A survey designed study on cost effectiveness of Amnisure - Should Amnisure replace Ferning and Nitrazine as the test of choice for diagnosis of rupture of membranes?

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Abstract- Rupture of membrane (ROM) is most commonly diagnosed clinically with physical examination; however, approximately 20% of women present very subtly, thus, making diagnosis difficult. An accurate diagnosis is important as it will influence subsequent management and care plan. The current diagnostic standard uses ferning and/or nitrazine, which performs at very low cost but with a low specificity value. This is a survey-designed study, information collected are used to estimate cost in introducing Amnisure in a selective fashion and for every patient with query PROM, to inform better decision making when using in triage unit at The Women's Hospital.

Methods- From March 14, 2015-April 14 2015, data will be gathered from triage unit in Women's Hospital including women that presents with query SROM. An assessment by medical staff if an Amnisure kit would have been useful in establishing diagnosis

Results- We determined from this study that approximately 25%-37.5% of PROM would benefit from Amnisure use in diagnosis of PROM. The cost of Amnisure per year is $7192.8-$10,789.2 in selective use and $28,171.8 in standard use.

Conclusion- Amnisure is an accurate method for diagnosing rupture of fetal membranes with high sensitivity, specificity, negative predictive and positive predictive values. Amnisure also concluded in this study to be cost effective.

Introduction

Premature rupture of fetal membranes (PROM) is the disruption of fetal membranes prior to the onset of labor. This is a common complication in obstetrics that occurs in approximately 10% of pregnancies [1]. If this disruption of membranes occurs prior to 37 weeks of gestation, it is then defined as preterm premature rupture of membranes (PPROM) and this represent one third of all premature births [2].

Despite the number of occurrences each year, for many, the etiology of PROM and PPROM remains unknown. The amniochorionic membrane forms when amnion and chorion concomitant fuses during the first trimester [1]. This amniochorionic membrane then fuses to decidua capsularis and decidua parietalis for the remaining course of the pregnancy. Any cause of weakening of this membrane prior to onset of labor will lead to PROM or PPROM. Women's lower genital tract can serve as a reservoir to many bacteria that causes bacterial vaginosis, including *Gardenerella vaginialis, ureaplasma urealyticum, Bacteroides, and Mycoplasma hominis*, which can ascend the cervical tract through the opening os and invade the

choriodecidual space [3]. Monocytes located in the fetal membrane and decidua are activated to produce inflammatory cytokines and proteolytic enzymes like collagenases and elastase. The cytokines triggers production and release of prostaglandins which cause inflammation and contractions, while the proteases acts to attack and cause weakening of local membranes [1,3]. There are several factors found to be associated with PROM, some of these include local inflammation and ascending infection, poor maternal nutrition, mechanical stress, maternal smoking, and collagen deficient syndromes [4]. Specifically in cases of PPROM, choriodecidual inflammatory syndrome (CoDIS) is found to be strongly associated as a leading etiology [4]. There are also risk factors that causes spontaneous PPROM, an intra-amniotic fluid infection and placental abruption that occurs prior to term may cause increased level of proteases release into the surrounding amniotic fluid and decidual tissues which lead to membrane rupture. However, the actual reason for PPROM remains unknown [1].

During the course of pregnancy, fetus is surrounded by the amniotic fluid throughout its growth in the uterus. The amniotic fluid production can reach approximately 1 L by the end of pregnancy course, and is contained by a sac made up of two membranes, amnion and chorion [5]. These membranes usually maintain intact until pressure from the labor builds high enough causing it to burst, usually near the end of labor. The two membrane sac serve to protect the baby from infections and acts as a cushion to prevent trauma from the contractions. Rupture of the membranes can occur at any remote site, but more often it occurs near the internal cervical os [5]. When this membrane ruptures, it is most commonly described as a gush of fluid released from the uterus. The amount of amniotic fluid released varies from 50ml to more than 300ml, this difference depends on the amount of fluid produced in the sac and how well the head of fetus

comes through the pelvis [1]. If the head fits through the pelvis tight, it will act like a plug and contain most of the fluid in the sac until post-delivery.

