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Non-invasive Prenatal Testing for Hemoglobin Bart's Hydrops Fetalis Syndrome (SEA Deletion) Using Cell-Free Fetal DNA in Maternal Plasma: Systematic Review and Meta-analysis

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KEYWORDS Fetal Cell. Hb Bart's Hydrops Fetalis. Maternal Plasma. Non-invasive Prenatal Testing. Thalassemia

ABSTRACT Non-invasive prenatal testing (NIPT) is a current technique for prenatal diagnosis. This technique uses cell-free fetal DNA in maternal plasma for detection. The objective in this study is to estimate the effectiveness of NIPT for screening of Hb Bart's hydrops fetalis syndrome (SEA deletion). A total of 343 studies were selected and reviewed. Elimination of article was done using inclusion criteria and exclusion criteria respectively, eight articles were included for full text review in this study. The combined five studies (a total of 271 samples) show the sensitivity of ninety-nine percent (95% CIs: 94-100%) and the specificity of seventy-three percent (95% CIs: 66-79%), respectively. The current study represented that NIPT of Hb Bart's hydrops fetalis syndrome (SEA deletion) is highly accurate. The most common detection methods are quantitative of detection SEA deletion fragment and informative SNPs or microsatellite of non-deleted paternal allele. These methods could be initial screening before continuing with invasive conventional methods

INTRODUCTION

Invasive Prenatal Diagnosis

Prenatal diagnosis consists of prevention and control of inherited disorders. Risk of severe disease to fetus should be investigated before birth. Conventional prenatal diagnosis involves the invasive technique of sampling fetal tissues (Wieacker and Steinhard 2010; Simpson 2012). Invasive prenatal diagnosis can be performed at 10-12 weeks or first trimester of gestation. Specimen collection for invasive prenatal diagnosis is different and depends on gestational age (Li and Yang 2017). For the first trimester, chorionic villus sampling (CVS) is performed using a catheter or needle to remove placental tissues for biopsy. Limitations of CVS sampling are suggested only between 10-12 weeks or first trimester of gestation. Risk of abortion from CVS tran-cervical sampling is greater than from trans-abdominal sampling. During the second trimester, amniocentesis (amniotic fluid) and cordocentesis (fetal blood) sampling are recommended. However, late genetic abnormalities identified for choosing pregnancy termination present greater emotional and physical risks to women than during the first trimester. Amniocentesis should be performed during 10-18 weeks of gestation because the concentration of DNA derived from amniotic cell is also less than CVS (Govender et al. 2015; Li and Yang 2017). Fetal blood sampling is preferred in cases of late investigation, more than 18 weeks of gestation. Some genetic diseases, severe thalassemia disease used the fetal blood for prenatal diagnosis from pattern of hemoglobin (Hb) type using automation detection (Srivorakun et al. 2009; Liao et al. 2012). Recent studies for prenatal diagnosis were introduced using non-invasive techniques to reduce fetal risk (Tungwiwat et al. 2007; Zafari et al. 2016; Hudecova and Chiu 2017; Skrzypek and Hui 2017; Chiu et al. 2018).

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Non-invasive Prenatal Testing (NIPT)

Non-invasive prenatal testing (NIPT) is a current technique for prenatal diagnosis of fetal

abnormalities using cell-free fetal DNA (cffDNA) in maternal plasma (Tungwiwat et al. 2007; Zafari et al. 2016; Hudecova and Chiu 2017; Skrzypek and Hui 2017; Chiu et al. 2018). NIPT is a non-invasive technique; however, it does not guarantee an affected pregnancy for positive cases. Conventional invasive techniques can confirm positive cases or those with unsure information (Hudecova and Chiu 2017). NIPT using cffDNA has been offered as a potential screening tool to increase accuracy in non-invasive prenatal diagnosis (Hudecova and Chiu 2017). Development of NIPT was established after the discovery of cffDNA in 1997 (Lo et al. 1997). Circulating fragments of cell-free DNA are short fragments of DNA found in the blood circulation of pregnant women. During pregnancy, cell-free DNA fragments that circulate in maternal plasma are also derived from both maternal and fetal cell-free DNA fragments. Short fragments consist of about 150-200 base pairs with a half-life of 4-30 minutes (Kolialexi et al. 2004; Swanson et al. 2013; Zafari et al. 2016). The cffDNA or fetal fractions are produced from fetal trophoblast cells. Fetal fractions are transferred directly to maternal circulation via the placenta. The cffDNA in maternal circulation can be detected at 4-5 weeks of gestation and increase during pregnancy, to ten percent of total plasma (Kinnings et al. 2015; Hudecova and Chiu 2017). The amount of fetal fractions relates to several factors of pregnant women including gestation age, weight, smoking status, ethnicity, fetal chromosome abnormalities and preeclampsia during pregnancy (Lo et al. 1999; Nicolaides et al. 2014; Kinnings et al. 2015; Hudecova and Chiu 2017). Shortened half-life cffDNA was completely removed in maternal circulation after birth. NIPT was offered by researcher for diagnosis fetal aneuploidies as a primary test to all pregnant women. Screening of fetal aneuploidies using cffDNA has been reported by researchers to include a paternally inherited Y chromosome, Patau syndrome (trisomies 13), Edwards's syndrome (trisomies 18) and Down syndrome (trisomies 21) (Gil et al. 2015). Monogenic disease as point mutation was also developed for NIPT including thalassemia, cystic fibrosis, and congenital adrenal hyperplasia (Vermeulen et al. 2017).

