orally for one day then

250 mg orally once daily for 6 days.

Data collection included demographic and baseline information: maternal age, gravidity, parity, marital status, underlying diseases, history of previous preterm delivery, total number of prenatal care visit, gestational age on admission, and duration from membranes rupture to admission, investigation results on the day of admission: amniotic fluid index, hemoglobin level, white blood cell count, percentage of neutrophils, urine analysis, urine culture, and cervical swab culture, and receiving interventions: tocolysis, and steroids to promote fetal lung maturity. The primary outcome of this study was percentage of women being success on prolongation of the latency period. Latency period was determined by the time interval from membranes rupture to delivery and the latency period of more than 48 hours defined as success. The secondary outcomes were the latency period, maternal complications and neonatal complications. Maternal complications included placental abruption, prolapsed umbilical cord, acute chorioamnionitis, sepsis and death. Neonatal outcomes focused on birthweight, Apgar scores, RDS, use of ventilator, neonatal intensive care unit (NICU) admission, neonatal sepsis, mortality and length of hospital stay.

The sample size was calculated by using the formula for a binary outcome in single population[15]. According to Phupong et al study[16], the proportion of women who success in prolongation of the latency was 0.647. Eighty-eight women were needed when an alpha error was 0.05 and an acceptable error was 0.1. Statistical analysis was performed with SPSS software package version 22.0 (IBM Corp., Armonk, NY, USA). Quantitative data were presented as mean and standard deviation (SD) or median and interquartile range (IQR) and a comparison was done by student t-test or Mann-Whitney U test. Percentages were used to describe qualitative data and compared by chi-square test or Fisher exact test. P value of less than 0.05 was considered statistically significant.

Results

From January 2010 through December 2016, 88 women were met the criteria and included in the study. Seven women having amniocentesis during second trimester for prenatal diagnosis were excluded. Baseline maternal characteristics were shown in Table1. Mean maternal age was 30.4 years and mean gestational age on admission was 31.3 weeks. Most of the women were nulliparous. Median duration from membranes rupture to admission was 4.4 hours.

Table 1. Baseline characteristics of the pregnant women.

Characteristics	N = 88
Age (years)[a]	30.4 ± 5.6
Gravidity[b]	
1	41 (46.6%)
2	23 (26.1%)
3	15 (17.1%)
4	8 (9.1%)
5	1 (1.1%)
Parity[b]	
Nulliparity	55 (62.5%)
Multiparity	33 (37.5%)
Number of prenatal care visit (times)[c]	6 (4-7)
Gestational age on admission (weeks)[a]	31.3 ± 2.5
Duration from membrane rupture to admission (hours)[c]	4.4 (3.0-9.0)
Amniotic fluid index[c]	6.5 (2.9-9.1)

[a] presented as mean ± standard deviation, [b] presented as number (%), [c] presented as median (interquartile range)

Cervical swab cultures were noted in 78 women and most of the cases reported negative results. Among cases with positive culture (N=19), group B streptococcus and Escherichia coli (E. coli) was found in 5 cases and 4 cases, respectively (Table 2).

To promote fetal lung maturity in pregnant women complicated with preterm labor or PPROM at KCMH, a regimen of dexamethasone 6 mg intramuscularly every 12 hours for 4 doses is used. Dexamethasone were prescribed in all study women, but 88.6% of cases received completed course and 62 women (70.5%) received tocolytic agents (Table 3). Tocolysis was used in the cases having regular uterine contraction after admission without any signs of chorioamnionitis, non-reassuring fetal status, or cervical dilatation ≥ 4 centimeters.

Seventy-six women (86.4%) were success to prolong the latency period while the median latency period was 96 hours. Chorioamnionitis occurred in 7 cases (8.0%) who having the latency period between 3 and 596 hours. One out of 88 women showed clinical sepsis. E.coli was found positive in her cervical swab and urine culture. No maternal death was noted.

Table 2. Microbiology of cervical swab culture on admission.

Organism	Number (%) (N = 78)
Yeast	6 (7.7%)
Group B streptococcus	5 (6.4%)
Escherichia coli	4 (5.1%)
Streptococcus viridans	2 (2.6%)
Acinetobacter baumannii	1 (1.3%)
Group D streptococcus	1 (1.3%)
No growth	59 (75.6%)

Table 3. Interventions, pregnancy and maternal outcomes.