In PPROM, the membrane ruptures prior to onset of labor between 23-37 weeks gestation [6]. On presentation, we look at the gestational age, whether maternal/fetal infection is present, maternal cervical status, and the well-being of fetus, fetal lung maturity. These will guide the key decision making, which is to induce labor, or perform Cesarean section, or carry out pregnancy expectantly. Gestational age has an inverse relationship with neonatal morbidity, therefore, premature fetus will benefit from prolongation of pregnancy [6, 7]. However, this benefit needs to be weighted with risks of PPROM. Latency is the time interval from rupture of membrane to onset of labor [6]. As latency period increases in length, the risk of maternal and fetal infection increases as well [6, 7]. There are many factors that can affect the latency period; these include gestational age, degree of oligohydraminos, myometrial thickness, number of fetuses, and pregnancy complications such as placental abruption, intra-amniotic infection, or active labor. Once the membrane ruptures, there is no barrier protection between fetus and the outside environment, thus, the risk of having an infection in the fetus or the mother increases directly with the length of latency period [7].

Bacterial Group B Streptococcus (GBS) transmission and colonization from mother to fetus occurs most often after membrane rupture and it is the most common cause of neonatal sepsis [8]. We may be able to reduce risk of baby developing neonatal sepsis by reducing delay between membrane rupture and delivery of baby. In management of women with PPROM who are GBS colonized, we can decrease the risk of neonatal sepsis by immediately induce the delivery post PPROM. A study from the Royal College of Obstetricians and Gynecology looked at GBS colonization in woman with PPROM between 34-37 weeks of gestation [8]. They found 14% of

the women enrolled in the study were colonized with GBS and the risk of these women having a baby with neonatal sepsis was 15.2%. However, this percentage is much reduced to 1.8% in women with PPROM and GBS colonization and have had immediate induced delivery. In women present with positive GBS, or when results are unknown, prophylactic IV antibiotics of ampicillin 2 g are administered every 6 hours for total of 48 hours [8]. Other studies indicate prophylactic antibiotics are usually not administered in PPROM with gestational age less than 26 weeks [8].

In PROM at term gestation or greater than 37 weeks of gestation, an assessment is done to confirm ROM, determine gestational age, fetal position, well-being of fetus and maternal evaluation for medical and obstetric complications. In uncomplicated term PROM, the subsequent management is to induce labor or perform Cesarean section in order to reduce rate of infection, prevent risks of cord prolapse, cord compression, and cord abruption [9]. A randomized trial study done in 2006 by Dare et al. compared pregnancy outcome of planned intervention versus expectant management and planned intervention resulted in fewer maternal infections (6.8% versus 9.8% chorioamnionitis, 2.4% versus 8.3% endometritis), fewer neonatal intensive care unit admissions (12.6% versus 17%) and possible reduction in neonatal infection [10].

Diagnosis of rupture of membranes can be difficult in those present with small amount of amniotic fluid or intermittently passing amniotic fluid. Majority of women present with obvious amount of fluid leaked from the cervix and visualizing pooling of amniotic fluid in the posterior vaginal fornix on speculum examination. However, not all rupture of membranes have obvious visual signs for diagnosis [2]. Approximately 20% of women with membrane rupture are subtle and have intermittent leakage of amniotic fluid [2]. These cases are often tricky to make

diagnosis of ruptured membrane based on visualization alone. Conventional bedside use of Nitrazine paper testing on sample vaginal fluid showing a pH greater than 6.5, or visualization of ferning under microscope on air-dried microscope slide can confirm rupture of membranes, but both methods yield a high false-negative and false-positive results[11]. False-negative results can occur if the sample collected is contaminated with semen, blood, dilution with vaginal fluid, or presence of bacterial vaginosis; whereas presence of topical antiseptics can produce false-negative results caused by contamination of fingerprints on the microscope slide [11].

Preterm deliveries are associated with an increased risk of fetal morbidity and mortality [12]. Thus, it is critical to accurately diagnose rupture of membranes in order to allow proper obstetrical care and intervention to occur. Accurate diagnosis also prevents against unnecessary interventions and potential harms to both maternal and fetus such as hospitalization and medication intake [12].

