Conservative Management of Ectopic Pregnancy: A Provincial Hospital Case Series of Medically Managed Ectopic Pregnancies

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Background: Conservative treatment of ectopic pregnancy using methotrexate for medical management has been considered an alternative to surgical intervention.

Objective: To present and discuss cases of ectopic pregnancies seen in our institution from 2007 to 2010 successfully treated using single dose methotrexate.

Methods: All patients diagnosed with ectopic pregnancy seen in our institution from 2007 to 2010 who met our selection criteria for medical treatment were included in the study. Cases were included only when the diagnosis was made either on serial βhCG or with ultrasonographic features of an unruptured ectopic pregnancy: 1) an empty uterine cavity, 2) adnexal mass <3 to 4cm with no fetal heart activity and 3) no free fluid in the cul de sac. Our criteria for medical therapy include a hemodynamically stable clinical condition, no evidence of rupture on ultrasound, normal liver and renal function and patients’ compliance with follow-up. Majority of our patients were treated on an outpatient basis using 50mg single dose intramuscular methotrexate and treatment response measured using serial βhCG monitoring measured on days 4 and 7 and weekly thereafter until they were less than 5mIU/ml. Repeat blood count, liver and renal function tests were carried out on days 4 and 7. Success of medical management was defined as the resolution of the βhCG level to less than 5mIU/ml. Surgical intervention for any reason was viewed as failure of treatment.

Results: All of the nine cases of unruptured ectopic pregnancies treated using 50mg single dose intramuscular methotrexate were successfully resolved without need for surgical intervention. There was a significant reduction in serum βhCG after the single dose of treatment with minimal side effects. Complete βhCG resolution was achieved (100% success rate) in all medically treated cases with a minimum of 4 days and a maximum of 35 days of serial βhCG monitoring.

Conclusion: Conservative management using single dose methotrexate is a safe and effective option to surgical intervention in the treatment of unruptured ectopic pregnancies. However, it is reserved for patients that satisfy the strict criteria for medical treatment. This mode of treatment offers minimal side-effects, has the advantage of avoiding invasive surgery and a cost effective method of treatment.

Key words: ectopic pregnancy, conservative management, methotrexate

In most instances, an egg is fertilized in the fallopian tube then travels to the implantation site and anything that interferes with the implantation of the ovum in the endometrial cavity could predispose to the development of an ectopic pregnancy. Ectopic pregnancy refers to any pregnancy occurring outside of the uterine cavity.1

Ectopic pregnancy is currently the leading cause of pregnancy-related deaths during the first trimester, accounting for 10% of all maternal deaths.2-3 Locally, annual statistics revealed that cases of ectopic pregnancy increased from 13% in 2005 to 17% in 2009.4 In our institution, the incidence is 1.3-1.5% per year based on a 5-year review of our statistics.5 Mortality rate associated with ectopic pregnancy is 10% from the world literature2-3 and 0.01% to 0.03% in the Philippines.6 In addition to the immediate mortality and morbidity caused by this condition, the woman’s future ability to reproduce may be adversely affected as well. Luckily, as its prevalence has increased, mortality and morbidity have declined.
due to the development of new diagnostic modalities and earlier detection and high index of suspicion in cases of ectopic gestation.\textsuperscript{7,8}

Several case reports over the past few decades revealed that the management of ectopic pregnancy has been revolutionized.\textsuperscript{7,8,9,11,14,15,16,19,20,21} This was brought about by the emergence of several non-surgical options to what had once been thought to be a solely surgically treatable condition. A high index of suspicion coupled with early clinical diagnosis supported by transvaginal ultrasound and quantitative baseline serum $\beta$-hCG has paved the way for increased chances of success of medical treatment thereby minimizing the morbidity, mortality and financial burden created by this health problem.\textsuperscript{7,8,9,10,11}

A successful medical treatment of a case of a cornual pregnancy done in our institution\textsuperscript{12} has geared us toward a more conservative approach, in accordance with recommendations from previous studies.\textsuperscript{7,8,9,10,11} We began to treat cases of unruptured ectopic pregnancy medically using single dose methotrexate injection and monitoring patient’s response to chemotherapy using serum $\beta$hCG assay monitoring and serial ultrasound. The objective of this paper is to present cases of unruptured ectopic pregnancy in our institution successfully treated with single dose methotrexate. The specific objectives are: a) to review adherence to guidelines for the medical treatment of ectopic pregnancy, and b) to present a viable option of conservative treatment of ectopic especially for patients desirous of preserving future fertility.

**MATERIALS AND METHODS**

All patients seen at our institution diagnosed with ectopic pregnancies from 2007 to 2010 who met the selection criteria for conservative treatment were included in the study and to ensure that all patients were identified, the admission list to the Gynecology Ward was also checked. Cases were included only when the diagnosis was made either on serial $\beta$hCG or on ultrasonographic features of unruptured ectopic gestation: 1) an empty uterine cavity, 2) adnexal mass <3 to 4cm with no fetal heart activity\textsuperscript{8,15,16,17} and 3) no free fluid in the cul de sac.\textsuperscript{8}

Our criteria for medical therapy included a stable clinical condition, no evidence of hemoperitoneum on ultrasound, normal liver and renal function, and patient reliability for follow-up. In all of our cases, conservative treatment included the following: management options were meticulously explained to the patient and relatives. Informed consent was secured in all of our subjects. Baseline laboratory values for renal and hepatic as well as baseline serum $\beta$hCG were determined before initiating therapy. Our patients were treated with 50mg single dose intramuscular methotrexate, majority on an outpatient basis, depending on the fall of $\beta$hCG levels following the protocol introduced by Pisarska in 1998 (Index A). $\beta$hCG levels were then measured on days 4 and 7 and weekly thereafter until they were less than 5 mlU/ml. Repeat full blood count and liver and renal function tests were carried out on days 4 and 7. A successful medical treatment was defined as the resolution of the $\beta$hCG level to less than 5 mlU/ml. Treatment failure was defined as the need for surgical intervention for any reason.

**Selection Criteria**

- Positive pregnancy test, hemodynamically stable patient, baseline serum $\beta$hCG <15000mlU/ml, ultrasound finding of an unruptured ectopic gestation <3 to 4cm with no fetal heart rate activity\textsuperscript{8,15,16,17,18}

- Cases were reviewed by our consultants

- Conservative treatment was explained to the patient and informed consent was secured

- Patient fully understands treatment and compliant to follow up

**RESULTS**

A series of 9 unruptured ectopic pregnancies were managed at our institution over this period. All said cases fulfilled the criteria for methotrexate therapy. None of the cases needed surgical intervention after medical intervention with single dose methotrexate.

Table 1 summarizes the profile of our index patients treated with single dose intramuscular methotrexate. Majority of our patients belonged to the reproductive age group, mostly primigravid and primarily developed tubal ectopic gestations. Average size on ultrasound scanning done on all our cases were all less than 4cm. Baseline quantitative serum $\beta$hCG, on the other hand, were all below 15000mlU/ml and fulfilled our selection criteria for methotrexate administration.
Table 2 depicts the number of doses of methotrexate given in each case. Only the second case of recurrent ectopic pregnancy required a re-treatment with single dose methotrexate. To date, all of our patients treated with single dose methotrexate are still in contact with our institution except for 2 cases that opted to settle abroad.

Our very first case of conservative management of a single dose treatment of intramuscular methotrexate was for a cornual pregnancy. A 33% reduction in serum $\beta$hCG was observed 4 days after medical treatment later followed by a 75% decrease in $\beta$hCG levels on day 7 with return to undetectable levels on day 35. She remained asymptomatic and began having normal regular monthly menstrual cycles 3 months post methotrexate treatment. Repeat scan 5 months after conservative treatment revealed complete disappearance of the cornual mass. Confirmatory hysteroscopy 6 months post treatment revealed a normal uterine cavity with patent tubal ostia. Patient was advised for a hysterosalpingography (HSG) for confirmation of tubal patency, however, she later settled abroad with her husband.

The second case was of a recurrent ectopic pregnancy previously managed conservatively using methotrexate. She had a negative pregnancy test with absent sonologic and clinical findings after her first medical treatment. She opted to undergo a non-surgical treatment for the second time since she was undesirous of surgical intervention. Serial $\beta$hCG level declined by more than half from baseline on day 7 with return to negligible levels on day 28. Transvaginal scan on day 28 revealed complete disappearance of the recurrent ectopic mass.

**Table 1. Summary profile of patients treated conservatively with single dose methotrexate.**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Gravidity/Parity</th>
<th>Location of Ectopic Gestation</th>
<th>Size of Ectopic</th>
<th>Baseline Serum $\beta$hCG (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>G4P2 (2012)</td>
<td>Cornual</td>
<td>3.6cm x 3.4cm x 3.4cm</td>
<td>1430</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>G2P0 (0010)</td>
<td>Recurrent Tubal</td>
<td>1.3cm x 1.2cm x 1.2cm</td>
<td>1184</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>G4P2 (2012)</td>
<td>Cesarean Scar</td>
<td>3.0cm x 2.5cm</td>
<td>15.9</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>G2P0 (0010)</td>
<td>Tubal</td>
<td>2.2cm x 1.7cm</td>
<td>314</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>G1</td>
<td>Tubal</td>
<td>3.26cm x 1.9cm</td>
<td>874.9</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>G2P1 (1001)</td>
<td>Tubal</td>
<td>2.2cm x 2.1cm x 1.5cm</td>
<td>305.7</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>G1</td>
<td>Tubal</td>
<td>2.7cm x 2.2cm x 1.9cm</td>
<td>137.1</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>G2P1 (1001)</td>
<td>Tubal</td>
<td>2.3cm x 3.3cm x 1.7cm</td>
<td>269.2</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>G1</td>
<td>Cervical</td>
<td>1.2cm x 1.2cm x 0.9cm</td>
<td>49.8</td>
</tr>
</tbody>
</table>

**Table 2. Summary of outcome of conservative treatment.**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Number of Dose of Methotrexate</th>
<th>Decline in Serum $\beta$hCG on Day 4</th>
<th>Decline in Serum $\beta$hCG on day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Cornual Pregnancy)</td>
<td>single</td>
<td>33%</td>
<td>75%</td>
</tr>
<tr>
<td>2 (Recurrent Ectopic Pregnancy)</td>
<td>single</td>
<td>*</td>
<td>54%</td>
</tr>
<tr>
<td>3 (Cesarean Scar Pregnancy)</td>
<td>single</td>
<td>67%</td>
<td>89%</td>
</tr>
<tr>
<td>4 (Tubal Pregnancy)</td>
<td>single</td>
<td>*</td>
<td>60%</td>
</tr>
<tr>
<td>5 (Tubal Pregnancy)</td>
<td>single</td>
<td>*</td>
<td>77%</td>
</tr>
<tr>
<td>6 (Tubal Pregnancy)</td>
<td>single</td>
<td>52%</td>
<td>91%</td>
</tr>
<tr>
<td>7 (Tubal Pregnancy)</td>
<td>single</td>
<td>25%</td>
<td>58%</td>
</tr>
<tr>
<td>8 (Tubal Pregnancy)</td>
<td>single</td>
<td>*</td>
<td>100%</td>
</tr>
<tr>
<td>9 (Cervical Pregnancy)</td>
<td>single</td>
<td>94%</td>
<td>100%</td>
</tr>
</tbody>
</table>

* Day 4 $\beta$hCG not done
resolution of the tubal ring. Patient was later placed on oral contraceptives for the next 6 months and had resumption of menses a month post methotrexate treatment.

The third case was of a cesarean scar pregnancy successfully treated with single dose methotrexate. She presented with vaginal bleeding after a period of amenorrhea and a positive pregnancy test. Ultrasound done revealed a highly vascular complex mass in the anterior wall of the lower uterine segment with loss of myometrial wall between the mass and the bladder wall. Both the uterine cavity and cervical canal were empty. A diagnosis of gestational trophoblastic neoplasia versus CS scar pregnancy was entertained. However, baseline \( \beta hCG \) geared a consideration of a CS scar pregnancy more than gestational trophoblastic neoplasia. Serial \( \beta hCG \) levels reached normal levels on day 4 after medical treatment. Repeat ultrasound done 14 days post treatment showed disappearance of the mass previously described.

The fourth case of conservative treatment was seen at our emergency room due to a history of on and off vaginal spotting. Ultrasound done revealed a normal size anteverted uterus with a complex mass lateral to the right ovary, ectopic gestation considered. Repeat \( \beta hCG \) on day 7 revealed a 60% decrease from baseline reaching normal levels on day 17 post treatment using single dose methotrexate. Repeat ultrasound on day 22 revealed complete disappearance of the previously seen mass.

Our fifth case of medical treatment of methotrexate was of a G1, suffering from primary infertility for 4 years with polycystic ovarian syndrome diagnosed with an unruptured ectopic pregnancy by ultrasound. The patient and her husband had been trying to conceive and even underwent infertility work-up. Our patient had a hysterosonosalphingography (HSSG) last September 2009 with normal results. Semen analysis done on her husband last 2008, on the other hand, revealed normal findings as well. Ultrasound revealed a left adnexal mass, ectopic pregnancy considered and a normal sized anteverted uterus with thickened endometrium. Quantitative \( \beta hCG \) done on day 4 revealed a 77% decrease from baseline reaching normal levels on day 21 after conservative treatment. Undetectable levels of \( \beta hCG \) were seen on day 91. She and her husband are presently trying to conceive their first child.

Our sixth case of conservative treatment was of a G2P1 (1001), 28 year old seen at our institution last April 2010. Ultrasound done revealed an ectopic gestation at the left adnexal mass measuring 2.2cm x 2.1cm x 1.5cm and she consented to medical treatment of methotrexate. There was a noted 52% decrease from baseline of \( \beta hCG \) with resolution of \( \beta hCG \) levels on day 33. Ultrasound done 3 months post methotrexate treatment revealed a live intrauterine pregnancy of 7 weeks and 4 days with no adnexal masses appreciated in the said scan.

The seventh and eighth cases of unruptured ectopic were all tubal pregnancies, all of which had remarkable decline in serum \( \beta hCG \) after medical treatment of single dose methotrexate.

Our last case was of a conservative treatment of a cervical pregnancy on a 14 year old with a history of heavy menstrual bleeding for 2 months later complicated by severe anemia. Patient was diagnosed with dysfunctional uterine bleeding and started on oral contraceptive medications by a private physician. Patient was later brought to our institution for anemia correction however routine pregnancy tests done on admission revealed a positive result. Ultrasound done on the patient revealed a normal size anteverted uterus, thickened endometrium which appears decidualized with a cystic-like structure within the cervix measuring 1.2cm x 1.2cm x 0.9cm for which a cervical pregnancy was considered. The patient and her parents consented for medical treatment using methotrexate. Serial monitoring of serum \( \beta hCG \) done on day 4 revealed normal results. Repeat serum \( \beta hCG \) on day 10 were of undetectable levels.