Characteristics	N = 88
Tocolytic use[a]	62 (70.5%)
Number of dexamethasone received[a]	
1 dose	4 (4.5%)
2 doses	2 (2.3%)
3 doses	4 (4.5%)
4 doses	78 (88.6%)
Latency period (hours)[b]	96 (60-192)
Prolonged latency period > 48 hours[a]	76 (86.4%)
Chorioamnionitis[a]	7 (8.0%)
Sepsis[a]	1 (1.1%)

[a] presented as number (%), [b] presented as median (interquartile range)

Regarding neonatal outcomes, the mean gestational age at birth was 32.3 weeks with the mean birthweight of 1,837 grams (Table 4). Most of the neonates had Apgar scores at 1 and 5 minutes equal to or greater than 7; however, 29.5% of them needed NICU admission. One-third (29/88) of the

newborns complicated with RDS and ventilation support was needed in 12 cases. Neonatal sepsis was diagnosed in 54.5% of all cases and there were three neonatal deaths. Fetal lung hypoplasia were suspected in all non-survived cases because they were born at between 27 weeks and 31 weeks of gestation with extremely low birth weight after complicated by severe oligohydramnios. Among surviving babies, the median length of stay was 10 days.

Table 4. Neonatal outcomes.

Characteristics	N = 88
Gestational age at birth (weeks)[a]	32.3 ± 2.0
Birthweight (grams)[a]	1,837 ± 425
Apgar score at 1 minute < 7[b]	18 (20.5%)
Apgar score at 5 minute < 7[b]	9 (10.2%)
Neonatal intensive care unit admission[b]	26 (29.5%)
Respiratory distress syndrome[b]	29 (33.0%)
Ventilation support[b]	12 (13.6%)
Neonatal sepsis[b]	48 (54.5%)
Neonatal death[b]	3 (3.4%)
Length of stay (days)[c]	10.0 (7.0-25.5)

[a] presented as mean ± standard deviation, [b] presented as number (%), [c] presented as median (interquartile range)

Discussion

Because neonatal morbidities and survival are associated with gestational age at birth[5], one of the goals in the management of PPROM is to extend latency period[17]. The present study found that ampicillin plus azithromycin could prolong the latency period of more than 48 hours in 86.4% of women presented with PPROM between 24 and 33^{+6} weeks of gestation. The success rate in the present study seem to be higher than those (64.7%) in the prior report[16] that reviewed cases with PPROM between 28 and 34 of gestation at KCMH from 1997 to 2009. During that time period, the antibiotic regimen for PPROM at KCMH consisted of ampicillin plus one of macrolide group: erythromycin, azithromycin or roxithromycin[16].

Although the guidelines[8-10] recommend antibiotic regimen including ampicillin plus erythromycin, an increasing use of oral azithromycin in place of erythromycin has been reported[16,17]. These antibiotics have similar antimicrobial coverage, but azithromycin is easier for administration than erythromycin. Two retrospective studies of women with PPROM between 23 and 34 weeks of gestation reported no difference in latency period between those who received ampicillin plus erythromycin and those who received ampicillin plus azithromycin[18,19]. The median latency period in women receiving ampicillin plus a single oral dose of 1 gram azithromycin in the study by Finneran et al[19] was 5.9 days (IQR 3.1-12.1 days) that comparable to those found in the present study (4 days, IQR 2.5-8 days). The study by Pierson et al[18] did not found difference of maternal and neonatal outcomes between women receiving erythromycin and women receiving azithromycin while cesarean section rate and positive neonatal blood culture were higher in women receiving erythromycin in Finneran et al study[19].

Clinical chorioamnionitis complicated in 8.0% of the women in the present study that similar to 8.6% of women receiving azithromycin reported in the study by Pierson et al[18]. However, the percentage of neonatal sepsis in the present study was much higher than those in the previous studies[18,19]. This disparity may be

explained by diverse criteria used for diagnosis. The neonatologists at KCMH defined neonatal presumed sepsis as neonatal sepsis that were 54.5%, but only 3.4% of all neonates were found positive blood culture. On the other hand, Finneran et al study used blood culture positive for bacteria as criteria to diagnose neonatal sepsis that reported in 4.1% of neonates delivered from women receiving azithromycin.