Thalassemia

Thalassemia is an inherited disorder of the globin gene resulting in the reduction or absence of globin chain production. It is the most common genetic disorder affecting about seven percent of global population. Some 300,000-400,000 infants with severe thalassemia are born each year. Genetic classification divides thalassemia into two major groups as α-thalassemia and β-thalassemia which depend on the extent of the globin gene defect (Fucharoen and Winichagoon 1992; Harteveld and Higgs 2010; Higgs and Gibbons 2010). Severe thalassemia diseases (thalassemia major) occur with no production of Hb A ($\alpha 2\beta 2$ combination) as homozygous α^0-thalassemia (Hb Bart's hydrops fetalis) or homozygous β^0-thalassemia. Compound heterozygous β^0- thalassemia and Hb E (β^E; beta globin gene mutation of codon 26) are also classified as severe thalassemia disease (Sanchaisuriya et al. 2005). The severity of thalassemia major leads to various symptoms including death after birth (Hb Bart's hydrops fetalis) and severe anemia which can be either blood transfusion dependent or non-regular blood transfusion dependent. Laws in several countries allow the termination of a fetus diagnosed with severe thalassemia during prenatal examination (Sanchaisuriya et al. 2005; Rosatelli and Saba 2009).

Hb Bart's Hydrops Fetalis Syndrome (SEA Deletion)

Infants with Hb Bart's hydrops fetalis syndrome usually die in utero, or soon after birth. This genetic defect is caused by a homozygous state of α^0-thalassemia. In Southeast Asia and Southern China, α^0-thalassemia (SEA deletion type) is the only predominant α^0-thalassemia. This defect is caused by deletion of duplicated alpha globin genes in *cis*-elements. Deletion of this α^0-thalassemia sequence extends about 20 kb from the 3' end of the $\theta 1$ gene to a region close to the third exon of the $\varphi\zeta$ gene (Fucharoen and Winichagoon 1992; Weatherall and Clegg 2001). The homozygous state is a serious public health problem in this region. Molecular techniques for defect detection are gap-PCR convention technique and real-time PCR technique (Fucharoen and Fucharoen 1994; Jomoui et al. 2017). Investigation of severe thalassemia disease was performed on the parent with a carrier of α^0-thalassemia. Conventional prenatal diagnosis of severe thalassemia was conducted by invasive techniques including chorionic villus sampling, amniocentesis and cordocentesis (Li and Yang 2017). However, several researchers reported screening Hb Bart's hydrops fetalis

syndrome caused by SEA deletion using NIPT (Hudecova and Chiu 2017).

Objective

To estimate the effectiveness of NIPT for screening Hb Bart's hydrops fetalis syndrome (SEA deletion).

METHODOLOGY

Study Design

This systematic review was conducted by multi-university researchers including WJ (Srinakharinwirot University), RK (Ubonratchathani University), and KW (Mahasarakham University). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist was also utilized (Liberati et al. 2009). A systematic review approach was conducted as follows:

Define the review title as, Non-invasive prenatal testing (NIPT) for Hb bart's hydrops fetalis syndrome (SEA deletion) using cell-free fetal DNA in maternal plasma.

Define the Review Objective and Benefit Outcomes

cffDNA in maternal plasma could be used to diagnose Hb Bart's hydrops fetalis syndrome (SEA deletion) and reduce the use of invasive techniques in prenatal diagnosis.