Amnisure, is a newly developed easy to use, non-invasive diagnostic test of PROM and PPROM that combines both high sensitivity with low false positive results [13, 14]. It is designed as a dipstick format highly sensitive for detecting presence of placental alpha microglogulin-1, a specific protein present in the amniotic fluid. PAMG-1 was selected as a marker to use in Amnisure due to its unique characteristic present in high levels in amniotic fluid, low level in blood, and extremely low in cervico-vaginal secretions [14]. The test contains highly sensitive monoclonal antibodies that is designed to detect as low as 5ng/ml of PAMG-1 in cervico-vaginal secretion after a rupture of membranes [13]. Amnisure swab is inserted into vagina for 1 minute, then swab is rinsed in the solvent provided and then a test strip is dipped into the solvent for 5-10 minutes before the results get interpreted. If one line present on strip, this indicate negative PROM, two lines indicate a positive PROM, and no line on strip indicate invalid test [13].

Amnisure has been studied by many researchers and it yields a low false positive result. According to Ibrahim's study, Amnisure has a sensitivity of 97.33% and specificity of 98.67% in detecting PROM, and a positive predicative value (PPV) of 98.64% and negative predictive value (NPV) of 97.37% [15]. It is much more sensitive when compared to conventional standard of tests, ferning with 84% sensitivity and 78.67% specificity and Nitrazine test with 86.67% sensitivity and 81.33% specificity. Lee Si Eun and colleagues also found that PAMG-1 was more accurate than the conventional methods for PROM detection [16]. Tagore et al. and Chen et al concluded Amnisure to have the highest sensitivity, specificity, NPV, as well as PPV than other methods of PROM detection [14].

The objective of this study is to estimate the cost effectiveness of using Amnisure in all cases versus in selective cases of premature rupture of membrane diagnosis, via a data collection survey which documents the use of Amnisure within a one month period, from March 15, 2015 to April 15, 2015, at The Women's Hospital in Winnipeg, Manitoba.

Method

This study conducted search of database in PubMed, EM Base, Cochrane, and Scopus to identify all published study articles up to December 2014 relating the subject of Amnisure, with limitation of human studies, without language restrictions and using a combination of the following predefined search terms: Placental Alpha Microglobulin-1, PAMG-1 test, Amnisure, Spontaneous Rupture of Membranes, Preterm Premature Rupture of Membranes, Preterm Rupture of Membranes.

All study articles were reviewed and selected based on abstract content and any articles funded by Amnisure Company were excluded from this study. All articles selected were published in the year 2011- Dec 2014.

This survey study was collected in a 32 day period; from March 15th to April 15th; from the triage unit in Women's Hospital at Winnipeg. The Women's hospital Data gathered include all women that presents with query PROM. Data collected include gestational age, history of rupture of membranes, whether there is obvious fluid in the vagina, result of ferning test if performed, if a referral to fetal assessment unit was done, whether the patient went on to induction, and an assessment by medical staff if an Amnisure kit would have been necessary. This data is then entered into a spreadsheet and analyzed statistically to come up with an estimated cost of Amnisure in the one month period with selective use versus standard use for all women presenting with PROM.

Results

From an initial 35 identified manuscripts, all titles and abstracts were screened to identify any repeated or non-specific content, leading to a total of 15 relevant articles included for use in this study.

A sample size of 35 were captured in this survey study, out of these 35, 25.71% presented with query ROM (26 people were documented as obvious SROM and 9 people documented as query SROM). Out of the 35 people, 20 people presented with query PROM, 2 unknowns; and 4 out of the 20 people presented with query PPROM. In total, Amnisure was documented to be useful in clinical decision making for determining ROM 22.86% of the cases and useful 50% of the cases in determining PPROM. 25%-37.5% of the cases documented Amnisure as useful were similar

in conditions, all presented with poor history of SROM, not in labor, no visible pooling and negative or difficult ferning result.