Two-thirds of the neonates delivered from women in azithromycin group from the previous studies were complicated with RDS[18,19] while it was noted in 33.0% of neonates in the present study. This discrepancy may be due to more advanced gestational age at birth of the newborns in the present study (mean gestational age 32.3 weeks) when compared to the previous studies (30-31 weeks of gestation)[18,19]. Nevertheless, neonatal death rates were not different between the present study (3.4%) and previous studies (2.2% and 4.0%)[18,19].

Drug administration of azithromycin practically is more convenient than erythromycin. If it is not inferior to erythromycin in term of clinical effectiveness, it may be a good choice for an antibiotic regimen in women complicated by PPROM. A recent study focusing on cost analysis of using azithromycin compared with erythromycin in treatment of pregnancies with PPROM demonstrated that azithromycin substituted for erythromycin in the antibiotic regimen had a potential for substantial cost reduction[20].

There was limited information of azithromycin use for antibiotic regimen in the treatment of PPROM. The present study reported outcomes of the regimen including ampicillin plus 7-day course of azithromycin. Since it was a retrospective study, a number of confounding factors may impact the interested outcomes such as tocolytic use. Other limitations of the study were incomplete data of the reviewed medical records and not having a control group. Although cervical swab culture is a routine practice for the management of PPROM at King Chulalongkorn Memorial Hospital, there were 10 medical records (11.4%) in the present study that cervical swab culture results are not available. A randomized controlled trial comparing outcomes between regimen including azithromycin and regimen including erythromycin should be conducted to confirm the non-inferiority of azithromycin. Furthermore, advantages of azithromycin including fewer side effects, better compliance and cost-effectiveness should be investigated.

Conclusion

In conclusion, antibiotic regimen including ampicillin and azithromycin was effective to prolong latency period in most women presented with PPROM. However, one-third of neonates complicated with RDS and more than half of them were presumed sepsis. Further study is needed to identify regimen that may improve neonatal outcomes.

Potential conflicts of interest

The authors declare no conflict of interest.

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OBSTETRICS

Pregnancy-associated Plasma Protein A Levels with Pregnancy Outcomes: A preliminary study

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ABSTRACT

Objectives: In the first trimester serum screening pregnancy-associated plasma protein-A (PAPP-A) levels are estimated in pregnant women. Its low values are leading to more risk of preterm delivery, isolated intrauterine growth restriction (IUGR), intrauterine death (IUD) or neonatal death, pregnancy-induced hypertension (PIH), and intrahepatic cholestasis of pregnancy (IHCP). The objective of this study was to find the correlation of PAPP-A levels with pregnancy outcomes and complications.

Materials and Methods: This retrospective study was done on the patients visiting the antenatal outpatient department for first trimester screening (11-13 weeks). Ultrasonographic nuchal translucency scan and blood sample test for double marker were performed. Based on the multiple of median (MOM) value of PAPP-A, two groups were made. MOM value ≥ 0.5 (normal PAPP-A levels) was considered as the control group and MOM value < 0.5 (low PAPP-A levels) was considered as the study group. Data were collected and analyzed. Pregnant women were followed-up until delivery. Pregnancy outcomes and complications were recorded.

Results: A total of 141 patients qualified and included in the study, 126 patients had normal (control group) and 15 had low PAPP-A values (study group). The study group had significant higher complications when compared to control group as IHCP (46.6% vs 14.3%, p = 0.002), IUGR (26.6% vs 8.7%, p = 0.034), preterm delivery (46.67% vs 19.84%, p = 0.017), IUD (13.3% vs 0.79%, p = 0.001) and fetal distress (13.3% vs 1.58%, p = 0.009). The patients of study group having more gestational diabetes (20% vs 16.6%, p = 0.744), both PIH and oligohydramnios (13.3% vs 7.93%, p = 0.482) and premature rupture of membranes (6.66% vs 0.79%, p = 0.069) that were insignificantly higher as compared to control groups.

Conclusion: The PAPP-A levels measurement is a valuable marker during the first -trimester screening for predicting adverse outcomes and complications, as low PAPP-A level was associated with a high chance of preterm delivery, IUGR, IHCP, and adverse fetal outcome.

Keywords: PAPP-A level, pregnancy, outcome, complications.