Table 3 presents the time taken for resolution of \( \beta hCG \) with a minimum of 4 days and maximum length of 35 days.

<table>
<thead>
<tr>
<th>Case</th>
<th>( \beta hCG ) Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Cornual Pregnancy)</td>
<td>Day 35</td>
</tr>
<tr>
<td>2 (Recurrent Tubal Pregnancy)</td>
<td>Day 28</td>
</tr>
<tr>
<td>3 (Cesarean Scar Pregnancy)</td>
<td>Day 4</td>
</tr>
<tr>
<td>4 (Tubal Pregnancy)</td>
<td>Day 17</td>
</tr>
<tr>
<td>5 (Tubal Pregnancy)</td>
<td>Day 21</td>
</tr>
<tr>
<td>6 (Tubal Pregnancy)</td>
<td>Day 33</td>
</tr>
<tr>
<td>7 (Tubal Pregnancy)</td>
<td>Day 25</td>
</tr>
<tr>
<td>8 (Tubal Pregnancy)</td>
<td>Day 7</td>
</tr>
<tr>
<td>9 (Cervical Pregnancy)</td>
<td>Day 4</td>
</tr>
</tbody>
</table>
Ectopic pregnancy presents a major life threatening condition for reproductive age women. It is the result of a flaw in human reproductive physiology that permits the conceptus to implant and mature outside the endometrial cavity, which ultimately ends in death of the fetus and can be a devastating situation for the mother if without timely diagnosis and treatment.

Historically, the treatment of ectopic pregnancy has been limited to surgery because cases were usually diagnosed in an emergency situation. However, to minimize the morbidity, mortality and financial burden created by this rapidly growing health problem, non-surgical alternatives are increasingly being investigated. Due to the emerging studies and evolving experience with methotrexate, the treatment of ectopic pregnancy has been revolutionized. Conservative management of ectopic pregnancy is appealing over surgical intervention for a number of reasons that include eliminating morbidity from surgery and general anesthesia, potentially less tubal damage, less cost and need for hospitalization. The overall success rate of medical treatment using methotrexate in properly selected women is nearly 90%. Conservative treatment with methotrexate is a especially attractive option when the pregnancy is located on the cervix, ovary, interstitial or cornual portion of the tube since all are often associated with increased risk of torrential hemorrhage resulting in hysterectomy or oophorectomy. Our case series supports the use of methotrexate as a safe and highly effective alternative treatment for unruptured ectopic pregnancies.

If the diagnosis of ectopic pregnancy can be made earlier non-invasively, conservative treatment with systemic intramuscular methotrexate is an alternative option after meticulously informing patients about the risks and benefits of the available treatment options. As recommended by our selection criteria, the following should be satisfied: 1) hemodynamically stable women, 2) unruptured ectopic gestation, 3) no signs of active bleeding, and 4) low initial serum hCG concentrations. The classical triad of ectopic pregnancy has become less common when good facilities for early diagnosis are available. Early ectopic gestations tend to be smaller and have lower baseline hCG levels, thus more time is available for conservative management with comparable outcomes to that of surgical treatment. There is an inverse association between hCG levels and successful medical treatment of an ectopic pregnancy. In viable pregnancies, hCG levels rise in a curvilinear fashion until they plateau at approximately 100,000mIU/ml with mean doubling time of 48 hours. With ectopic pregnancies, levels of hCG rise at much slower rates. In a systematic review by Menon, et al. of 503 women, it confirmed that there is a substantial increase in failure rate when the initial hCG is above 5000mIU/ml. Relative contraindications to treatment are hCG levels greater than 5000mIU/ml, fetal cardiac activity, large ectopic size greater than 3cm to 4cm, and sonographic findings of free peritoneal fluid. On the other hand, absolute contraindications to treatment include documented hypersensitivity to methotrexate, hemodynamically unstable patient, coexistent viable intrauterine pregnancy, breastfeeding, renal or pulmonary disease, blood dyscrasia and peptic ulcer disease. Severe side effects from methotrexate treatment are usually related to long term treatment use such as in cancer treatments. It is important to counsel the patients for prolonged follow up, need for a second injection or emergency surgery and distinct likelihood of increased pelvic pain. None of the mentioned serious side effects in the literature were seen in all of our patients. In most series, more than half of all patients experienced increased abdominal pain occurring 2 to 3 days after methotrexate injection which is believed to be caused by the separation of the pregnancy from the implanted site. It can be differentiated from tubal rupture in that it is milder, of limited duration and is not associated with signs of acute abdomen or hemodynamic instability. Fortunately for all of our cases, none needed a follow up dose of intramuscular methotrexate and none needed emergency laparotomy. Only two of our patients complained of mild abdominal pain later relieved spontaneously.

Tanaka, et al. first described the successful resolution of ectopic pregnancy with systemic methotrexate in 1982. Forty-five percent of all ectopic pregnancies were managed with methotrexate and it effectively treated ectopic pregnancy in 82%-90% of selected cases. In a review by Slaughter and Grimes of 17 studies including 400 patients, the overall success rate was 90%. Approximately, 5% of patients required surgery for failed treatment as compared to 15% in another study. In our institution, we geared to a more conservative management of ectopic pregnancy as most of our patients included in the treatment are
amenable to medical intervention and desirable of future fertility.

The most commonly used approach is the single dose methotrexate protocol. The average success rates for the single dose regimen are reported to be from 88% to 94%. The recommended dose is 1mg/kg/BSA, administered intramuscularly at a dose of 50mg/m² after normal laboratory work up. Serum βhCG is measured on days 1, 4 and 7. A second dose is given if serum βhCG level declines less than 15% between days 4 and 7. Serum βhCG is measured weekly once the level declines by 15% or more between days 4 and 7, until the level is less than 15mlU per milliliter. It can be noted that four of our cases had failure to follow up on day 4 however repeat serum βhCG done on day 7 revealed declining levels. Data from a study of βhCG values on days, 1, 4 and 7 suggest that day 4 serum βhCG levels is not an accurate test to predict treatment success. It is common to note an increase in βhCG levels in the first several days post treatment. This can be attributed to the continue hCG production by the syncitiotrophoblast despite cessation of production by the cytotrophoblast. Studies have also shown that up to 50% of ectopic gestational masses that are treated medically with methotrexate and monitored ultrasonographically increase in size and these masses may persist even if serum βhCG levels have decreased to <15mlU/ml. These masses should not be interpreted as treatment failure since these are probably resolving hematomas rather than persistent trophoblastic tissue. Weekly βhCG monitoring is continued until the level is undetectable and studies have shown that βhCG resolution usually declines to less than 15mlU/ml by day 35 post treatment but may take as long as 109 days. Although it is called single dose methotrexate protocol, approximately 20% of women require more than one cycle of treatment. Luckily, for all of our cases, a follow-up dose was not needed since declining levels of serum βhCG were observed after only a single dose of methotrexate. In a study done by Stovall and Ling, 133 patients (94%) were treated successful, 4(3.3%) of whom needed a second dose and no adverse effects were encountered. In the largest single center series done in 1999 by Lipscomb and colleagues, they reported a 91% success rate in 350 women given methotrexate therapy, 80% of whom required only a single dose. Fortunately, for all our nine cases, all resulted to 100% success rate.

In a study done by Barnhart, et al., 14% of patients who received single dose regimen needed two or more doses and 10% of those who received multiple dose received a single dose. A recent meta-analysis including data from 26 trials demonstrated the success with the single-dose regimen to be 88.1% while the success with the multiple dose regimen was 92.7%. A small randomized clinical trial also demonstrated the single-dose regimen to have a slightly higher failure rate. Although multiple dose protocols appear to cause more adverse side effects. The single dose regimen is less expensive, requires less intensive monitoring and does not require folinic acid rescue. It is for this reason that we adapted the single dose regimen and results were successful. The overall success rate of conservative treatment for both single and multiple dose protocols is about 90% reported in the literature. In a systematic review of two randomized trials comparing single dose with multiple dose regimen revealed that there was no significant difference between both regimen which ranged from 89-91% for single dose therapy and 86-93% for multidose treatment. A hybrid protocol, involving 2 equal doses of methotrexate (50mg/m²) given on days 1 and 4 without the use of leucovorin has been shown to be an effective and convenient alternative to the existing regimens with success rates reported at 87%. In all our cases, a 100% success rate was achieved.

The evidence in the literature supporting treatment of ectopic pregnancy with subsequent reproductive outcome is limited mostly to observational data and randomized trials comparing the various treatment options. Determination of successful treatment and future reproductive outcome with diverse treatment alternative is often influenced by selection bias. Comparing expectant management of ectopic pregnancy to a patient who was treated conservatively using methotrexate or to a patient who had a laparoscopic salpingectomy is complicated. A patient who presents with vaginal spotting, no abdominal pain and a low baseline βhCG level that is decreasing in value may be managed expectantly or medically. However, patient who presents with hemodynamic instability, an acute abdomen, and high initial βhCG levels must be treated surgically. These two patients probably signify different degrees of tubal damage and comparing their future reproductive outcomes would be flawed. There is no evidence however of adverse effects of methotrexate treatment of ectopic pregnancy on future pregnancies and it does not...
appears to compromise ovarian reserve. Attempts to conceive may be resumed after hCG level is undetectable although it is not surprising that ectopic pregnancy can be followed by infertility and recurrent ectopic gestations with the incidence approximately 15% rising to 30% following two ectopic pregnancies. Studies have shown that the risk of recurrence is both for surgical and medical interventions. To date, only one of our nine cases successfully treated with methotrexate had a recurrent ectopic pregnancy successfully treated again conservatively and one had an intrauterine pregnancy and we are presently closely monitoring her pregnancy.

The average successful pregnancy rates using the multiple dose regimen are in the range of 91-95%, demonstrated by multiple investigators. One study of 77 patients desiring subsequent pregnancy showed intrauterine pregnancies in 64%, and recurrent ectopic pregnancy occurred in 11%. Other studies have demonstrated similar results, with intrauterine pregnancy rates ranging from 20-80%. On the other hand, the average success rates for the single-dosage regimen are reported to be from 88-94%. In a study by Stovall and Ling, 87.2% of patients achieved a subsequent intrauterine pregnancy, whereas 12.8% experienced a subsequent ectopic pregnancy. Other studies have reported similar results with some mild adverse effects and lower reproductive outcomes. The success rates of intrauterine pregnancies after conservative treatment are comparable with laparoscopic salpingostomy, assuming the selection criteria mentioned above are observed.

Studies done on the effect of systemic methotrexate on pregnancy suggests that the threshold dose of methotrexate required to produce defects is 10mg weekly and that the vulnerable period of gestation is between 6 and 8 weeks. Methotrexate is widely distributed in body tissues, the highest concentrations being in the kidneys, gallbladder, spleen, liver and skin after administration. Its presence in the liver has been reported up to 116 days after exposure, although the amount of drug retained does not appear to be related to the dose received. There is thus, a theoretical risk of fetal exposure in babies of mothers who were given the drug up to 4 months prior to conception. It is for this reason that we are closely monitoring all our patients especially our sixth case who conceived three months post methotrexate treatment. Patients wishing to continue with their pregnancy following exposure to methotrexate in the first trimester should be informed that there is a chance of abnormality in the fetus on the basis of data from cases of methotrexate exposure. Women planning to continue the pregnancy should be offered treatment with folinic acid for at least 5 months in order to minimize methotrexate effects on the fetus. Methotrexate treatment is unlikely to have a major effect on short or long term fertility in men and women, but a washout period of 6 month cessation of treatment prior to conception is advisable to prevent the small chance of chromosomal abnormalities in offspring.

CONCLUSION

Ectopic pregnancy remains to be a potentially life threatening predicament in a woman’s life. As the ability of a physician to diagnose ectopic pregnancy improves, early invention and prompt treatment is indeed possible. Therefore, serious complications are avoided and a woman’s future fertility is preserved. We have presented this paper to emphasize that conservative treatment of ectopic pregnancy is an attractive option for the management of selected cases of ectopic pregnancy. Medical management is an appealing alternative since it is associated with reduced morbidity from surgery, potentially less tubal damage, less cost and need for hospitalization. It is indeed noteworthy to conclude that intramuscular single dose methotrexate is a safe and cost effective option to surgical intervention in selected cases of ectopic pregnancy. The results presented are promising and shows conservative management using methotrexate a viable option in clinically stable patients desirous of preserving future fertility. It should always be emphasized, in employing this mode of treatment, that rigorous monitoring of physician and compulsive compliance of patients are keys to successful treatment.

LIMITATIONS OF THE STUDY

Our study may suffer from some problems inherent in any case series review. Since our report is based solely from the patients seen from 2007 to present, the limited number of subjects in a single center such as our institution would not allow accurate estimation of risks of morbidity and mortality.
RECOMMENDATIONS

Large multicenter studies should be advocated to determine further the impact of conservative treatment of ectopic pregnancy using systemic methotrexate. Comprehensive follow up of successfully treated cases is recommended to determine future pregnancy outcome.

REFERENCES

5. Bulacan Medical Center, Department of Obstetrics and Gynecology Annual Census 2005 to August 2010
6. Maternal Mortality, leading causes, number and rate per 1,000 livebirths 5 year average (2003-2007) and 2008. www.doh.go.ph
A Randomized Double-blind Controlled Trial of Oral Probiotics versus Oral Metronidazole for the Treatment of Symptomatic Bacterial Vaginosis Among Non-Pregnant Women

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Objective: A randomized double blind controlled trial was undertaken to compare the efficacy of oral probiotics and oral metronidazole in the treatment of symptomatic bacterial vaginosis (BV) among non-pregnant women.

Methods: A total of 66 patients were included in this investigative study. Thirty five patients were assigned to oral metronidazole (500mg twice a day for 7 days) and 31 patients to oral probiotics (1 capsule three times a day for 10 days). Symptoms of abnormal vaginal discharge, vaginal pruritis, vaginal burning sensation, and dyspareunia were recorded. Diagnosis of BV was based on Amsel's criteria: homogenous vaginal discharge; pH of vaginal fluid >4.5; positive whiff test; and presence of clue cells. Patients were seen 10 days from initiation of treatment. Review of symptoms and Amsel's criteria were re-evaluated including a Gram stain for Nugent's score.