Define the Eligibility of the Study

This study focused only cell-free fetal DNA in maternal plasma for prenatal diagnosis of Hb Bart's Hydrops Fetalis Syndrome (SEA deletion).

Search Strategy

The researchers used keywords to search for related articles including free fetal dna and thalassemia or maternal plasma dna and thalassemia or non-invasive prenatal and thalassemia. Medical and medical sciences databases were selected as main sources including PubMed, Sciences Direct, Clinical Key and Biomedical Center.

Exclusion Criteria: Review Article, Duplicated Articles

All papers were selected using keywords and initially scanned using inclusion criteria based on title, abstract and keywords contained. WJ was the first reviewer to scan articles based on inclusion criteria and rejected articles that did not comply. Full texts of the selected articles downloaded for evaluation were evaluated by RK and KW as second reviewers based on exclusion criteria. If there was disagreement between the two reviewers, the assessment process was referred to WJ for a decision.

Data Collection

The researchers defined specific data extracted in this study including first author, published year, place of study, sample size, gestation age for specimen collection, volume of specimen collection, nucleic acid extraction method and detection method.

Data Analysis and Selection Issues

Each article was independently reviewed by two researchers (WJ and KW). Issues were classified after completing the review and data listed in one summary data table. Any disagreements resolved by RK.

Interpretation of the results (discussion/conclusion/recommendation) was performed using calculation of sensitivity, specificity, false positive and false negative of each original article. The forest plot with confident intervals for sensitivity and specificity were done. The summary receiver operating characteristics (sROC) curve was operated on overall article for meta-analysis.

RESULTS

Study Characteristics

After searching key words: free fetal DNA and thalassemia or maternal plasma DNA and thalassemia or non-invasive prenatal and thalassemia on four data bases, a total of 343 studies were selected and reviewed. Elimination of article was done using the inclusion and exclusion criteria respectively. Eight articles were included for a full text review. Figure 1 shows a flow chart of the search strategy of systematic review and meta-analysis. Non-invasive prenatal testing (NIPT) for Hb Bart's hydrops fetalis syndrome (SEA deletion) using cell-free fetal DNA was related to full text of final eight articles pub-