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Introduction

The double marker screening test consists of two biochemical markers, pregnancy-associated plasma protein -A (PAPP- A) and free β-human chorionic gonadotrophin (hCG) level. Along with the nuchal translucency ultrasound examination, it is used to assess the risk for trisomy 21 and other fetal aneuploidies in the first trimester. How PAPP-A can be associated with bad pregnancy outcomes, the answer can be as following. PAPP- A, a protease, helps to release free insulin like growth factor (IGF) for its action[1]. Studies show that IGF helps in the activation of cell division, differentiation and decidual invasion by trophoblasts[2]. It affects fetal growth by regulating the use of amino acids and glucose in the trophoblast[3]. The low levels of maternal serum PAPP-A will lead to low levels of active IGF and finally affect fetal growth. This effect on fetal growth may also cause other adverse pregnancy complications, such as preterm delivery, intrauterine growth restriction (IUGR), pregnancy-induced hypertension (PIH), stillbirth and neonatal death[4-6]. The circulating PAPP-A is formed in syncytiotrophoblast during pregnancy[7]. One has to give more attention to patients who have low PAPP-A levels during the first trimester of pregnancy.

Hence, we hypothesize the use of low PAPP-A, as an important marker of pregnancy and useful to predict outcome and complications in pregnant women. The primary objective of this study was to find the correlation between low levels of PAPP-A with pregnancy outcomes and complications. The secondary objective was to compare the normal to low PAPP-A levels to pregnancy outcome and complications.

Materials and Methods

This retrospective study was performed from January 2017 to December 2018 on pregnant patients visiting for antenatal clinic (ANC) checkup at the department of Maternal and Reproductive Health, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, UP, India. We advised all patients to visit the antenatal out patient department (OPD) for first trimester screening at 11-13 weeks 6 days of gestation, for nuchal translucency ultrasound and blood sample for double marker test. The double marker test included the free β-HCG and PAPP-A levels.

The selection of patients was done based on inclusion criteria from pregnant patients with singleton pregnancy presented between 11-13 weeks 6 days and having nuchal translucency ultrasound and double marker with complete follow up to delivery and complications. We screened 605 patients, who attended the OPD for ANC checkup at first trimester during the study period, but only 141 patients had a complete dataset in terms of follow-up, outcome and complications that were found eligible as per inclusion criteria to include in this study and analyzed.

Blood samples were collected in a vacutainer tube without anticoagulant and sent for analysis to the molecular medicine laboratory of our institute. The samples were analyzed on the device-Siemens-IMMULITE 1000 automated immunoassay system, using automated chemiluminescent immunoassays. The risk calculation was done with Siemens software PRISCA, which uses biochemical markers, ultrasound measurements, and demographics data to make calculations. Data of the double marker test of all the patients were collected and analyzed. PAPP-A, multiple of median (MOM) value was taken to analyze the results. Based on previous studies results from the patients with PAPP-A MOM value 0.5 were taken as the cut of value[22]. The patients were divided into two groups as per the results, the patients having MOM value ≥ 0.5 was considered to control group (normal PAPP-A levels) and MOM value < 0.5 was considered as a study group (low PAPP-A level). The results were presented in absolute values and percentages. The data of the patients were collected on proforma that includes demographic, obstetric, and other details. All the pregnant women were followed-up till delivery and the outcome of the baby was examined in the neonatal period by a neonatologist. All the new born were without any congenital malformation and infections.

Pregnancy outcomes as of preterm and term deliveries were determined and adverse findings in fetus and mother including fetal congenital anomalies, PIH, and oligohydramnios, gestational diabetes noted. Pregnancy complications spontaneous abortion, stillbirth, premature rupture of membranes (PROM), fetal distress, IUGR, and intrahepatic cholestasis of pregnancy (IHCP) were compared between two study groups. The definition of the above outcome and complications of pregnancies studied were taken as per standard guidelines of the American College of Obstetricians and Gynecologists (ACOG)[18].

Exclusion criteria were patient with comorbidities as a history of diabetes, chronic hypertension, renal and liver diseases, autoimmune and metabolic disease and other medical diseases. The other criteria were the presence of congenital infection, anomalies and chromosomal abnormalities, and patient coming before, and after 11-13 weeks 6 days of gestation or having multiple gestations

The continuous variables presented as mean ± standard deviation (SD), whereas categorical variables were represented as frequency (%). Independent samples t-test was used to compare the mean age between patients. Chi-square test or Fisher exact test were used to compare the proportions between two groups. Adjacent bar diagram was used to compare the age (≤ 30 years, > 30 years) distribution of the patients between two patient groups. The p value < 0.05 was considered as statistically significant. Statistical package for social sciences, version 23 (SPSS-23, IBM, Chicago, USA) was used for statistical analysis.