Results: There were significantly more patients still with symptoms of abnormal vaginal discharge and presence of homogenous vaginal discharge on examination in the oral probiotics group (77.8% vs 28.57%, P = 0.001, RR = 5.25). Based on the Nugent's score, there were significantly more patients evaluated with positive results for BV in the oral probiotics group (77.14% vs 33.33%, P = 0.001, RR = 7.4) after treatment. Giving oral probiotics shows a relative risk of 309%, a negative RRR and a negative ARR.

Conclusion: Based on resolution of symptoms and Amsel's criteria, oral metronidazole was superior to oral probiotics. Oral probiotics were not beneficial in eradicating the organisms causing BV. Oral probiotics should not be offered as treatment for bacterial vaginosis.

Key words: bacterial vaginosis, probiotics, Lactobacillus

Bacterial vaginosis (BV) is the most common cause of genital discomfort in women of reproductive age. It has been associated with various gynecological and obstetrical complications including pelvic inflammatory disease, post partum endometritis, premature rupture of membrane, chorioamnionitis, and preterm labor.1

Bacterial vaginosis is an infection of the female genital tract characterized by both decreased or absent H₂O₂-producing Lactobacillus sp. and increased concentrations of potential pathogenic bacteria. It is the result of alterations in the vaginal ecosystem, the environment of the vagina shifts from a predominance of Lactobacilli to a predominance of anaerobic bacteria (e.g. Prevotella sp. Mobiluncus sp.), G. vaginalis, and Mycoplasma hominis. Moreover, the characteristic changes include high vaginal pH, formation of clue cells, odor due to increased vaginal fluid concentrations of amines, polyamines, and organic acids, an up regulation of inflammatory cytokines, absence or rare presence of white blood cells in the vaginal discharge, and a decrease in naturally protective molecules like secretory leukocyte protease inhibitor.2,3,4

Among symptomatic non-pregnant women, the established benefits of treatment are to relieve vaginal symptoms and signs of infection and reduce the risk for infectious complications after abortion or hysterectomy. Other potential benefits of treatment might include a reduction in risk for other infections such as HIV and other STDs. All women who have symptomatic disease require treatment. Recommended regimens include: metronidazole 500mg orally twice
a day for seven days or metronidazole gel, 0.75%, one full applicator (5g) intravaginally, once a day for five days or clindamycin cream, 2%, one full applicator (5g) intravaginally at bedtime for seven days. The recommended metronidazole regimens are equally efficacious. Alternative regimens include clindamycin 300mg orally twice a day for seven days or clindamycin ovules 100mg intravaginally once at bedtime for three days.2

Treatment of this condition using recommended antibiotics is often associated with failure and high rates of recurrence. This led to the concept of replacing the depleted lactobacilli using probiotic strains as treatment approach.5 Several studies have evaluated the clinical and microbiologic efficacy of using lactobacillus intravaginal suppositories to restore normal flora and treat BV. However, no currently available lactobacillus suppository was determined to be better than placebo one month after therapy for either clinical or microbiologic cure.2

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.”6 Probiotic doses are usually standardized in terms of the amount of living bacteria per unit of volume.7 The concept of probiotics came from belief that a disrupted microflora in the host could be restored by exogenous application of bacteria commonly found in that niche.8

In terms of probiotics specifically designed for women’s health, by far the most evidence comes from work on Lactobacillus rhamnosus GR-1 in combination with Lactobacillus fermentum B-54 and RC-14. These organisms are antagonistic to the growth and adhesion of various intestinal and urogenital pathogens that include Gardnerella vaginalis. Of greater importance, the administration of the lactobacilli by mouth and intravaginally has been shown to be safe and reduce the risk of urinary tract infection, bacterial vaginosis and yeast infection.9

Lactobacillus are normally present in the vagina and those strains producing hydrogen peroxide and other inhibitory substances are widely assumed to offer protection against the overgrowth of pathogens. Restoration of a normal vaginal flora has been tried by others with some degree of success, as reviewed by Sieber and Dietz.10 A number of probiotic products have been thoroughly researched and good clinical evidence of their efficacy is currently available. According to Sobel and Reid and Heinemann women with chronic or recurrent bacterial vaginosis associated with diminished vaginal lactobacilli would be prime candidates for biotherapy.11,12 Hiller, et al. found a relationship between the presence of hydrogen peroxide-producing lactobacilli and a reduced risk of bacterial vaginosis in pregnant women.13

Efforts have been made to identify suitable Lactobacillus strains for urogenital use. Reports show that Lactobacillus rhamnosus GR-1 and Lactobacillus fermentum RC-14, which colonize the intestine and vagina, administered orally restore and maintain uro vaginal health.8

Certain Lactobacilli strains can safely colonize the vagina after oral and vaginal administration, displace and kill pathogens including Gardnerella vaginalis and Escherichia coli, and modulate the immune response to interfere with the inflammatory cascade. Additional attributes of probiotics include their potential to degrade lipids and enhance cytokine levels. There is a strong case to be made that hydrogen peroxide (H2O2) production is a key factor in resisting bacterial vaginosis. The H2O2 had been shown to be toxic to BV-causative agents, namely, Gardnerella vaginalis and Prevotella bivia.

The mechanism of action of probiotic lactobacilli begins by adhering to the vaginal epithelium and interfering with pathogen adhesion, invasion/ translocation, growth and survival. Probiotic lactobacilli enhance anti-inflammatory cytokins, via the intestine and vagina, that block the pathway to COX-2 and prostaglandins and increases lgA which in turn inhibits pathogen colonization. Lactobacilli reduce the pH to make the vaginal environment more conducive to lactobacilli growth and better able to prevent BV recurrence. If certain strains of probiotic lactobacilli are given orally, there may be additional benefits such as degradation of lipids and increase in conjugated linolenic acid as well as modulation of inflammation and reduction in pathogen emergence from the rectum to the vagina.9

The actual mechanisms of action of probiotics in the vagina have not been proven and are probably multifactorial. The production of lactic acid, bacteriocins, and hydrogen peroxide are important. Modulation of immunity and cell-to-cell communication is another probable mechanisms of action.15 Mastromarino, et al. analyzed ten Lactobacillus strains belonging to four different Lactobacillus species for properties relating to mucosal
colonization. Adherence to epithelial cells varies greatly among the *Lactobacillus* species and among different strains belong to the same *Lactobacillus* species.¹⁶

In a prospective randomized study by Anukam, results showed cure of BV in significantly more probiotic treated subjects compared to metronidazole.

On the other hand, in a systematic review done by Senok, et al., analysis suggested beneficial outcome of microbiological cure with the oral metronidazole/probiotic regimen and the probiotics/estriol preparation. There was no conclusive evidence that probiotics were superior to or enhance the effectiveness of antibiotics in the treatment of BV. There was insufficient evidence to recommend the use of probiotics either before, during or after antibiotic treatment as a means of ensuring successful treatment or reducing recurrence. Larger, well-designed randomized controlled trials with standardized methodologies were still needed to confirm the benefits of probiotics in the treatment of BV.⁵

The oral capsules were formulated after the discovery that *Lactobacillus* strains GR-1 and RC-14 can be ingested daily, pass through the gut, and ascend from the rectum to colonize the vagina and/or enhance the indigenous vaginal lactobacilli numbers.¹⁴ There were no failures but based on ongoing studies, they estimated that at least 50% to 90% of women would have healthier vaginal flora within 1-2 weeks of treatment.¹⁴ Moreover, daily oral ingestion of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 significantly improved the vaginal flora, lowering the yeast and coliform counts compared with placebo.¹⁸

With the recent advent of probiotics in the local market, it is important to evaluate these probiotics, their *Lactobacillus* strain composition and their role in maintaining female urogenital health.

**General Objective**

To compare the efficacy of oral probiotics and oral metronidazole in the treatment of symptomatic bacterial vaginosis among non-pregnant women.

**Specific Objectives**

- To compare the efficacy of oral probiotics and oral metronidazole in the treatment of symptomatic bacterial vaginosis among non-pregnant women according to symptomatic relief, Amsel’s criteria and Nugent’s score.
- To be able to identify any adverse reaction to oral probiotics and oral metronidazole in the treatment of symptomatic bacterial vaginosis among non-pregnant women.

**Definition of Terms**

**Oral Probiotics**

Oral probiotics used in this study is of a formula that was meticulously processed using natural-temperature fermentation for five years rendering bacteria of high potency which are able to live longer. It is vegetable-based and non-diary making it suitable for vegetarians and those who are intolerant of lactose or allergic to casein (protein) in milk. The oral probiotic formula contains twelve strains of natural live lactic acid bacteria proven to be 6.25 times stronger than others. There are 59 million live and viable friendly bacteria per soft gel. The oral probiotic formula was manufactured at an encapsulation company which manufactures pharmaceutical products in compliance with strict Japanese Good Manufacturing Practices (GMP). Recommended regimen for bacterial vaginosis: two capsules once a day for two weeks or one capsule three times a day for ten days; the capsule must be taken before meals.¹⁹

**Oral Metronidazole**

Metronidazole is a nitroimidazole medication, an antibiotic, amoebicide and antiprotozoal. Oral metronidazole regimen for bacterial vaginosis is 500mg orally twice a day for seven days.²

**Bacterial Vaginosis**

Bacterial vaginosis (BV) is a polymicrobial clinical syndrome resulting from replacement of the normal H₂O₂-producing *Lactobacillus* sp. in the vagina with high concentrations of anaerobic bacteria (e.g. *Prevotella* sp. and *Mobiluncus* sp.) *G. vaginalis*, and *Mycoplasma hominis*. BV can be diagnosed by the use of clinical criteria or Gram stain.
Definition of Outcome Measures

Symptoms

Patients commonly come in for abnormal or malodorous or foul-smelling vaginal discharge; vaginal pruritus or vaginal irritation; vaginal burning sensation, a symptom caused by vaginal irritation; and dyspareunia or pain on sexual intercourse.

Amsel’s Criteria

Amsel’s criteria require three of the following signs or symptoms: 1) vaginal discharge - homogenous, thin, white discharge that smoothly coats the vaginal walls; 2) pH of vaginal fluid > 4.5 - the vagina is normally slightly acidic (with a pH of 3.8 - 4.2) which helps control the bacteria. A pH > 4.5 is loss of acidity and a positive result for bacterial vaginosis; 3) positive whiff test - a fishy odor of vaginal discharge before or after addition of 10% KOH; and 4) presence of clue cells - a classic finding on the wet smear showing vaginal epithelial cells with clusters of bacteria adherent to their external surfaces. The bacteria give the clue cells a granular or stippled appearance by obscuring their cellular borders.2,3

Nugent’s Score

When a Gram stain is used, determining the relative concentration of lactobacilli (long Gram-positive rods), Gram-negative and Gram-variable rods and cocci (i.e., G. vaginalis, Prevotella, Porphyromonas, and peptostreptococci), and curved Gram-negative rods (Mobiluncus) characteristic of BV is considered the gold standard laboratory method for diagnosing BV. In this scale, a score of 0-10 is generated from combining three other scores: 0-3 is considered negative for BV; 4-6 considered intermediate; and 7+ is considered indicative of BV. At least 10-20 high power (1000 x oil immersion) fields are counted and an average determined.2,20

MATERIALS AND METHODS

This was a prospective randomized double blind controlled trial conducted at the Department of Obstetrics and Gynecology of our institution from June 2009 to January 2010. Permission to conduct the study was obtained from the Ethics Committee of the Department of Obstetrics and Gynecology. Included in the study were non-pregnant women coming in for consult at the Out Patient Clinic for abnormal vaginal discharge. Inclusion criteria were patients aged 18 years or more, eligible to give informed consent and able to comply with treatment requirements who were consulting for abnormal vaginal discharge, may be malodorous or foul-smelling or non-foul smelling. Included in the study were those diagnosed with bacterial vaginosis by Amsel’s criteria (requires three of the following signs or symptoms: 1) homogenous vaginal discharge as seen on speculum examination; 2) pH of vaginal fluid >4.5 using colometric strips; 3) positive whiff test after addition of 10% KOH; and 4) presence of clue cells on wet smear as read by a registered medical technologist blinded to clinical findings). Patients were excluded if any of the following is present: menstruation; amenorrhea with possible early pregnancy; last normal menses more than two weeks prior to consult (might not be able to complete the course of treatment due to subsequent menstrual cycle); post abortion status; history of antibiotic treatment in the last two weeks; or with mixed or confounding vaginal infection such as Candida or fungal infection.

If the patient fulfills the inclusion criteria and none of the exclusion criteria on consult, the patient were asked to read and understand the subject information sheet which explains the study objectives, scientific rationale, reasons for treatment, method of administration, possible risks, discomfort and inconveniences that may occur. Adverse effects of both experimental and control therapies were explained. Conditions for withdrawal from the study were likewise explained. Patients were dropped from the study if there was sexual intercourse, onset of menstruation or use of vaginal douche during the course of treatment and thus patients were advised...
Oral Probiotics versus Oral Metronidazole for the Treatment of Symptomatic Bacterial Vaginosis Among Non-Pregnant Women / Cotaco and Zamora

Once included in the study and after obtaining the informed consent, patients were randomized using drawing of lots to one of the two treatment groups. Group A - oral metronidazole given as 500mg/tab twice a day for 7 days and Group B - oral probiotics given as 1 capsule three times a day for 10 days. Allocation to the treatment group was undertaken by an independent senior resident (not involved in the investigation). All oral medications have been transferred into similar sterile plastic containers, labelled according to the letter of group assignment and packed in brown paper bags by an independent senior resident which also included an instruction sheet regarding oral medication intake and details of return appointment. Neither the OPD resident/primary investigator nor the patient was aware of the randomization. The primary investigator and the assigned registered medical technologist who read the wet smears and did the Nugent’s score were blinded to the group assignments.

Baseline demographic data were collected. A detailed review of the past and present medical histories was conducted. Symptoms were recorded accordingly: burning sensation; and dyspareunia. Clinical diagnosis based on Amsel’s criteria was likewise tabulated. A patient’s database form was filled up. Patients were given their complete treatment supply of oral medications during the initial consult with an instruction sheet regarding oral medication intake and details of return appointment inside the brown paper bag. Follow up for all patients was after 10 days from start of treatment.

On follow up consult with the primary investigator, a review of history was conducted with details on the symptoms. Physical examination which included speculum exam and evaluation for Amsel’s criteria was again performed. Wet smear for determination of clue cells were sent to the same registered medical technologist (blinded to the treatment allocation). For confirmation of presence or absence of bacterial vaginosis after treatment, specimen (vaginal discharge or vaginal wall swab/smear) for Gram stain was also sent to the same registered medical technologist for Nugent’s scoring (gold standard).