The cost of a single Amnisure kit listed by Amnisure is $49.95 [17], the total cost for the 8 cases where Amnisure was documented to be useful in determining ROM in this study is $399.6. There were 5 cases where Amnisure was thought to be useful in making a clinical decision for query PROM, and the cost for this selective use in determining PROM in this study is calculated to be $249.75. From delivery report of Health Sciences Women's Hospital, there were a total of 467 deliveries in the month of March in 2015 [18]. If 10% of these pregnancies presents with PROM [1], and we were to use Amnisure for each case of PROM diagnosis this would require 47 (46.7) Amnisure kits and an estimated cost of $2347.65 for the month of March 2015. Since we determined from this study that approximately 25%-37.5% of PROM would benefit from Amnisure use in diagnosis of PROM, we can estimate 25%-37.5% of the total 47 Amnisure kits be used selectively in making diagnosis- 12 (11.75)- 18 (17.63) Amnisure kits, this corresponds to $599.4-$899.1 for March 2015 when used selectively in PROM diagnosis.

Table 1. Amnisure documented to be useful in clinical decision making of ROM

Gestation Complete Week	In Labor	Good History-of SROM	Visible Pooling	Ferning	Admission
32	No	Yes	Yes	No	Yes
35	No	No	Yes	Yes	Yes
37	No	No	No	No	No
38	No	No	No	No	No
39	Yes	Yes	No	N/A	Yes
39	Yes	Yes	No	Yes	Yes
40	No	Yes	Yes	Yes	Yes
40	No	No	No	Yes - On 2nd test	Yes

Table 2. Documented query PROM

Gestation Complete Weeks	Good History of SROM	Visible Pooling	Ferning	Induction	Admission	Amnisure useful in clinical decision making
n/a	No	No	No	Yes	Yes	No
32	No	No	No	No	No	No
32	Yes	Yes	No	No	Yes	Yes
35	No	Yes	Yes	Yes	Yes	Yes
36	Yes	No	No	No	No	No
37	No	No	No	No	No	No
37	No	No	No	No	No	Yes
38	No	No	No	No	No	No
38	No	No	No	No	No	No
38	Yes	No	No	No	No	No
38	No	No	No	No	No	Yes
39	Yes	Yes	Yes	No	Yes	No
39	Yes	N/a	N/a	Yes	Yes	No
39	Yes	No	No	No	No	No
40	Yes	N/a	N/a	Yes	Yes	No
40	No	No	Yes	No	Yes	Yes
40	Yes	N/a	N/a	Yes	No	No
41	Yes	Yes	Yes	Yes	Yes	No
41	Yes	Yes	Yes	No	No	No
41	Yes	No	Yes	No	No	No

Discussion

Main Findings

The analysis of results reported here show that Amnisure is most useful in clinical diagnosis of

mothers presenting with query PROM when they are not in labor, does not have a good history

of SROM, no visible pooling and negative or difficult ferning, confirmed with three out of the

eight cases in this study. This correspond to a cost of $599.4-$899.1 when Amnisure is used

selectively for clinical diagnosis of PROM in March 2015, versus $2347.65 when it's used for

everyone presenting to triage with query PROM. The study acknowledge that this is a subjective

survey study, with no parameters or guidelines on filling out the survey form, so my finding should be validated before they are applied in clinical practice.

Strengths and Limitations

One strength in this study being this is a survey designed study, Amnisure itself was never used, all the information gathered comes from third party resources, unrelated or funded by Amnisure Company. This study and its result is not in any way under the influence of Amnisure Company. Second strength of this study is all the parameters are reproducible; the study method can be easily replicated if the study needs to be repeated. Another strength of this study is that the survey evaluate effects of treatment in real-world settings; a positive result can inform the practice because it provides evidence that the intervention is effective in its practice.

The major limitation of this study is that the data collected are subjective surveys with limited information. There was no guidelines stating how or who should be filling out these surveys. Data collected was done in triage unit by inconsistent triage nurses, each have their own understanding of the survey form. Some information were filled out incompletely, which we have disregarded in this study. Also not all women presented with query SROM were documented on the survey, this study only captured a very small sample size of all the query SROM presented to triage. Thus any discrepancies in documentation can influence the results significantly. Another major limit of this study is due to experimental conditions. The study unfortunately was unable to gain approval from Research Ethics Board (REB) due to time limitation; therefore, we weren't able to gain access to patient's old chart to review and verify information collected in the survey. If to repeat this experiment again, I would obtain approval from REB and look into each patient chart to gather the missing information missed on the survey and document whether or not Amnisure was actually used along with its result and any

intervention that occurred for the patient. I would also set guidelines to ensure consistency in filling out the survey in order to produce more complete and accurate data along with larger sample size in the study.