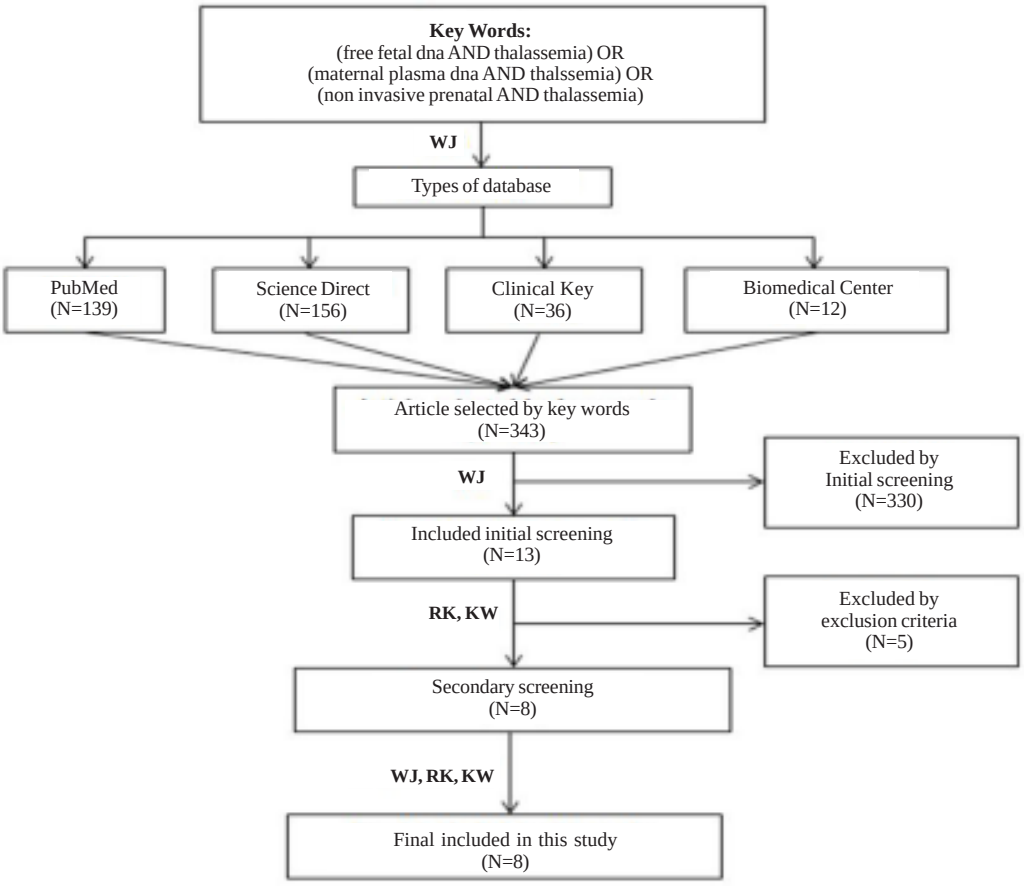

Fig. 1. A flow chart of the search strategy of systematic review and meta-analysis in this study

lished between 2006 and 2017. Place of study involved three countries including four articles from Thailand, three from China and one from Singapore (Table 1).

Study characteristics of non-invasive prenatal testing (NIPT) for Hb Bart's hydrops fetalis syndrome (SEA deletion) using cffDNA of the eight original papers is listed in Table 1. Gestation age for blood collection ranged between 8 and 31 weeks. Total maternal blood collection for the studies amounted to almost 10 ml (5 original articles). Most nucleic acid extraction methods were performed by a kit; one study did not shown the kit for extraction DNA (Pornprasert et al. 2012). Quantitative relative using real-time PCR were performed in five studies and remaining three using a sequencing analyzer. Prediction or detection methods were related to detection of SEA deletion fragment in four studies and prediction of paternal SNPs/microsatellite markers in SEA deletion breakpoints in four studies, two of these using target region capture sequencing. In total 321 samples were included with sample size ranging between 1 and 158.

Calculation of sensitivity, specificity, false positive and false negative of five original articles is shown in Table 1 (Tungwiwat et al. 2006; Ho et al. 2010; Pornprasert et al. 2010; Yan et al. 2011; Pornprasert et al. 2012). For the remaining three articles these parameters could not be calculated because –one article had only one sample and another two articles could not distinguish between Hb Bart's hydrops fetalis syndrome and non-diseases (wild type or trait) (Sirichotiyakul et al. 2012; Ge et al. 2013; Wang et al. 2017). Sensitivity and specificity of five original

Table 1: The characteristics of non-invasive prenatal testing (NIPT) for hemoglobin bart's hydrops fetalis syndrome (SEA deletion) using cffDNA. The calculation of sensitivity, specificity, false positive, and false negative in this study. NC; not calculation

	First authors	*Published year*	*Place of study*	*Gestation age for blood collection (weeks)*	*Blood collection (ml)*	*Nucleic acid extraction methods*	*Methods for detection*	*Prediction/ detection*	*ample size (n)*	*Sensitivity (%)*	*specificity (%)*	*False positive (%)*	*False negative (%)*	*Reference*
1	Tungwiwat W	2006	Thailand	8-20	2	QIAmp Blood Mini Kit	Semi-nested real-time q PCR	SEA deletion fragment	13	100	100	0	0	29
2	Sherry SY	2010	Singapore	16	6	High Pure Template DNA Purification Kit	Quantitative fluore-scence PCR (QF-PCR)	Microsatellite markers within the breakpoints	30	100	35.7	64.3	0	30
3	Pornprasert S	2010	Thailand	7-31	10	ChargeSwitch® kit technology	Taqman real-time qPCR	SEA deletion fragment	25	NC	NC	NC	NC	31
4	Yan TZ	2011	China	8-25	10	QIAamp DNA blood Mini Kit	Multiplex PCR mini sequencing technique	Paternal SNPs within the breakpoints	65	100	75	25	0	32
5	Sirichotiyakul S	2011	Thailand	12-22	10	QIAamp DNA blood Mini Kit	Taqman real-time qPCR	SEA deletion fragment	158	98.4	79.2	20.8	1.6	33
6	Pornprasert S	2012	Thailand	7-31	10	Charge Switch® kit	Semi-nested real-time q PCR	SEA deletion fragment	24	NC	NC	NC	NC	34
7	Huijuan G	2013	China	17-30	ND	Two-step centrifu-gation protocol	Target region capture sequencing	Pathogenic CNVs	5	100	100	0	0	35
8	Wenjuan W	2017	China	22	10	QIAamp Circulating Nucleic Acid Kit	Target region capture sequencing	Parental haplotype	1	NC	NC	NC	NC	36