The database form for each patient was completed at the end of the study. The specific drug administered to each patient was not known until all the data were collected and recorded. The intake of the oral medication was discontinued in any event of adverse drug reaction such as gastrointestinal irritation, diarrhea, skin rash or skin allergic reaction, dizziness or headache or nausea and vomiting. It was only at this point that the blinding was opened to determine the drug assignment. The patient was dropped off from the completion of the study and was medically managed accordingly. Necessary medications were provided. Patients who were symptomatic and/or with bacterial vaginosis by Amsel’s criteria and/or by Nugent’s scoring were given the standard oral metronidazole regimen for bacterial vaginosis and advised to follow up accordingly.

This study should include at least 30 patients per group for a 95% level of confidence and 80% power of study. There were 35 patients assigned to Group A (oral metronidazole) and 31 patients assigned to Group B (oral probiotics).

All data were entered and recorded in MS Excel format and computation and analysis were carried out using Chi-square and Fisher’s tests were used to determine association among categorical variables. For all the tests, a 95% confidence level was considered significant (P<0.05). All statistical tests were analyzed using the SPSS software. Risk, Relative Risk (RR), Relative Risk Reduction (RRR). Absolute Risk Reduction (ARR) and Number Needed to Treat (NNT) were computed based on standard formulas and definitions.

**RESULTS**

There were a total of 66 patients included in this investigative prospectiive study who were randomized into the two treatment groups, 35 patients for the oral metronidazole group and another 31 patients for the oral probiotics group. All patients from the oral metronidazole group completed the study up follow up consult. On the other hand, there were 4 patients who dropped out of the completion of the study from the oral probiotics group, all of whom did not come back for follow up consult.

Table 2 shows the characteristics of the patients included in the study according to their symptoms and diagnosis by Amsel’s criteria. All patients came in for abnormal vagina discharge of which for both treatment groups, there were almost similar proportions of symptoms of malodorous or foul-smelling and non-foul-smelling vaginal discharge (42.85/57.14 in the oral metronidazole group and 41.93/58.06 in the oral probiotics group). For both treatment groups, the second most common symptom was vaginal pruritus of equal incidence (about 94% for both treatment groups). For the oral metronidazole
group, there were more patients with vaginal burning sensation as compared to the oral probiotics group (62.85% vs 45.16%). There was similar incidence of symptom of dyspareunia for both treatment groups. As to Amsel’s criteria, all patients had homogenous vaginal discharge. For both treatment groups, the second most common finding was pH of vaginal fluid > 4.5 although it was more common among those assigned to the oral probiotics group than the oral metronidazole group (96.77% vs 88.57%). Presence of a positive whiff test and presence of clue cells on wet mount were nearly similar in both treatment groups.

Table 3 shows the presence of symptoms among patients after their respective treatment courses. In the oral metronidazole group, 28.57% (from 100%) of the patients still had abnormal vaginal discharge but none of them complained of vaginal pruritus, vaginal burning sensation and dyspareunia anymore. In the oral probiotics group, about three fourths or 77.78% (from 100%) of the patients still had abnormal vaginal discharge and about 15%-19% still had vaginal pruritus, vaginal burning sensation and dyspareunia. The difference in resolution of vaginal pruritus, vaginal burning sensation and dyspareunia between the two groups was not statistically significant. However, as to the persistence of abnormal vaginal discharge after the treatment course, there was a statistically significant difference (P=0.001) rendering the patients from the oral probiotic group about 5 times at increased risk of still having abnormal vaginal discharge despite complete treatment. Thus, oral probiotics were not beneficial for the resolution of abnormal vaginal discharge.

Table 4 shows the result of the Amsel’s criteria of the patients after their respective treatment courses. In the oral metronidazole group, 28.57% (from 100%) of the patients still had homogenous vaginal discharge and about 6%-17% still had vaginal fluid pH > 4.5, positive whiff test and presence of clue cells on wet smear. In the oral probiotics groups, about three-fourths or 77.78% (from 100%) of the patients still had homogenous vaginal discharge and about 15-30% still had vaginal fluid pH > 4.5, positive whiff test and presence of clue cells on wet smear. The difference in resolution of the basic vaginal pH, disappearance of whiff test and absence of clue cells on wet smear between the two group

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**Table 2. Patients’ symptoms and Amsel’s criteria on initial consult.**

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Oral Metronidazole Group</th>
<th>Oral Probiotics Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 35</td>
<td>n = 31</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal vaginal discharge</td>
<td>35 100%</td>
<td>31 100%</td>
</tr>
<tr>
<td>Foul-smelling</td>
<td>15 42.85%</td>
<td>13 41.93%</td>
</tr>
<tr>
<td>Non-foul-smelling</td>
<td>20 57.14%</td>
<td>18 58.06%</td>
</tr>
<tr>
<td>Vaginal pruritus</td>
<td>33 94.28%</td>
<td>29 93.54%</td>
</tr>
<tr>
<td>Vaginal burning sensation</td>
<td>22 62.85%</td>
<td>14 45.16%</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>16 45.71%</td>
<td>16 51.16%</td>
</tr>
<tr>
<td><strong>Amsel’s Criteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogenous vaginal discharge</td>
<td>35 100%</td>
<td>31 100%</td>
</tr>
<tr>
<td>pH of vaginal fluid &gt; 4.5</td>
<td>31 88.57%</td>
<td>30 96.77%</td>
</tr>
<tr>
<td>(+) whiff test</td>
<td>23 65.71%</td>
<td>24 77.41%</td>
</tr>
<tr>
<td>Presence of clue cells</td>
<td>28 80.00%</td>
<td>24 77.41%</td>
</tr>
</tbody>
</table>

---

**Table 3. Patients’ characteristics after treatment: Symptoms.**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Oral Metronidazole Group</th>
<th>Oral Probiotics Group</th>
<th>P value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 35</td>
<td>n = 27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal Discharge</td>
<td>10</td>
<td>21</td>
<td>0.001</td>
<td>5.25</td>
</tr>
<tr>
<td>Vaginal Pruritus</td>
<td>0</td>
<td>5</td>
<td>0.013</td>
<td>–</td>
</tr>
<tr>
<td>Vaginal Burning Sensation</td>
<td>0</td>
<td>4</td>
<td>0.028</td>
<td>–</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>0</td>
<td>4</td>
<td>0.028</td>
<td>–</td>
</tr>
</tbody>
</table>
was not statistically significant. However, as to the persistence of homogenous vaginal discharge after the treatment course, there was a statistically significant difference (P=0.001) rendering the patients from the oral probiotic group about 5 times at increased risk of still having abnormal vaginal discharge despite complete treatment. Thus, oral probiotics is not beneficial for the resolution of abnormal vaginal discharge.

Table 5 shows the comparative results of the Nugent’s score that serve as the gold standard in the evaluation of presence or absence of bacterial vaginosis (BV) after their respective complete treatment courses. About three-fourths (77.14%) of patients from the oral metronidazole group were negative for BV while only 33.33% of the patients from the oral probiotics group were negative for BV. This difference was statistically significant (P=0.002) rendering the patients from the oral probiotics group about 7 times at risk of having intermediate or positive results for bacterial vaginosis despite complete oral therapy. Thus, oral probiotics was not beneficial in eradicating the organisms causing BV.

Table 6 shows the grouping of Nugent’s score of intermediate and positive results for BV against those with negative results for BV findings. The four drop out subjects (lost to follow up) from the oral probiotics group were considered not treated or still with presence of bacterial vaginosis. About three-fourths of 77.14% of the patients from the oral metronidazole group were negative for BV while only about one-fourth or 29.03% of the patients from the oral probiotics group were negative for BV.

Table 7 shows that the relative risk of having an intermediate/positive results for bacterial vaginosis on Nugent’s score post-treatment was 309%. Negative values obtained for the Relative Risk Reduction (RRR) and Absolute Risk Reduction (ARR) suggest that the treatment, oral probiotics, might have even caused harm rather than give benefits. The measures of effect size of the intervention of giving oral probiotics suggest no offering the treatment.

Table 8 shows the adverse drug reaction to treatment among the patients included in the study. There were only 3 patients (8.57%) who reported an adverse drug reaction all categorized under gastrointestinal irritation described as epigastric discomfort but tolerable enough to complete the treatment. There were no other reports of adverse drug reaction in the oral metronidazole group. There were no adverse drug reactions reported among the patients from the oral probiotics group.

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Table 4. Patients’ characteristics after treatment: Amsel’s criteria.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Oral Metronidazole Group</th>
<th>Oral Probiotics Group</th>
<th>P value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenous vaginal discharge</td>
<td>10</td>
<td>21</td>
<td>0.001</td>
<td>5.25</td>
</tr>
<tr>
<td>pH of vaginal fluid &gt; 4.5</td>
<td>2</td>
<td>4</td>
<td>0.311</td>
<td>-</td>
</tr>
<tr>
<td>(+) whiff test</td>
<td>2</td>
<td>4</td>
<td>0.311</td>
<td>-</td>
</tr>
<tr>
<td>Presence of clue cells</td>
<td>6</td>
<td>8</td>
<td>0.390</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5. Patients’ characteristics after treatment: Nugent’s score.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Oral Metronidazole Group</th>
<th>Oral Probiotics Group</th>
<th>P value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative for BV</td>
<td>27</td>
<td>9</td>
<td>0.001</td>
<td>7.4</td>
</tr>
<tr>
<td>Intermediate</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive for BV</td>
<td>3</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Presence or absence of bacterial vaginosis (BV) by Nugent’s score.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Oral Metronidazole Group</th>
<th>Oral Probiotics Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative for BV</td>
<td>27 (77.15%)</td>
<td>9 (29.03%)</td>
</tr>
<tr>
<td>Intermediate and Positive for BV</td>
<td>8</td>
<td>22*</td>
</tr>
</tbody>
</table>

* drop out patients from this treatment group were considered as not treated or still with presence of bacterial vaginosis (BV).
Discussion

Bacterial vaginosis is a polymicrobial clinical syndrome that results from the overgrowth or replacement of the normal hydrogen peroxide producing bacterial species with high concentrations or anaerobic bacteria. Patients commonly come in for abnormal or malodorous or foul-smelling vaginal discharge; vaginal pruritus or vaginal irritation; vaginal burning sensation, a symptom caused by vaginal irritation; and dyspareunia or pain on sexual intercourse.

Clinical diagnosis requires three of the following symptoms or signs: homogenous, thin, white discharge that smoothly coats the vaginal walls; presence of clue cells on microscopic examination; pH of vaginal fluid >4.5; and a fishy odor of vaginal discharge. The Nugent’s scoring, a Gram stain procedure, is the gold standard laboratory method for diagnosing bacterial vaginosis.2

In this study, all patients came in for abnormal vaginal discharge of which there were almost similar proportions of symptoms of malodorous or foul-smelling and non-foul smelling vaginal discharge. The second most common symptom was vaginal pruritus followed by similar incidences of symptoms of vaginal burning sensation and dyspareunia. As to Amsel’s criteria, all patients had no homogenous vaginal discharge. The second most common finding was pH of vaginal fluid >4.5 with presence of a positive whiff test and presence of clue cells on wet mount at almost similar incidences.

The most common recommended regimen used on an outpatient consult is metronidazole 500mg orally twice a day for seven days. Recently however, Lactobacilli have been used with varying degrees of success in the treatment of vaginal infections.21 Lactic acid and other fatty acids produced by lactobacilli may contribute to the maintenance of a low vaginal pH and the production of hydrogen peroxide by lactobacilli important in inhibiting the overgrowth of other bacterial species in the vagina.22 There is a strong case to be made that hydrogen peroxide \( \text{H}_2\text{O}_2 \) production is a key factor in resisting bacterial vaginosis. The \( \text{H}_2\text{O}_2 \) had been shown to be toxic to BV-causative agents, namely, Gardnerella vaginalis and Prevotella bivia. Therefore, it is argued that an advantage to the organisms would be gained by increased production of \( \text{H}_2\text{O}_2 \). This is the apparent basis for selection of Lactobacillus as a probiotic strain to colonize the vagina and treat bacterial vaginosis.9

Among symptomatic non-pregnant women, the established benefits of treatment are to relieve vaginal symptoms and signs of infection.

Ried, et al. studied a group comprised of 10 women whose flora were abnormal and who had suffered repeated bacterial vaginosis, yeast infections and/or UTIs. The regimen consisted of ingesting a capsule containing greater than 10^9

| Table 7. Relative Risk (RR), Relative Risk Reduction (RRR), Absolute Risk Reduction (ARR) and Number Needed to Treat (NNT) of oral probiotics as intervention. |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Relative Risk RR = EER/CER | Relative Risk Reduction RRR = CER-EER/CER | Absolute Risk Reduction ARR = CER-EER | Number Needed to Treat NNT = 1/ARR |
| (22/31) (8/35) | (8/35 - 22/31) 8/35 | 8/35 - 22/31 | 1/-0.48 |
| 0.71/0.23 | 0.71 - 0.23 / 0.23 | 0.71 - 0.23 | -2.09 |
| 309% | -2.09 | -0.48 | -2.08 |

| Table 8. Adverse drug reaction related to treatment. |
|----------------------------------|----------------------------------|
| Adverse Drug Reactions | Oral Metronidazole Group n = 35 | Oral Probiotics Group n = 27 |
| GI irritation | 3 (8.57%) | 0 |
| Diarrhea | 0 | 0 |
| Skin rash/allergic skin reaction | 0 | 0 |
| Dizziness/headache | 0 | 0 |
| Nausea/vomiting | 0 | 0 |
| None | 32 | 27 |

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viable Lactobacillus GR-1 and RC-14 bacteria each day. Vaginal irritation and other symptoms such as dysuria, frequency and urgency disappeared and the patients remained healthy for many months following this treatment regimen. Moreover, in their subsequent randomized placebo-controlled study of 64 women, daily oral ingestion of Lactobacillus rhamnosus GR-1s and Lactobacillus fermentum RC-14 significantly improved the vaginal flora, lowering the yeast and coliform counts compared with placebo.

In a prospective randomized study by Anukam, et al, 40 women diagnosed with bacterial vaginosis by discharge, fishy odor, salidase positive test and Nugent Gram scoring were randomized to receive either two direct capsules containing Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 each night for five days or 0.75% metronidazole gel applied vaginally twice a day. Follow-up at day 6, 15 and 30 showed cure of BV in significantly more probiotic treated subjects compared to metronidazole (P=0.016 at day 6; P=0.002 at day 15 abd P=0.056 at day 30). This was the first report of an effective (90%) cure of BV using probiotic lactobacilli.