Results

In this study, a total of 141 patients were included and analyzed. The control group had 126 patients (normal PAPP-A level) and the study group had 15 patients (low PAPP-A level) all were observed till delivery for outcome and complications. PAPP-A level with ≥ 0.5 MOM was considered normal (n=126, 89.3%), while levels < 0.5 MOM was marked as low (n=15, 10.7%). Mean (SD) age of the patients with study and control group were 30.11 (4.72) and 30.21 (4.73) years, respectively (p = 0.462). Similarly, proportions of patients with age ≤ 30 years were almost equal between control and study group (55.56% vs 53.30%, p=0.870) (Fig. 1).

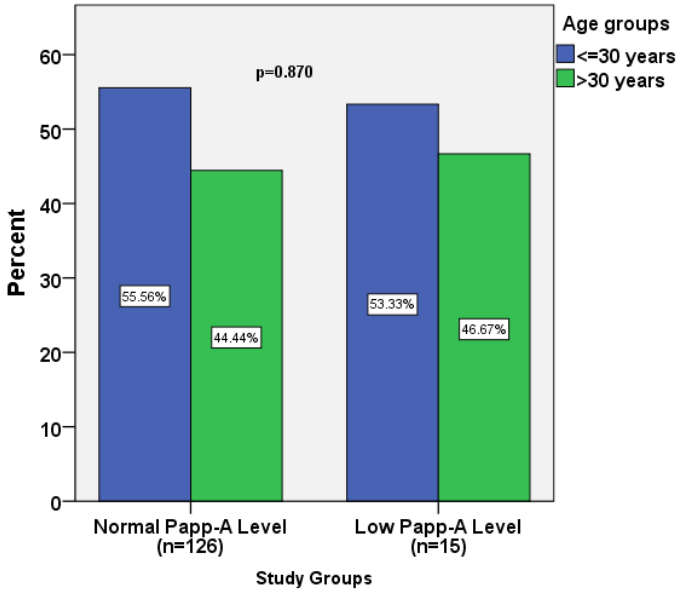

Fig. 1. Distribution of age between the study groups (low multiple of median (MOM) and control MOM).

Pregnancy outcome in the control group 80.16% patients and study group 53.33% had a delivery at ≥ 37 weeks of pregnancy (term delivery) (Table 1). The patients delivered at 28 - 36 weeks 6 days of pregnancy were 40% in the study group and were 18.25% in control group (Table 1). In the control group overall 19.84% of patients and in the study group 46.6% of patients delivered at < 37 weeks of pregnancy (preterm labor), which was significant (Table 2).

Pregnancy complications in the control group: 21 patients (16.6%) had gestational diabetes, 11 (8.73%) had IUGR and 10 (7.93%) had PIH (Table 2). In study group: 3 patients (20%) had gestational diabetes, 7 (46.6%) had IHCP, and 4 (26.6%) had IUGR (mostly asymmetrical) due to an increase in uteroplacental resistance. There was a significantly high incidence of IHCP, preterm delivery, and fetal growth restriction in the study group as compared to the control group (Table 2).

The study group patients had significant higher complications as compared to control group as IHCP (46.6% vs 14.3%, p = 0.002), IUGR (26.6% vs 8.7%, p = 0.034), preterm delivery (46.67% vs 19.84%, p = 0.017), intrauterine death (IUD) (13.3% vs 0.79%, p = 0.001) and fetal distress (13.3% vs 1.58%, p = 0.009). The patients of study group having more gestational diabetes (20% vs 16.6%, p = 0.744), both PIH and oligohydramnios (13.3% vs 7.93%, p = 0.482) and PROM (6.66% vs 0.79%, p = 0.069) that were insignificantly higher as compared to control groups (Table 2).

Table 1. Distribution of gestational age between the two groups.

Gestational age	Control group (n = 126)		Study group (n = 15)	
	Frequency	%	Frequency	%
< 28 Weeks	2	1.59	1	6.67
28-36 weeks 6 days	23	18.25	6	40.00
≥ 37 weeks	101	80.16	8	53.33

Table 2. Pregnancy outcomes between two study groups.