Interpretation of results

"With rupture of membranes, **the clock of infection starts to tick**; from this point on isolation and protection of the fetus from external microorganisms virtually ceases…Fetal mortality, largely due to infection, increases with the time from rupture of membranes to the onset of labor." (Shubeck et al., 1966) [22]

Even with advancement in health care and medical precaution developed today, infection is still high at risk for women presenting with PROM, the longer they wait the higher the risk of infection and other complications involving the mom and fetus. Thus it's essential for proper diagnosis of ROM upon presentation, however diagnosis of ROM remains difficult when there is no clear leakage or pooling of amniotic fluid. In these uncertain cases, a tool like Amnisure, where it's specific, non-invasive and able to be performed at bedside becomes useful in detecting ROM. Besides Amnisure, there are other tools developed to detect biochemical markers in cervical discharge to help diagnose uncertain ROM such as alpha-fetoprotein, vaginal prolactin, fetal fibronectin, beta-subunit of human chorionic gonadotropin, creatinine, urea, lactate, and insulin-like growth factor binding protein-1 (IGFBP1). However, none of these biomarker turned out to be the optimal marker for ROM, as some are gestational age dependent, like fetal fibronectin or require a more invasive speculum examination such as insulin-like growth factor binding protein-1 [21]. A study done by Tagore et al. in 2010 has demonstrated that Amnisure performed equal or even superior in detecting ROM than IGFBP1 rapid strip test (Actim PROM) with sensitivity of 92.7% versus 87.5% and a specificity of 00% versus 94.4% [14]. This result

was confirmed in a more recent study by Montse et al. in 2014, which concluded Amnisure showed a higher specificity and positive predictive value than Actim PROM [13].

Amnisure is proven to be useful in diagnosis of PROM but because it is an expensive piece of equipment, this study aim to examine the cost of Amnisure in selective use versus standard use for all women presenting with PROM. At present the standard test for ROM diagnosis is Nitrazine and Ferning, the cost for each test is approximately $0.18 and $0.13 respectively [19, 20]. The current standard tests are cheap to use but they also carry low sensitivity, specificity and a high false positive value. This can lead to missed diagnosis involving complications and risks when presenting with an undiagnosed ROM; and false positive diagnosis of ROM which cause unnecessary interventions, like hospitalizations, and procedures like Cesarean-section or induction of labor. In both cases, it will generate a substantial amount of cost in medical care and hospitalizations for the health care system which could be avoided with use of a more accurate diagnostic tool. When this amount is compared to the cost of Amnisure in one year, $7192.8-$10,789.2 in selective use and $28,171.8 in standard use, Amnisure tests appears less costly in either case.

Conclusion

Amnisure is an accurate method for diagnosing rupture of fetal membranes with high sensitivity, specificity, negative predictive and positive predictive values. This survey study analyzes the cost of using Amnisure in Women's Hospital here in Winnipeg if used selectively to be $599.4-$899.1 versus $2347.65 when it's used for all women presenting to triage with query PROM in a one month period.

Appendix

Gestation Complete Weeks	Good History of SROM	Visible Pooling	Ferning	Induction	Admission	Amnisure useful in clinical decision making
n/a	No	No	No	Yes	Yes	No
32	No	No	No	No	No	No
32	Yes	Yes	No	No	Yes	Yes
35	No	Yes	Yes	Yes	Yes	Yes
36	Yes	No	No	No	No	No
37	No	No	No	No	No	No
37	No	No	No	No	No	Yes
38	No	No	No	No	No	No
38	No	No	No	No	No	No
38	Yes	No	No	No	No	No
38	No	No	No	No	No	Yes
39	Yes	Yes	Yes	No	Yes	No
39	Yes	N/a	N/a	Yes	Yes	No
39	Yes	No	No	No	No	No
40	Yes	N/a	N/a	Yes	Yes	No
40	No	No	Yes	No	Yes	Yes
40	Yes	N/a	N/a	Yes	No	No
41	Yes	Yes	Yes	Yes	Yes	No
41	Yes	Yes	Yes	No	No	No
41	Yes	No	Yes	No	No	No

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