Results of this study differed remarkably from that of Reid, et al. and Anukam, et al. In this study, there were significantly more patients still with abnormal vaginal discharge in the oral probiotics group as compared to the oral metronidazole group (77.78% vs 28.57%, P = 0.001, RR = 5.25). Although about 15-19% of the patients in the oral probiotics group still complained of vaginal pruritus, vaginal burning sensation and dyspareunia after treatment as compared to none from the oral metronidazole group, their difference was not statistically significant. Based on resolution of symptoms oral metronidazole was superior to oral probiotics since oral probiotics still rendered the patients at 5 times increased risk of still having abnormal vaginal discharge despite complete treatment.

As to the clinical signs of bacterial vaginosis based on the Amsel’s criteria, there were significantly more patients still with homogenous vaginal discharge in the oral probiotics group as compared to the oral metronidazole group (77.78% vs 28.57%, P = 0.001, RR = 5.25). Although about 15-30% of the patients in both groups still had vaginal fluid pH > 4.5, positive whiff test and presence of clue cells on wet smear, their difference was not statistically significant. Based on Amsel’s criteria, oral metronidazole was superior to oral probiotics since oral probiotics still rendered the patients at 5 times increased risk of still having homogenous vaginal discharge despite complete treatment.

Based on the Nugent’s score, the confirmatory gold standard test done after treatment, there were significantly more patients evaluated with positive results for BV in the oral probiotics group as compared to the oral metronidazole group (77.14% vs 33.33%, P = 0.001, RR = 7.4) after treatment. The oral probiotics were not beneficial in eradicating the organisms causing BV.

Moreover, even if there were no reported adverse drug reactions for the use of oral probiotics, the incidence in the use of oral metronidazole was low and tolerable.

Results of this study concur with the the systematic review of randomized controlled trials done by Senok, et al. (2009) They made use of probiotics for the treatment of women diagnosed with bacterial vaginosis, regardless of diagnostic method used or preparation type/dosage/route of administration but there was no conclusive evidence that probiotics were superior to antibiotics in the treatment of BV. In addition, results of their study showed that there was insufficient evidence to recommend the use of probiotics either before, during or after antibiotic treatment as a means of ensuring successful treatment or reduced recurrence. Similarly, Rosentstein, et al. conducted a joint study by scientists in UAE, Belgium and Italy, and they concluded that there was insufficient evidence to recommend the use of probiotics either before, during or after antibiotic treatment as a means of ensuring successful treatment or reduced recurrence.

In a more recent publication of Reid, it was stated that daily oral probiotic intake may prove to be a natural method for women to restore and maintain urogenital health, however, oral therapy for symptomatic bacterial vaginosis is less likely effective unless it follows an antibiotic treatment with vaginal probiotic capsules.

According to Reid, Burton, et al., the actual mechanisms of action of probiotics in the vagina have not been proven and are probably multifactorial. The production of lactic acid, bacteriocins, and hydrogen peroxide seems to be important, but these substances have not been measured in healthy women and compared with those obtained in women immediately before and during a BV event. A plausible mechanism is modulation of immunity, in bacterial vaginosis, IL-1 and IL-8 levels are elevated compared with levels in healthy (lactobacilli-dominated) vaginas. Lactobacilli have been shown to produce biosurfactants and collagen-
binding proteins that inhibit pathogen adhesion and to some extent displace pathogens.24-25 This might be a possible explanation why vaginal mucosa dominated by lactobacilli could be less receptive to pathogens. Furthermore, cell-to-cell communication is another probably mechanism of action. This involves the signalling of mucus production, which acts as a barrier to pathogens, or signalling of anti-inflammatory cytokine production.26,27,28

CONCLUSION

In this study, all patients came in for abnormal vaginal discharge with homogenous vaginal discharge on Amsel's criteria. Patients also complained of vaginal pruritus, vaginal burning sensation and dyspareunia. Findings on Amsel's criteria also included vaginal fluid pH > 4.5 with presence of a positive whiff test and presence of clue cells on wet mount.

There were significantly more patients still with abnormal vaginal discharge in the oral probiotics group as compared to the oral Metronidazole group (77.78% vs 28.57%, P = 0.001, RR = 5.25). Resolution of symptoms of vaginal pruritus, vaginal burning sensation and dyspareunia after treatment were not statistically different between the two treatment groups. Based on resolution of symptoms oral metronidazole was superior to oral probiotics. Oral probiotics were not beneficial.

As to the clinical signs of bacterial vaginosis on the Amsel's criteria, there were significantly more patients still with homogenous vaginal discharge in the oral probiotics group as compared to the oral metronidazole group (77.78% vs 28.57%, P = 0.001, RR = 5.25). Presence of vaginal fluid pH > 4.5, positive whiff test and presence of clue cells on wet smear after treatment were not statistically different between the two treatment groups. Based on Amsel's criteria, oral metronidazole was superior to oral probiotics. Oral probiotics were not beneficial.

Based on the Nugent’s score, the confirmatory gold standard test done after treatment, there were significantly more patients evaluated with positive results for BV in the oral probiotics group as compared to the oral metronidazole group (77.14% vs 33.33%, P = 0.001, RR = 7.4) after treatment.

LIMITATIONS OF THE STUDY

At our institution, Gram’s stain for Nugent’s scoring is not available and thus the registered medical technologist who participated in this investigative study was outsourced from another diagnostic unit/institution. Thus, requesting for the Nugent’s score before and after treatment would cost most and would be less feasible to be carried out. The investigators suggest that Nugent’s scoring, the gold standard in the diagnosis of bacterial vaginosis, be used at the initial assessment of the presence of bacterial vaginosis and not just as a confirmatory test after the treatment. This would confirm the presence or absence of bacterial vaginosis prior to the treatment.

RECOMMENDATIONS

Awareness of recent and ongoing research on probiotics has encouragingly changed the medical community’s perception of probiotic use and seems to be promising. The provision of good scientific evidence and clinical data and the availability of high quality probiotics are still critical. Further studies should be done to identify, recover and document the properties of Lactobacilli that make them effective probiotic agents; show their mechanisms of action that would prevent and treat disease; and determine the optimal dosage, duration and mode of Lactobacilli delivery.

More research is needed to further understand the rationale of probiotics in bacterial vaginosis. Larger, well-designed randomized controlled trials with standardized methodologies are still needed to confirm the benefits of probiotics in the treatment of bacterial vaginosis.

The investigators of this study would like to recommend further clinical studies on the potential use of probiotics in the treatment and prevention of other infections concerning urogenital health such as recurrent vaginal candidiasis and urinary tract infections.

REFERENCES

Oral Probiotics versus Oral Metronidazole for the Treatment of Symptomatic Bacterial Vaginosis Among Non-Pregnant Women / Cotaco and Zamora


A Case of Harlequin Ichthyosis

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Harlequin fetus is rare and is the most severe form of congenital ichthyosis, inherited as autosomal recessive trait with an incidence of about 1 in 300,000 births. It is characterized by hyperkeratosis and desquamation of the epidermis which begins prenatally. The skin barrier is severely compromised, leading to excessive water loss, electrolyte imbalance, temperature dysregulation and an increased risk of life threatening infection. In our institution, we report a case of a pregnant woman with a previously born child affected with Harlequin Ichthyosis. For this second pregnancy, they were expecting to have a normal baby since the congenital sonographic scan done prenatally was unremarkable. However, the mother delivered at 29-30 weeks age of gestation with the fetus showing signs of harlequin ichthyosis. The child was a 1200 gram female neonate born prematurely by partial breech extraction. Clinical manifestations of harlequin ichthyosis were present at birth. Furthermore, the fetus suffered from respiratory distress few hours after delivery and died few days after birth. Prenatal diagnosis of HI is made with the use of ultrasound guided fetal skin biopsy, imaging technique with 2D and 3D real time sonography as well as genetic mutational analysis. Prenatal genetic counseling is very essential in her case because of the serious implications to consider for her offspring. The complex management of our patient can be best achieved by a multidisciplinary approach characterized by strong communication, both among the medical team and with the family.

Key words: Harlequin ichthyosis, congenital, prenatal diagnosis.
Objectives

General Objective:

To present a rare case of two consecutive harlequin ichthyosis in the offspring of a secundigravida.

Specific Objectives:

To discuss the pathogenesis of harlequin ichthyosis.

To review current literature in the prenatal diagnosis based on the following:

1. Ultrasonographically guided fetoscopic skin biopsies
2. Two and three dimensional real-time sonography
3. Molecular ABCA12 mutational analysis

To discuss genetic counseling, therapeutic options and updates in the management of harlequin ichthyosis.

THE CASE

A 30 year-old gravid 2, para 1, (0100) on her 29-30 weeks age of gestation with non-consanguinous marriage, presented in labor with chief complaint of vaginal bleeding. Condition started few hours prior to admission, the patient experienced watery vaginal discharge accompanied with hypogastric pain. She regularly followed up at nearby health center and had an uncomplicated prenatal course. She had previous child diagnosed with harlequin ichthyosis syndrome and shortly died 2 days after delivery in our institution. (Figures 1 & 2). Since the probability to have harlequin baby was likely, we advised her to undergo genetic study for chromosomal abnormality however due to financial constraints, it was not done.

Latest ultrasound revealed a single, live frank breech presentation 25 weeks and 6 days age of gestation. The volume of amniotic fluid was normal. No gross fetal anomalies were seen. Likewise, a congenital scan was also performed on her 25th week of pregnancy. Other routine laboratory examinations such as CBC, urinalysis and thyroid function test were unremarkable.

Patient was diagnosed with hypothyroidism ten years ago and maintained on thyroxine for years. She denied exposure to any radiation, skin diseases and illicit drug use. No history of intrauterine fetal death nor chronic dermatologic problem in the family. Patient is non-smoker and non-alcoholic beverage drinker. No allergies to food and drugs were noted. She is a college graduate and works as a government employee.

Upon admission, premature rupture of membranes was confirmed. The internal examination revealed a fully dilated cervix breech presentation ruptured bag of waters, station 0. Fundic height was 25cm, and fetal heart tones were noted in right paraumbilical area. Normal vital signs were recorded. Initial laboratory investigation included complete blood count which showed marked leukocytosis (WBC of 33.3 x 10^9/L).

Figure 1. Patient’s first born baby affected with HI. Preterm 28 weeks AOG by LMP, NSD, born on June 13, 2009.

Figure 2. Patient’s second baby with harlequin ichthyosis. Preterm, 29-30 weeks AOG by LMP, PBE, live baby girl, born last April 10, 2010.
She was immediately brought to the delivery room where she delivered via partial breech extraction to a live preterm baby girl, with Apgar score: 7,8, Ballard score: 29 weeks, birth weight: 1.2 kg, appropriate for gestational age. At birth, the infant was noted to have generalized edema with thick hyperkeratotic plates over her entire body and associated with deep erythematous tissues. The baby also had face without mimicry, nasal hypoplasia with eroded nasal alae, small pinnae, eclabium and a fixed, open mouth and severe ectropion. The appearance of skin lesions was consistent with the diagnosis of harlequin ichthyosis. Within hours of delivery, the infant developed respiratory distress. She was transferred to our neonatal intensive care unit where aggressive interventions were done. However, despite these efforts, the child eventually died of septic shock 2 days after birth.

**DISCUSSION**

Congenital (harlequin) ichthyosis is a rare and devastating disorder. More than 100 cases have been reported worldwide. This disorder is inherited as an autosomal recessive trait characterized by defective keratinization and desquamation of the epidermis. There is mutation in ABCA12 causing defective lipid transport that significantly affects the normal development and function of the skin barrier. The ABCA12 works as an epidermal keratinocyte lipid transfers lipids from the cytosol into lamellar granules and discharges their content into the intracellular space, forming lipid lamellae of the stratum corneum. This provides an effective skin barrier. The development of the HI phenotype is initiated by the onset of hair canal keratinization at 17 weeks of gestation and is expressed in the entire hair-covered skin from 20 weeks of gestation onward. In 1750, Rev Oliver Hart reported the first case who suffered from thickened and cracked skin often dividing into polygonal plaques over the whole body. The name of the disease derives from the typical facial expression of a child’s face and the triangular, diamond – shaped pattern of hyperkeratosis. The mouth of a child is open and similar to a clown's smile.

It is a fatal disorder in which neonates die in a few days after birth. As is true in our patient, the usual cause of demise in the perinatal is sepsis. On the other hand, respiratory failure, poor nutrition and electrolyte imbalances were the common complications observed. The fetuses have eclabium, ectropion, and scaling of the skin with resultant akinesia, the same features in our patient’s babies. Consequently, neonates are often born prematurely like in our patient’s offsprings. The child is usually in serious condition, with low Apgar score. Luckily the second baby delivered with good Apgar score but eventually died due to sepsis.

A few cases of prenatal diagnosis of HI have been reported. The fetal diagnosis of HI can be established through analysis of ultrasonographically guided fetoscopic skin biopsies. These biopsies showed premature hyperkeratosis, most marked around hair follicles and sweat ducts, forming plugs of hyperkeratotic debris. Diagnosis by skin biopsy can be done at 20-22 weeks’ gestation although recently, diagnosis was achieved at 17 weeks’ gestation using electron microscopy of pillous follicles, whose cornification occurs a few weeks before that of the epidermis. However, prenatal diagnosis of HI by fetal skin biopsy is technically difficult, requires excellent skin biopsy site selection, and is time-consuming.

According to M Mihalko, et al., two dimensional sonographic demonstration of anomalies associated with HI was possible, including cystic protuberance over the orbits and absence of normal lips, which indicate ectropion and eclabium, respectively (Figure 3). The abnormally thickened fetal skin, in the absence of hydrops, also aids in the prenatal diagnosis of HI. The most constant sonographic findings are large gaping mouth (Figure 4), dysplastic or swollen hands and feet, aplasia of the bulging eyes. The “snowflake sign” reflecting skin particles floating in the amniotic cavity, intra-amniotic debris or floating membranes might be another indirect signs. Hence, the diagnosis of HI in the early trimester can be established using this method. Likewise, a three dimensional ultrasound imaging will confirm the diagnosis showing a more detailed typical appearance of HI. HI was not diagnosed in our case using 2-D sonography.