Pregnancy outcomes	Control group (n = 126)		Study group (n = 15)		p value
	Number	%	Number	%	
No complications	34	26.9	3	20	0.759
Gestational diabetes	21	16.6	3	20	0.741
Intrahepatic cholestasis of pregnancy	18	14.28	7	46.6	0.002
Intrauterine growth restriction	11	8.73	4	26.6	0.034
Pregnancy-induced hypertension	10	7.93	2	13.3	0.482
Oligohydramnios	10	7.93	2	13.3	0.482
Preterm delivery	25	19.84	7	46.67	0.017
Premature rupture of membranes	1	0.79	1	6.66	0.069
	1	0.79	2	13.3	0.001
Placental abruption	0	0	0	0	-
Fetal distress	2	1.58	2	13.3	0.009
Abortion	1	0.79	0	0	0.731

Discussion

In the antenatal period, if an ultrasound scan is normal, even then the possibility of adverse pregnancy outcomes cannot be ruled out[11]. A low PAPP-A level is not very sensitive test, but it is associated with more adverse pregnancy outcomes, that can be predicted with accuracy[8-13]. In this retrospective study found that low PAPP-A level was associated with a high chance of preterm delivery, IUGR, IHCP, and adverse fetal outcome. PAPP-A is synthesized by syncytiotrophoblasts in the placenta[14]. The PAPP-A, activate the IGF by releasing its binding protein from its cell receptor. The early development and vascularization of the placenta with trophoblast invasion occurs with the help of IGF[15]. When PAPP-A level is low, IGF level will be low and due to its low availability, it can lead to abortions, IUGR, PIH, IUD, preterm labor[16]. The rate of the cesarean section may be high due to fetal or maternal complications.

In this study, a total of 141 patients were included and analyzed. The control group had 126 patients (normal PAPP-A level) and the study group had 15 patients (low PAPP-A level, which is only 10.64 % of total patients). In the study group (low PAPP-A levels): more patients delivered at < 37 weeks of pregnancy (preterm delivery) as compared to the control group (normal PAPP- A level). Similar results were found by Cowan and Spencer analyzed PAPP-A in the first trimester of pregnancy without chromosomal abnormality and found a threefold increase risk of pregnancy loss with low PAPP-Alevels[17]. In another study, they find a linear relationship between low values and morphological small babies. In the first and second trimester evaluation of risk trial found that low PAPP-A were associated with more chances of pregnancy loss and other associated complications. So overall low PAPP-A levels appeared to be a strong independent marker of aneuploidy and a risk factor for spontaneous abortion but not a risk factor for structural anomalies[19] .

In the study group, patients had a higher incidence of IHCP, IUGR, fetal distress, PROM, and IUD. The gestational diabetes, PIH and oligohydramnios were also more in the study group, but statistically not significant. Low PAPP-A and the associated adverse outcomes are supposed due to poor placental function, leading to morphologic and histopathological anomalies and changes. Low PAPP-A was predictive of adverse pregnancy outcomes. The normal PAPP-A levels were almost having normal fetal growth, term delivery and favorable pregnancy outcomes, similar as per the results of this study[20, 21].

In a recent study explaining the high association of a low PAPP-A level and pregnancy outcome with complications (as pregnancy loss, IUGR, preterm delivery, PIH), as seen by this study[22, 23]. These results were also comparable to findings of low level of PAPP-A was associated with increased risk of IHCP as compared to average PAPP-A levels[24]. Physiological and hormonal changes during pregnancy, abnormal biliary transport and excretion, genetic, environmental and other multiple factors may be responsible for the pathogenesis of IHCP[25]. However, there is no specific cause is known for IHCP, it may be multifactorial and not yet fully explained. In previous studies, PAPP-A has been suggested as an early marker of IHCP development[26].

To make a strong recommendation to use PAPP-A MOM value to predict the pregnancy outcome, a large number of patients should be included, which was the limitation of this study. The more precise cut-off values of PAPP-A should be taken, that has a significant impact on pregnancy outcome. For example, Australian national policy recommends for follow-up and management of patients with low PAPP-A values[27]. With an extensive follow-up of patients with low PAPP -A levels, as a warning sign, so we can prevent an adverse pregnancy outcome and further decreasing maternal and fetal morbidity and mortality.

The PAPP-A levels measurement is a valuable marker during the first trimester screening for predicting adverse outcomes and complications. Lower the PAPP-A MOM value, higher is the chance and incidence of adverse outcomes. In present study, we found an increased chance of preterm delivery, IHCP, and IUGR in low PAPP-A group. We recommend the

larger study to establish a strong association between PAPP-A level and pregnancy outcomes.

Conflict of interest

The authors declare that there is no conflict of interest

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