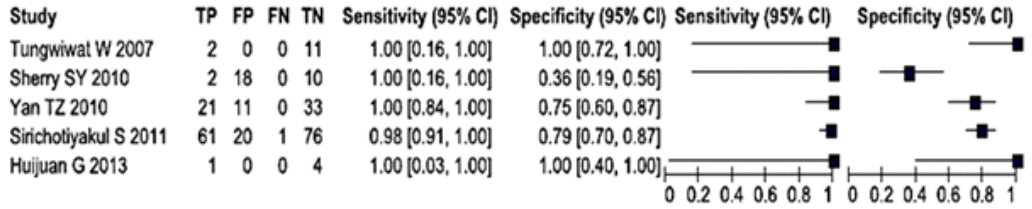

Fig. 2. The forest plot shows the variation of different studies with confident intervals for sensitivity and specificity

articles ranged between 98.4 and hundred percent, and 35.7 and hundred percent, respectively. Two out of the five studies gave hundred percent for both sensitivity and specificity. False positive was reported at 20.8 percent, twenty-five percent and 64.3 percent in three studies and false negative was reported at 1.6 percent in one study.

Meta-analysis

Diagnostic of Hb Bart's hydrops fetalis syndrome (SEA deletion) using cffDNA in maternal circulation was analyzed with ninety-five percent confident intervals (95% CIs) for sensitivity and specificity and created as a forest plot. The forest plot shows the variation of different studies with confident intervals for sensitivity and specificity that were used for meta- analysis. The summary receiver operating characteristics (sROC) curve was doneusing five articles for meta-analysis. The display shows the sensitivity and specificity of each study with different sized symbols representing different sample sizes. All analyses were performed using the Review Manager 5.3 program. Figure 2 and 3 show the statistical analyses of the diagnosis meta-analysis of Hb Bart's hydrops fetalis syndrome (SEA deletion) using cffDNA in maternal circulation. The combined five studies (a total of 271 samples) represented sensitivity at ninety-nine percent (95% CIs: 94-100%) and specificity at seventy-three percent (95% CIs: 66-79%) (Z-value = 6.338; p-value = 0.000). The sROC curve shows a ninety-five percent confident area regarding the non-invasive prenatal diagnosis of Hb Bart's hydrops fetalis syndrome (SEA deletion) using cffDNA in maternal circulation.

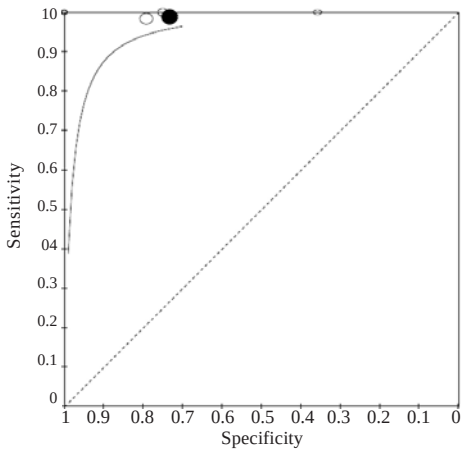

Fig. 3. Summary ROC curves with 95% confidence interval of NIPT for Hb Bart's hydrops fetalis syndrome (SEA deletion). A summary curve of the sensitivity versus specificity of cffDNA for prediction of NIPT for Hb Bart's hydrops fetalis syndrome (SEA deletion); The straight line represents the sROC curve, the round shape represents the point of the curve that corresponds to the average point estimates of sensitivity and specificity each study and the size of shape depend on sample size, the dark dot represents the summary operating point, the dashed line represents the 95% confidence area in which a new relevant study will be located

DISCUSSION

The challenge of non-invasive prenatal diagnosis involves overcoming invasive techniques in the field of hemoglobinopathy (Chiu et al. 2002). Use of cffDNA is one available choice in maternal circulation. The conceptual study mainly has focused on prediction or detection of paternal allele in a couple who were carriers of different mutations (Hudecova and Chiu 2017). Thalassemia majors including homozygous α^0-thalassemia, homozygous β^0- thalassemia and compound heterozygous β^0- thalassemia/Hb E were focused for prenatal diagnosis in Southeast Asia and Southern China (Fucharoen and Winichagoon 1992; Sanchaisuriya et al. 2005). A

systematic review and meta- analysis of beta thalassemia major indicated that sensitivity and specificity of NIPT were ninety-nine (95% CIs: 69-100%) and ninety-nine (95% CIs: 89-100%) percent, respectively (Zafari et al. 2016). Detection of paternal mutant allele using cffDNA was highly accurate in beta thalassemia major (Zafari et al. 2016; Hudecova and Chiu 2017). However, homozygous α^0-thalassemia was mostly caused by deletion in contrast with beta thalassemia. The NIPT base of homozygous α^0-thalassemia was investigated to detect SEA fragment using quantitative PCR to distinguish each genotype. Other proposes focused on informative paternal SNPs or microsatellites in non-deleted paternal allele (Hudecova and Chiu 2017).