Another diagnostic clue for HI as early as 22-24 weeks is sonographic measurement of femur-foot length ratio. Normally, the foot length is approximately equal to length throughout gestation. In several case reports, foot length was decreased compared with the femur length in patients affected with HI. The foot length is considerably shortened because of severe restrictive dermopathy. Although there is abnormal skin development all over the body, and scaling is present, the long bones are not affected. The phalanges and metacarpal and metatarsal bones are underossified and incurved because of tight wrapping of the skin, leading to decreased foot length ratio.
length. Hence, fetal foot length may be an early marker that may help in the prediction of harlequin ichthyosis, especially when there is a sibling history of this disorder.15

Recently, molecular genetic testing by analysis of DNA detects mutation on ABCA12 which was identified as the underlying gene causing HI.16 Owing to these discoveries, it has now become possible to undertake HI DNA-based prenatal diagnosis by chorionic villus sampling at approximately 10 to 12 weeks of gestation or amniotic fluid sampling usually performed at approximately 15 to 18 weeks of gestation. These procedures are technically more reliable and have a reduced burden on the mothers, as in other severe genetic keratinization disorders.17

In our patient, she was devastated to have a first born child with HI. For the second pregnancy, the fear of having a second harlequin baby was present. Hence, the mentioned procedures were offered to the parents. However, due to financial constraints, our patient was unable to avail of these tests.

Given the appearance of the neonate, it is not uncommon for our patient to harbor profound feelings of guilt, shame or grief. Thus, prenatal genetic counseling should be offered (including discussion of potential risks to offspring and reproductive options) to young adults who are at risk of having a child with HI. The Mendelian human genetic theory shows that each sibling of an affected individual has a 25% risk of being affected, a 50% chance of being an asymptomatic carrier and a 25% chance of being unaffected and not a carrier. Once risk of having a child with HI in the family members has been identified, carrier molecular genetic testing for mutations in ABCA12 is advised. Our patient had 2 consecutive HI, hence, it is appropriate to investigate further and find out factors such as consanguinous marriage and skin diseases in the family that might contribute for the development of such disorder.

According to Berg, et al., there are nine cases of prolonged survival of HI reported. Prolonged survival with better neonatal care is possible with development of neonatal care, multidisciplinary and targeted oral retinoid therapy.18 Isotretinoin, etretinate and acitretin were used for treatment of HI to achieve survival up to 8 years. However, the intensive treatment appears to necessitate a tertiary level of hospital care with consultants available who are familiar with the condition. The focus of intensive treatment is to ensure close monitoring for signs of sepsis and aggressive treatment of bacterial or fungal infections. In our case, treatment of manifestations and prevention of secondary complications were undertaken however, despite these interventions the child did not survive.

Once HI is detected during the early trimester of pregnancy by either DNA mutation analysis or fetal skin biopsies, termination of pregnancy is offered to parents in other countries. However, in our setting, we do not offer such intervention. Recently Akiyama, et al. showed that genetic correction of ABCA12 deficiency by gene transfer in patient’s
keratinocytes restored normal glucosylceramide cell distribution and lamellar granule formation. This raises the possibility of HI treatment using systemic administration of functional peptides with ABCA12-like properties or ABCA12 gene delivery approaches undertaken either prior to or after birth. Indeed, this new therapeutic discovery in the management and prevention of HI would greatly benefit our patient in a future pregnancy.

CONCLUSION

Harlequin ichthyosis is a severe disorder of the skin that was diagnosed in both consecutive offsprings of our secundigravida patient. This disorder presents a formidable challenge for the parents since there are serious implications to consider such as the high risk that their newborn will either survive for a short period of time or die soon.

Prenatal diagnosis of the syndrome which include ultrasonographically-guided fetoscopic skin biopsies, two and three dimensional real-time sonography and molecular mutational genetic analysis are essential to screen as well as to confirm the diagnosis.

The complex management of our patient can be achieved by using a multidisciplinary approach characterized by strong communication, both among the medical team and with the family.

While the present treatment of gene correction of ABCA 12 deficiency by gene transfer or systemic administration of functional peptide treatment is not yet available in our country, there is no doubt that we can expect her to have another harlequin baby with same prognosis as her previous offsprings, 25% chance to have harlequin baby, every pregnancy. Hopefully, these newly discovered treatments will be available in the near future. We will not discourage our patient to get pregnant again, since there is hope for her to have a normal baby.

REFERENCES

Beckwith-Wiedemann Syndrome: A Case Report

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Beckwith-Wiedemann Syndrome (BWS) is a rare congenital overgrowth disorder due to alterations in specific genes in chromosome 11p15. It has a variable clinical picture. Infants may exhibit a combination of the following characteristics: macroglossia, macrosomia, abdominal wall defects, ear creases or posterior helical pits, hypoglycemia, polyhydramnios and prematurity. Presented is a case of a 24-year-old gravida 3 para 2 (2002) who manifested with preterm labor and polyhydramnios. She delivered a preterm live baby girl who was diagnosed to have Beckwith-Wiedemann syndrome. The rarity of this condition, as well as the significant maternal and perinatal complications associated with it, is discussed in this paper.

Key words: Beckwith-Wiedemann syndrome, macroglossia, macrosomia

A 24-year-old gravida 3 para 2 (2002) from Quezon City came in with the chief complaint of labor pains. Her past and family medical histories were unremarkable. Her obstetric history included two uncomplicated term pregnancies for which she underwent cesarean sections for cephalopelvic disproportion. The two children were born healthy and appropriate for gestational age. Prenatal care was provided by a midwife at a local health center where the patient was seen four times. No ultrasound examinations or tests for gestational diabetes were done antenatally. Baseline prenatal laboratory tests were normal. There was no known exposure to teratogen or radiation and no intake of any drug other than ferrous sulfate during the course of her pregnancy.

The patient had essentially normal systemic findings. Her fundic height, however, was noted to be 40cm, which was too large for her menstrual age of 35 weeks and 2 days. Internal examination showed a cervix dilated to 3-4cm, 50% effaced, with intact bag of waters. A consideration of multiple gestation versus ovarian new growth in pregnancy was made. Biometry with biophysical profile was requested. The scan was significant for an estimated fetal weight above the 90th percentile (4514 grams by Hadlock, 4170 grams by Warsof) and polyhydramnios (amniotic fluid index of 56cm). No congenital anomaly scan (CAS) or targeted imaging for fetal anomalies (TIFFA) were performed. Baseline laboratory examinations (blood typing, complete blood count and urinalysis) were done, the last two of which revealed normal values. Specimen for capillary blood glucose (CBG) was tested, which revealed a value of 108mg/dl. Glycosylated hemoglobin was 5.8 percent.

The patient underwent a repeat low segment cesarean section. Intraoperatively, about 4.5 liters of clear amniotic fluid was evacuated. A live female infant, weighing 3,700 grams, 36 weeks by pediatric aging and cephalic in presentation, was delivered. The placenta was implanted posterofundally and measured 22cm x 17cm x 2cm. It weighed 690 grams and had a grossly normal three-vessel umbilical cord that measured about 25cm. There was no note of infarcts on gross examination. The placenta was sent to the Surgical Pathology Section for further examination. Histopathology results showed: third trimester singleton placenta with mild placentomegaly (690 grams); no diagnostic abnormality recognized, three-vessel umbilical cord and extraplacental membranes (Figure 1).

The infant, upon delivery, was immediately given newborn care at the transition nursery. Apgar scores were 6 at 1 minute and 7 at 5 minutes. At the sixth minute of life, however, chest retractions were noted, along with some flexion of extremities and poor cry. Respiratory rate was 50 bpm. The infant was intubated and then brought to the Neonatal Intensive Care Unit (ICU) for further work-up and management. Given a birthweight of 3,700 grams,
length of 51cm and a pediatric aging of 36 weeks, she was considered large for gestational age. Head circumference was 34.5cm, chest circumference was 36cm, and abdominal circumference was 36.5cm. Physical examination revealed the following (Figures 2 A-F):

Figure 1. Histopathology of the Placenta. (a) Scanning view showing small terminal villi and stem villi. Prominent congested villi are already visible at this magnification. Occasional islands of intervillous fibrin (pink amorphous material) are also present, but this is not pathologically or clinically significant. (b) Low power magnification showing that the vascularity of the villi is more conspicuous. Non-nucleated RBC’s can be seen both inside the vessels intravillously, and in the intervillous space. (c) High power magnification. The villi are lined by a single layer of trophoblastic epithelium, the syncytiotrophoblast. Note the pink continuous cytoplasm of the syncytium. The nuclei of the syncytiotrophoblast tend to cluster together on one side of the villi. The intravillous capillaries are located close to and just below the syncytiotrophoblastic epithelium. (d) Low power view of one of the umbilical arteries. There is no inflammation both in the vessel wall and the surrounding Wharton jelly. (e) Low power magnification of the extraplacental membranes shows 1) an intact amnionic epithelium, composed of a single layer of cuboidal cells with pink cytoplasm, 2) the light pink and hypocellular connective tissue beneath it, and 3) the more cellular chorion layer further below. There is no inflammation in all the layers.
Figure 2. (a) bilateral corneal opacity; (b) anterior ear lobe fissures (arrow), low set ears; (c) high-arched palate; (d) short neck, diastasis recti; (e) flat nasal bridge, macroglossia; (f) proximally inserted left fifth toe.
Given the aforementioned physical examination findings and course at the transition nursery, the initial impression was:

- Preterm, 36 weeks by pediatric aging, 3,700 grams, large for gestational age, cephalic presentation, delivered by repeat low segment cesarean section, live baby girl, Apgar score of 6 becoming 7
- Multiple congenital anomalies, rule out Beckwith-Wiedemann syndrome
- Rule out transient tachypnea of the newborn (TTN) versus neonatal pneumonia.

The infant’s initial hemoglobin value (Hgb) was 20mg/dl, which went up to 40mg/dl after administration of 10cc/kg of D10W. Baseline laboratory examinations included a chest radiograph, which confirmed pneumonia and pleural effusion on the right; and holoabdominal ultrasound, which revealed an enlarged left kidney with intact morphology and normal ultrasound of the liver, spleen and right kidney. The complete blood count, urinalysis, 12-lead ECG, and total, direct, and indirect bilirubin were all essentially normal. A chromosomal analysis and 2D-echocardiography were likewise requested but not done due to financial constraints. Specimen for blood culture studies was sent. Empiric treatment with Meropenem and Amikacin was started. She was co-managed with Genetics, Ophthalmology and Otolaryngology.

The infant’s course at the Neonatal ICU was initially unremarkable. On her 30th hour of life, however, the infant exhibited episodes of hypothermia for which thermoregulation was done. Multiple petechiae and bruises were likewise noted all over the abdomen, upper and lower extremities. Platelet count was noted to have dropped from a level of 133 x 10^9/L to 55 x 10^9/L. One aliquot of platelet concentrate was transfused. On the 45th hour of life, generalized mottling was observed, and the abdomen was noted to be distended, firm, and tympanitic. She had hypoactive bowel sounds. Bowel movement, however, was normal and there was no vomiting. Babygram showed gas-filled distended gastric bubble and bowel loops, which suggested ileus. On the 50th hour of life, bleeding per nasogastric tube was observed and the infant was noted to have no heart rate. Neonatal advanced life support was performed. However, the infant was not revived even after 25 minutes of resuscitation. Final diagnosis was disseminated intravascular coagulopathy secondary to neonatal sepsis; to consider Beckwith-Wiedemann syndrome.

Blood culture results came out five days after and showed moderate growth of *Klebsiella rhinoscleromatis*, resistant to Meropenem.

The mother had an unremarkable immediate postpartum course. She went home on her fifth postoperative day.

**DISCUSSION**

Presented is a case of a 24-year-old gravida 3 para 2 (2002) who manifested with preterm labor and polyhydramnios and delivered a live, large for gestational age baby girl who had macroglossia, diastasis recti, anterior ear lobe creases, hypoglycemia, and visceromegaly. Beckwith-Wiedemann was considered in the offspring. The rarity of this condition, as well as the significant maternal and perinatal complications associated with it, is a point of interest.

Beckwith-Wiedemann Syndrome (BWS) is rare but is the most common congenital overgrowth disorder in infancy. It was first described in 1963 by Dr. J. B. Beckwith, an American pediatric pathologist, and then by Dr. H. E. Wiedemann, a
German geneticist, in 1964. It was originally called EMG syndrome, based on three hallmark features: exomphalos, macroglossia, and gigantism.

**Epidemiology and Prevalence**

Beckwith-Wiedemann Syndrome affects various ethnic groups and occurs equally in males and females. It has a frequency of about 1 in 13,700 births. This figure is likely an underestimate, however, because milder phenotypes may be unknowingly omitted. An article published in 2007 cited that a little over 500 cases have been reported in literature. A search of the Philippine Pediatric Society (PPS) and the Institute of Human Genetics (IHG) database showed no records on the incidence of BWS in the Philippines. The IHG, however, reported that from 2007 to present, a total of 9 cases of BWS have been documented in the Philippine General Hospital. (Table 1).

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<th>Period</th>
<th>Number of BWS Cases</th>
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<tr>
<td>2007</td>
<td>4</td>
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<tr>
<td>2008 to June 2009</td>
<td>3</td>
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<td>July 2009 to April 2010</td>
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Only two factors have been cited to increase the incidence of Beckwith-Wiedemann syndrome. About 10-15% of BWS cases are associated with an autosomal dominant mode of inheritance, a positive family history is said to increase the risk of another child having BWS. The utilization of assisted reproductive technology (ART) has also been linked to an increased frequency of BWS. In a study by Maher, et al., 149 sporadic cases of BWS were reviewed. It was found out that six of 149 (4%) were born after ART; that is, three after intracytoplasmic sperm injection and three after in-vitro fertilization. This 4% was higher compared to the ~1.2% risk of BWS occurring in the general population.

**Etiology**

Alterations in specific genes on chromosome 11p15 have been implicated in the pathogenesis of Beckwith-Wiedemann Syndrome. They can be: 1. sporadic (85%) (1); that is, affected individuals represent single occurrences in the family, are chromosomally normal and have no family history of BWS (2), 2. inherited (10-15%) (1); that is, affected individuals have a positive family history and a normal karyotype (2), or 3. secondary to chromosomal abnormalities (1%); that is, the alteration has been found primarily on the IGF2 genes, which is a fetal growth factor, and in the H19 gene, which is thought to be a tumor suppressor gene. These modifications would then result in the macrosomia and increased tumor risk associated with BWS.

The family history of the index patient was unremarkable, which could point to a possible sporadic alteration. However, since chromosomal analysis was not done on the infant, the presence of any chromosomal abnormality could not be ruled out.

**Clinical Presentation**

Beckwith-Wiedemann syndrome presents in diverse forms. Its most common features are macroglossia (97%), pre- or postnatal gigantism (88%), abdominal wall defects such as omphalocoele, umbilical hernia and diastasis recti (80%), ear creases or posterior helical pits (76%), and hypoglycemia (63%). A history of polyhydramnios or prematurity (27%) are also often encountered. Other common features include a long umbilical cord and an enlarged placenta, averaging almost twice the normal weight for gestational age. As can be recalled, the patient in this case was admitted for preterm labor at 35 weeks age of gestation, and had polyhydramnios on ultrasound. Histopathology of the placenta also confirmed placentomegaly.

Macroglossia, as was seen in the infant in this case, is present in about 97% of BWS cases. It is usually the most evident physical feature in these patients. Tongue size can vary with more severe cases leading to difficulties in feeding, speech and sleep apnea. Macrognosia, on the other hand, can be manifested as whole body overgrowth or regional overgrowth affecting areas of the body or specific organs. Growth parameters typically show height and weight greater than the 90th percentile during the latter half of pregnancy and early years of age. Bone age is
significantly advanced, particularly during the first 4 years. Widening of the metaphyses and cortical thickening of the long bones can be observed. Growth velocity normalizes thereafter, with final height attainment usually within the 50th to 90th percentile. In the index case presented, the infant’s birth weight and length were above the 90th percentile for age.

Abdominal wall defects, which include omphalocoele, umbilical hernia, and diastasis recti, are very common findings in children with Beckwith-Wiedemann Syndrome. Omphalocoele complicates about half of patients. Visceromegaly involving any single or combination of organs (liver, spleen, pancreas, kidneys, and adrenals) has also been found. Cystomegaly and cysts in the adrenal cortex with hyperplasia in the adrenal medulla have been described in several cases. Cardiomegaly and structural cardiac defects occur in approximately 20-25%, but no specific defect has been prominent. About half manifest cardiomegaly that resolves spontaneously. Cardiomyopathy is rare. The infant in this case did not present with omphalocoele, although diastasis recti was noted. She was also diagnosed to have nephromegaly on the left based on holoabdominal ultrasound. No cardiomegaly was noted on babygram.

A recognizable facial expression is commonly observed in BWS. This may include midfacial hypoplasia (85%), prominent occiput (58%), and facial nevus flammeus or port-wine stains of the forehead and eyelids (54%). Anterior ear lobe creases and posterior helical pits (63%) are very distinctive, consisting of slit-like linear indentations. Indeed, the infant of the index patient had a flat nasal bridge and anterior ear lobe fissures.

Hypoglycemia occurs in more than 50% of BWS cases and is one of the features that play a significant role in the prognosis of BWS patients. In an article published in 2000, hypoglycemia was defined as less than 30mg/dL in full-term or less than 20mg/dL in preterm infants. Several investigators have attempted to explain the hypoglycemia of BWS at the molecular level but no consensus has been defined. In general, it is said to be caused by hyperinsulinism. Majority of infants with hypoglycemia will be asymptomatic and have resolution of the hypoglycemia within the first 3 days of life. Less than 5% will have hypoglycemia beyond the neonatal period. The infant in this case had Hgt value of 20mg/dL, which went up to 40mg/dL with administration of D10W. Her blood glucose was strictly monitored at the neonatal ICU. Untreated hypoglycemia could lead to neurologic disturbances such as seizures and adverse neonatal outcomes.

Other less commonly encountered laboratory findings, none of which were seen in the index case, include polycythemia (20%), hypocalcemia (5%) and hyperlipidemia (2%). Hypercalcuiaria is found in 22% of cases and may suggest primary structural abnormality in the kidneys. These findings have not been extensively studied yet.

Differential Diagnosis

The presentation of a newborn with large growth parameters, macroglossia, polyhydramnios, hypoglycemia, and other congenital structural defects should prompt a comprehensive clinical examination followed by relevant investigations, for example, for maternal diabetes mellitus. In the case presented, maternal diabetes was easily ruled out because of the normal blood glucose and glycosylated hemoglobin levels of the mother.

Other overgrowth syndromes that manifest with characteristics similar to those of Beckwith-Wiedemann syndrome may also be considered. Simpson-Golabi Behmel syndrome, Perlman syndrome, and Sotos syndrome. These genetic syndromes can be distinguished by clinical genetics consultation, ancillary tests (e.g., brain imaging, molecular and/or biochemical testing) and ongoing follow-up.

Simpson-Golabi Behmel syndrome (SGBS) is an overgrowth syndrome that shares the following characteristics with BWS: macrosomia, visceromegaly, macroglossia, ear lobe creases, renal anomalies and a risk of embryonal tumors. It is distinguished from BWS by the presence of characteristic facies, cleft lip, supernumerary nipples, and skeletal abnormalities, including polydactyly. SGBS also has a higher incidence of congenital heart disease. Perlman syndrome is an autosomal recessive disorder characterized by increased birth weight and length, macrocephaly, nephroblastomatosis and distinctive facial features such as full round face and deeply set eyes. It also has a high incidence of Wilms tumor, similar to that seen in BWS. Mortality during the neonatal period is common and those who survive beyond the neonatal period, significant intellectual handicap is usually seen.
Sotos Syndrome, also called cerebral gigantism, is a condition that presents with neonatal macrosomia, excessive growth during the first four years of life, advanced bone age, and recognizable facial characteristics which include macrodolichocephaly, ocular hypertelorism, and prominent mandible. It has been concluded that neuroimaging findings of Sotos syndrome are distinct enough to allow differentiation of this syndrome from other genetic syndromes and mental retardation syndromes that present with macrocephaly. Anomalies of the corpus callosum are almost universal.\textsuperscript{14}

**Diagnostic Approaches**

The diagnosis of BWS relies primarily on clinical findings.\textsuperscript{2} No set of criteria has been established as absolute requisite for the diagnosis, but it is generally accepted that the presence of at least three major findings, or two major findings and one minor finding support a clinical diagnosis (Table 2).\textsuperscript{3} In the index case, the infant exhibited macrosomia, macroglossia, anterior ear lobe creases and nephromegaly, thus satisfying four of the major criteria. There was also a history of polyhydramnios, neonatal hypoglycemia, characteristic facies, and diastasis recti, which are minor findings that support the diagnosis.

Molecular testing may be helpful in confirming the diagnosis of BWS. However, due to the heterogeneous expression of the disorder, the test cannot rule out BWS at the time. Cytogenetically detectable abnormalities involving 11p15 are also only found in 1\% or less of cases.\textsuperscript{2}

More recently, studies have been focusing on detecting Beckwith-Wiedemann syndrome prenatally. Not only will this help the obstetrician gynecologist in counseling the pregnant woman and planning the proper mode of delivery but will also allow preparation of a multidisciplinary team which can address the probable fatal problems of the neonate, including airway obstruction secondary to macroglossia, respiratory distress, and hypoglycemia, which may lead to mental deficiency, if left untreated.\textsuperscript{15}

About 20 of the 500 cases of BWS reported in the literature were actually diagnosed prenatally through ultrasonography.\textsuperscript{15} These reports have relied on a combination of ultrasound findings: macrosomia (usually accelerated growth starting at 22 weeks to large for gestational age during the third trimester), macroglossia, omphalocele, polyhydramnios, increased abdominal circumference, kidney to abdominal circumference ratio, and renal or liver enlargement. A single case report in 2001\textsuperscript{16} cited that a sonographic finding of a single umbilical artery may also suggest BWS. An article published in 2002\textsuperscript{17} concluded that in the absence of diabetes mellitus, the detection of advanced fetal growth, polyhydramnios, placentomegaly, and a distended abdomen or normal abdominal circumference in the presence of omphalocele should alert the obstetrician to the possibility of BWS. A targeted imaging for fetal anomalies should therefore be performed.

<table>
<thead>
<tr>
<th>Table 2. Major and minor findings associated with BWS.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major Findings</strong></td>
</tr>
<tr>
<td>□ Abdominal wall defect: omphalocele (exomphalos) or umbilical hernia</td>
</tr>
<tr>
<td>□ Macroglossia</td>
</tr>
<tr>
<td>□ Macrosomia (traditionally defined as height and weight &gt;97th percentile)</td>
</tr>
<tr>
<td>□ Anterior ear lobe creases and/or posterior helical pits (bilateral or unilateral)</td>
</tr>
<tr>
<td>□ Visceromegaly of intra-abdominal organ(s); for example, liver, kidney(s), spleen, pancreas, and adrenal glands</td>
</tr>
<tr>
<td>□ Embryonal tumor in childhood</td>
</tr>
<tr>
<td>□ Hemihiperplasia</td>
</tr>
<tr>
<td>□ Cytomegaly of adrenal fetal cortex, usually diffuse and bilateral</td>
</tr>
<tr>
<td>□ Renal abnormalities, including medullary dysplasia and later development of medullary sponge kidney (MSK)</td>
</tr>
<tr>
<td>□ Positive family history of BWS</td>
</tr>
<tr>
<td>□ Cleft palate</td>
</tr>
<tr>
<td><strong>Minor Findings</strong></td>
</tr>
<tr>
<td>□ Pregnancy-related findings of polyhydramnios, enlarged placenta and/or thickened umbilical cord, premature onset of labor and delivery</td>
</tr>
<tr>
<td>□ Neonatal hypoglycemia</td>
</tr>
<tr>
<td>□ Nevus flammeus</td>
</tr>
<tr>
<td>□ Cardiomegaly / structural cardiac anomalies/cardiomypathy</td>
</tr>
<tr>
<td>□ Characteristic facies</td>
</tr>
<tr>
<td>□ Diastasis recti</td>
</tr>
<tr>
<td>□ Advanced bone age</td>
</tr>
</tbody>
</table>

Legend: □ present in the index neonate


**Course and Prognosis**

The prognosis of Beckwith-Wiedemann syndrome is generally favorable, although a mortality rate of 20\% has been seen in affected infants because of complications of prematurity, neonatal hypoglycemia,
macroglossia, cardiomyopathy and omphalocoele. In the index case presented, macroglossia could have caused aspiration, which eventually could have led to aspiration pneumonia. The pneumonia, in turn, could have progressed to sepsis which eventually caused the demise of the neonate.

Among those who survive, the BWS facies often normalize across childhood. The growth of the child’s cranium would eventually allow accommodation of the enlarged tongue into the oral cavity, therefore resulting in regression of the macroglossia. Referral to feeding specialists and speech pathologists may be helpful and surgery may be undertaken for severe macroglossia. Neurodevelopment is usually normal unless there is chromosome 11p15.5 duplication or serious perinatal complications, including prematurity or uncontrolled hypoglycemia.

Of major concern in children with BWS is the increased incidence of tumor development in early childhood. Most of the tumors associated with BWS occur in the first 8-10 years of life with very few being reported beyond this age. Most common are Wilms tumor and hepatoblastoma. The overall risk for tumor development in children with BWS has been estimated at 7.5%. Clinical findings associated with higher risks of tumor development include hemihyperplasia, nephromegaly, and nephrogenic rests. During infancy and childhood, patients should be evaluated with a baseline abdominal CT or MRI, with subsequent abdominal ultrasound every three months up to age 8. Screening for malignancy should include include serum alpha-fetoprotein until 8 years of age.

CONCLUSION

In summary, this is a case of a 24-year-old gravida 3 para 2 (2002) who manifested with preterm labor and polyhydramnios. She delivered a preterm live baby girl who was diagnosed to have Beckwith-Wiedemann syndrome based on the following: macrosomia, macroglossia, characteristic facies, anterior ear lobe creases, visceromegaly, diastasis recti, polyhydramnios, enlarged placenta, and neonatal hypoglycemia. Prompt recognition of this disorder, both prenatally and upon delivery, will not only help in counseling the patient and her family but also allow adequate preparation to avoid the potentially fatal problems of the neonate.

REFERENCES

5. Interview with Dr. Eva Cutiongco-dela Paz, Director, Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila.
Fetal Brittle Bones
A Case Report on Fetal Osteogenesis Imperfecta

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This is a case report of a fetal osteogenesis imperfecta diagnosed through ultrasound findings of short femur with fractures seen in utero. This disease is quite rare with incidence of one patient per 25,000 to 40,000 live births. Prenatal diagnosis of specific skeletal dysplasia is difficult and can be imprecise. However, efforts should be geared towards accurate diagnosis for proper counseling of the parents and advanced coordination with the pediatric and orthopedic services for subsequent neonatal care as in this case. The diagnostic approach, management and ultimate course of the patient are discussed.

Key words: fetal osteogenesis imperfecta

Osteogenesis imperfecta or brittle bone disease is one of the most common among the skeletal dysplasias. It represents a wide spectrum of genetically and clinically heterogeneous disorder of bone and connective tissue mainly characterized by bone fragility accompanied by osteoporosis, hyperextensible joints, dentinogenesis imperfecta, blue sclera and adult-onset hearing loss.

Prenatal diagnosis of this disease can be challenging to obstetricians however efforts should be exerted to obtain accurate diagnosis for proper counseling of the parents. In the study by Sharony, et al., 50% of the cases with osteogenesis imperfecta type II were accurately diagnosed ultra-sonographically; in 30% a specific diagnosis was not attempted, and 20% were incorrectly thought to represent different specific skeletal dysplasias, mostly thanatophoric dysplasia. In the Philippines, in utero diagnosis of osteogenesis imperfecta has not been reported.

Subsequent management including parental counseling, mode of delivery and proper coordination with neonatal care will depend on the prognosis of the specific fetal condition.

This case is presented to highlight the diagnostic and management approach on this rare disease.

THE CASE

V.G. is a 36 year old primigravid, with findings of short femur starting 21-22 weeks during a congenital scan. Other extremities were also short with humerus, tibia and ulna (<10th percentile). There was also bowing of femur, with a “telephone receiver appearance” (Figure 1).

Figure 1. Bowing of femur “telephone receiver appearance”
She was a diagnosed case of systemic lupus erythematosus (1998) with lupus nephritis (2001) S/P cyclophosphamide therapy and currently maintained on Prednisone 2.5 mg/tab OD and calcium carbonate 1 cap BID. She started prenatal check-up in our high risk clinic at 10-11 weeks of gestation. Aspirin 80 mg/tab OD was started at 18 weeks AOG.

Due to a lag in the fundic height (cm) plotted against the gestational age (in weeks), serial fetal biometry was done to rule out intrauterine growth restriction (IUGR) (Table 1). Despite shortened limbs and a significant lag in the fundic height; the estimated fetal weight remained above the 10th percentile with a steady increase in weight and fetal abdominal circumference (Figure 2). The findings of shortened femur in the absence of IUGR pointed to the presence of skeletal dysplasia, notably thanatophoric dwarfism and achondroplasia.

The prenatal diagnosis of a specific skeletal dysplasia is admittedly a difficult and mostly inaccurate task to undertake. The possibility of having such fetal condition was very well explained to the patient and her partner.