Table 1 shows the gestation age of NIPT at between 8 and 31 weeks. However, cffDNA in maternal circulation was reported that detectable at 4-5 weeks of gestation age (Hill et al. 2012). Early detection in prenatal diagnosis of severe disease is beneficial for the patient to choose termination in severe cases. Late abortion presents greater physical and emotional risks to pregnant women (Govender et al. 2015). Homozygous α^0-thalassemia (SEA deletion) is predominant in Southeast Asia and Southern China and the studies of NIPT are also reported in Thailand, Singapore, and China (Fucharoen and Winichagoon 1992; Tungwiwat et al. 2006). Specimen collection for NIPT was recommended at 20 ml of maternal blood and cffDNA concentration amount at about 50-700 genome-equivalents for accurate NIPT analysis (Zafari et al. 2016). In this review, specimen collection was collected about 10 ml. All studies used commercial kit extraction methods appropriate for the concentration of cffDNA with no reports concerning problem specimens. In this review, two conceptual majors of NIPT in homozygous α^0-thalassemia (SEA deletion) were quantitative detection of SEA deletion fragments related to the real-time PCR method and informative SNPs or microsatellites of non-deleted paternal alleles related to sequencing analysis. Detection of SEA deletion using quantitative PCR showed higher sensitivity and specificity than detection of informative paternal alleles. However, quantitative PCR depended on the quantity of SEA deletion fragment and may result in misdiagnosis in the case of low concentration cffDNA. The use of NIPT in prenatal diagnosis should be screen tested before investigation by invasive or conventional methods. The use of informative paternal alleles could rule out non severe disease by detection of normal paternal alleles in maternal circulation. Using this method could eliminate cases before invasive testing by about fifty percent with increased accuracy and confidence in the results (Zafari et al. 2016; Hudecova and Chiu 2017).

The detection of cffDNA in maternal circulation for NIPT uses different methods. Allele specific real-time PCR is preferred in beta thalassemia mutation (Zafari et al. 2016). In contrast, SEA detection cannot use allele specific real time PCR; however, gap real-time qPCR could be available in deletion cases. The current approach recommends next-generation sequencing that the RHOD analysis could be used for multiple genomic regions with no need to optimize conditions. To decrease cost of sequencing in RHOD methods, target region capture sequencing is preferred (Lam et al. 2012; New et al. 2014). Here, two studies using target region capture sequencing represent hundred percent concordance of prenatal diagnosis of homozygous α^0-thalassemia (SEA deletion) (Pornprasert et al. 2012; Wang et al. 2017).

Meta-analysis shows diagnosis results for five articles concerning Hb Bart's hydrops fetalis syndrome (SEA deletion) using cffDNA in maternal circulation. The calculation of Z test in meta- analysis is 6.338 (p-value = 0.000) which represent the significant of the result of meta-analysis. Sensitivity of ninety-nine percent (95% CIs: 94-100%) gave similar results of systematic review to NIPT for beta thalassemia major (99% (95% CIs: 96-100%)). In contrast, the specificity in this review (73% (95% CIs: 66-79%)) was lower than systematic review of NIPT for beta thalassemia major (99% (95% CIs: 89-100%)) (Zafari et al. 2016). A systematic review of NIPT for thalassemia major, both α-thalassemia and β-thalassemia represents method of cffDNA detection and should be applied in the initial prenatal diagnosis before the adoption of invasive techniques. These methods could resolve fifty percent of cases that continue to the invasive process.

CONCLUSION

The NIPT for Hb Bart's hydrops fetalis syndrome (SEA deletion) is highly accurate. Two major detection methods are quantitative of detection SEA deletion fragments and use of infor-

mative SNPs or microsatellite of non-deleted paternal allele.

RECOMMENDATIONS

NIPT methods should be applied in the initial step before the adoption of invasive techniques. These methods could eliminate severe cases about fifty percent of the total cases. Nevertheless, more studies are required for systematic review into efficient, precise, and reliable methods.

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