She then underwent serial ultrasound with emphasis on the fetal biometric parameters (BPD, HC, FL and FAC). Thoracic: abdominal circumference ratio was computed to be 0.89 (NV 0.77-1), which ruled out lethality of the fetal condition from pulmonary hypoplasia. At 33-34 weeks of gestation, bilateral femoral fractures were noted (Figure 3). These practically clinched the diagnosis of osteogenesis imperfecta. We sought the help of a genetic counselor to further enlighten the couple of the facets of osteogenesis imperfecta and how this malady would impact on the eventual course of her pregnancy, the mode of delivery, and on how fracture in utero and eventual fractures at

Table 1. Expanded Sillence classification of osteogenesis imperfecta.

<table>
<thead>
<tr>
<th>Type</th>
<th>Frequency*</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Clinical features</th>
<th>Radiographic or histological features</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>50%</td>
<td>AD</td>
<td>COL1A1 COL1A2</td>
<td>Blue sclera, normal stature, fractures, hearing loss, presence of DI rare</td>
<td>Fractures</td>
</tr>
<tr>
<td>II</td>
<td>AD</td>
<td>COL1A1 COL1A2</td>
<td>Perinatal lethal, Blue-Gray sclera, small for gestational age, respiratory distress, limb deformities, &quot;frog leg&quot; positioning, soft calvarium</td>
<td>Multiple fractures &quot;crumpled&quot; appearance, beaded ribs, short thoracic cage, osteopenia, long bone deformities, limited calvarial mineralization</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>AD</td>
<td>COL1A1 COL1A2</td>
<td>Severe phenotype, short stature, multiple fractures, progressive deformities, usually non-ambulatory, may have DI, adolescent onset hearing loss</td>
<td>Osteopenia, multiple fractures, long bone deformity, thin ribs</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>AD</td>
<td>COL1A1 COL1A2</td>
<td>Milder than Ol III, typically ambulatory, DI is common, adult onset hearing loss</td>
<td>Intermediate appearance between Ol II and III</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>5%</td>
<td>AD</td>
<td>?</td>
<td>Mild to moderate, calcification of the interosseous membrane, radial head dislocation, hyperplastic callous formation</td>
<td>Histology: abnormal lamellae on polarized light microscopy.</td>
</tr>
<tr>
<td>VI</td>
<td>Rare</td>
<td>?</td>
<td>?</td>
<td>DI absent, similar to type III</td>
<td>Histology: abnormal bone mineralization</td>
</tr>
<tr>
<td>VII</td>
<td>&lt;10% for both types VII and VIII</td>
<td>AR</td>
<td>CRTAP</td>
<td>Overlap with types II and III. Milder forms also documented</td>
<td>&quot;Popcorn&quot; epiphyses often seen in severe forms</td>
</tr>
<tr>
<td>VIII</td>
<td>VII and VIII</td>
<td>AR</td>
<td>LEPRE1</td>
<td>Overlap with types II and III. Milder forms also documented</td>
<td>&quot;Popcorn&quot; epiphyses often seen in severe forms</td>
</tr>
</tbody>
</table>

This classification scheme likely to change as additional information is gathered on newly described forms caused by CRTAP and LEPRE mutations.

*Frequency refers to proportion of Ol cases thought to be accounted for by type. DI, Dentinogenesis Imperfecta; CRTAP, cartilage-associated protein.
birth and her subsequent neonatal, infancy and adolescent years could be optimally handled. Also taken up during the counseling sessions was the manner of transmission and the numerical figure for the recurrence of this heritable disorder.

Patient underwent primary low transverse cesarean section for breech presentation on her 37-38 weeks AOG. Complete breech extraction was done with the utmost gentle care to a live baby boy in left sacrum anterior position BW 2.3 kg BL 43cm

Figure 2. Fetal biometry results plotted on a normogram
CC 13 cm HC 31 cm AC 26 cm AS 5,8 MT 37-38 weeks SGA symmetric. Amniotic fluid was thinly meconium stained but adequate. Placenta was grossly normal. The uterus and adnexa were unremarkable. On physical examination of the neonate, the following features were noted: widened sutures (large anterior fontanelle), grayish-bluish sclera, high arched palate, saddle nose, with skin fold on the thighs and crepitation (Figure 4). The pediatric service assessment was osteogenesis imperfecta vs. achondroplasia. Hypophosphotasia was ruled out with normal alkaline phosphate level. Babygram (Figure 5) showed healed fractures of both femoral shafts and clavicles with plastic deformation of both tibias and fibulas (with stable callus formation). Cranial ultrasound was normal. He was referred to Orthopedics service and was advised close follow-up. Parents were instructed on proper handling of the neonate. Genetic testing for mutations in \textit{COL1A1} or \textit{COL1A2} genes was contemplated for confirmation however it was not done due to financial constraints and the clinical features of the neonate were already consistent with osteogenesis imperfecta.

Both the mother and neonate were discharged after 4 days with instructions for proper caring for the neonate.

On follow up, the baby incurred 2 new fractures during the 1st year of life, necessitating splinting. Motor skills were notably delayed, only being able to sit with support at 1 year old. Initiation of bisphosphonate therapy was advised but was not given due to financial constraints. He now has a younger sibling who is clinically healthy.

**Objectives**

The objectives of this paper were

1. Project ultrasound as a prenatal detection tool to substantiate the diagnosis of osteogenesis imperfecta in utero.

2. Underscore the vital role of genetic counseling in the management of osteogenesis imperfecta.

3. To explore the general features of each of the known clinical types of osteogenesis imperfecta and their respective mode of heritable transmission.

**DISCUSSION**

Osteogenesis imperfecta or “brittle bone disease” is a well studied heritable disease with a cardinal feature of bone fragility resulting from mutations in the genes that code for type I procollagen, COL 1A1 and COL 1A2. Both qualitative defects (e.g., an abnormal collagen I molecule) and quantitative defects (e.g., decreased production of normal collagen I molecule) resulted to poorly developed
Figure 4. Distinguishing features of the neonate with osteogenesis imperfecta: widened fontanelles, grayish-bluish sclera, triangular shaped face, saddle nose and short femur. Bilateral femoral fractures with callus formation on radiograph.

Figure 5. Babygram of the neonate showing healed clavicular and femoral fractures with plastic deformation of both tibias and fibulas.

bones. It exhibits a broad range of clinical severity, ranging from multiple fractures in utero and perinatal death to normal adult stature and a low fracture incidence. Accompanying symptoms include bluish sclera, dentinogenesis imperfecta and adult-onset hearing loss.

Incidence is reported to be 1 in 20,000–50,000 infants, but the true incidence is probably higher due to misdiagnosis due to its heterogenous presentation.28

Classification

The most popular classification is that proposed by Sillence and colleagues in 1979 into 4 types based on disease severity and progression.

- type I mild non-deforming
- type II perinatal lethal
type III severely deforming  
type IV moderately deforming

This classification has been more recently updated to reflect current pathogenetic understanding. Clinical features, mode of inheritance and genes involved were summarized by Basel, et al. as shown in Table 1.

In 2010, Barnes, et al. proposed a type IX OI as they studied a family with mutations in the gene encoding peptidyl-prolyl isomerase B (PPIB), which causes moderate osteogenesis imperfecta, with normal collagen helical modification and normal P986 3-hydroxylation. It is transmitted in an autosomal recessive fashion and is milder than types VII and VIII.

Although this classification is widely used, it must be kept in mind that not all patients always conveniently fall into one category only and most of the time, clinical features of patients represent a continuum of severity. Even affected members of the same, family may present with a dissimilar degree of severity.

The clinical geneticist and a group of perinatologists are convinced on sound clinical grounds that our case belongs to type III OI, the most severe non-lethal form. The outstanding clinical feature of this case is the presence of fractures in utero, affecting both femurs and clavicles. The bilateral fractures were appreciated as early as 33-34 weeks of gestation whereas the clavicular fractures were evident at birth by x-ray. The long bones are mild to moderately shortened but with marked angulation.

Genetics

Approximately 80% to 90% of the cases of osteogenesis imperfecta are caused by heterozygosity of dominant mutations in one of the two genes (COL1A1 and COL1A2) encoding the chains of type I collagen, with quantitative (mild or moderate forms) or qualitative (severe or lethal forms) defect of the synthesis of type I collagen. Other involved genes are those encoding for cartilage-associated protein (CRTAP), prolyl 3-hydroxylase 1 and cyclophilin B, that form an intracellular collagen modifying complex that 3-hydroxylates proline at position 986 in the alpha 1 chains of collagen (type I, II and V). Mutations in these essential cofactors cause alterations in the posttranslational chain modification and collagen folding that are responsible for autosomal recessive lethal or severe OI: type VII, type VIII and type IX. Probably other disease causing genes will be found for rare recessive OI forms; it should also be considered that types V and VI etiologies are still unknown.

DNA mosaicism in one parent was also implicated in families with multiple affected babies with type II OI and clinically healthy parents. Autosomal recessive inheritance was initially proposed as the manner of inheritance however, affected babies were heterozygous for their mutation. The parent with mosaicism, who usually was healthy clinically, carried the mutation in a small number of their gonadal and somatic cells. The overall risk of recurrence of OI Type II is approximately 7% with each pregnancy, however risk of recurrence is increased to 28% after 2 affected pregnancies. DNA mosaicism was also reported among OI type III and type IV.

Our case would probably take the autosomal recessive form of inheritance since both parents were clinically healthy. In this situation, the parents probably both carry the mutation in their genes. To inherit recessive OI, the child must receive a copy of the mutation from both parents. When a child has recessive OI, there is a 25% chance per pregnancy that the parents will have another child with OI. Siblings of a person with a recessive form of OI have a 50% chance of being a carrier of the recessive gene. Ideally, DNA testing should have been done to confirm the genetic mutation, however it is costly and not readily available. Pattern of inheritance were thoroughly explained to the parents.

Possible Role of Steroids and Cyclophosphamide

Although osteoporosis is a complication of chronic steroid use in adults, no direct effects on the human fetal skeleton have been reported in the literature. Animal studies on the effect of intravenous steroids using rat and mice models had conflicting results.

Cyclophosphamide use during the first trimester has been associated with missing or hypoplastic digits but there were no cases of OI.

Diagnosis

Diagnosis of OI is easy among patients with family history of OI however; it can be a challenging task for index cases who present with short limbs and fractures in utero.
Ultrasound

Ultrasound is the primary imaging modality due to its safety and availability. Accuracy of the 2D ultrasound ranges from 48-73% based on previous studies. Generally, it is considered a good screening tool, however diagnosis may still be missed because the phenotypic characteristics of some skeletal dysplasias do not manifest until the latter half of pregnancy. Schramm, et al. in 2009 studied the efficacy of ultrasound for investigation of 162 affected fetuses with confirmed clinical genetic, pathological or molecular diagnosis of skeletal dysplasias. The ability to achieve the correct specific diagnosis by prenatal ultrasound depends on the type of skeletal dysplasia. In the two most common disorders in this series, thanatophoric dysplasia and osteogenesis imperfecta (25% and 22% of all cases, respectively), typical sonomorphology accounts for the high rates of completely correct prenatal diagnosis (88% and 89%, respectively) at the first diagnostic examination. Determination of lethality caused mainly by pulmonary hypoplasia secondary to thoracic hypoplasia, in this study was excellent (99%). Other studies have varying rates of accuracy on determination of lethality, ranging from 80-100%.

The prospective study by Ruano, et al. have shown that combination of 3D-HCT and 3D-US identified significantly more abnormalities than did 2D-US (3DHCT: 94.3% (33/35); 3D-US: 77.1% (27/35); 2D-US: 51.4% (18/35); P < 0.01). The diagnosis was made between 27 and 36 weeks' gestation in all cases. The advantage of 3D-HCT over 3D-US was the possibility of imaging the entire fetus. However, it may be impractical to routinely use 3D HCT due to the radiation exposure, its cost and unavailability. Ulla, et al. have recommended that this modality has a complementary role to ultrasound, when fetal skeletal dysplasia is suspected and no definite diagnosis can be made using ultrasound alone. It was especially effective in confirming/excluding bone fractures, recognizing wormian bone and for detailed evaluation of the fetal spine in respect of vertebral body shape and inter-vertebral spaces. It also allowed visualization of the whole fetal skeleton without contamination from maternal anatomy. The images were clear and easily decipherable for experts in skeletal dysplasia not familiar with ultrasound.

Callen, et al. proposed a systematic approach to diagnosis of skeletal dysplasia. Initially, evaluate the long bones as to their individual measurements, presence of demineralization (visualization of unusually prominent falx and decreased echogenicity of spines), degree of long bone curvature (as seen in campomelic dysplasia), and fractures (mostly seen in OI types II and III).

Next important step is to determine the lethality of the skeletal dysplasia through prediction of pulmonary hypoplasia brought about by a hypoplastic thorax. This is important for proper counselling of the parents and for deciding the mode of delivery. Thoracic dimensions in fetuses with known gestational age can be evaluated thru available normograms. For those with unsure gestational age, the thoracic-to-abdominal ratio (normal: 0.77 to 1) and thoracic-to-head circumference ratio (normal 0.56 to 04) can be used. Lung volumetry using 2D and 3D ultrasound as well as femur length/abdominal circumference ratio (lethal – 0.16) can also be used. Hands and feet should be examined for polydactyly, missing digits, and postural deformities including clubfoot and hypoplastic or hitchhiker thumbs. Fetal cranium should be examined for frontal bossing, poor ossification or cloverleaf skull (which can be seen in thanatophoric dysplasia). The fetal face and spines as well as the interal organs should also be investigated. Associated anomalies including hydramnios, fetal hydrops, congenital heart defects and cystic renal malformation should also be noted. Although diagnosis may be determined during the initial sonogram, serial sonograms may help establish the diagnosis firmly.

With findings of persistent short femur in the absence of IUGR, initial considerations in this case were thanatophoric dysplasia and achondroplasia. Ultrasound features of heterozygous achondroplasia include short limbs and fingers, macrocephaly and frontal bossing, with or without hydrocephalus. Thanatophoric dysplasia have similar features but may present also with narrow thorax with short ribs, platyspondyly, polyhydramnios, agenesis of the corpus callosum, cardiac defect, and kidney malformation. Presence of a telephone receiver appearance of the femur pointed more to thanatophoric dysplasia type I. However, with the findings of fractures in utero on serial examination, osteogenesis imperfecta became the main diagnosis. Hypophosphatasia closely resemble our case which may also present with multiple fractures accompanied by shortening and bowing of long bones and marked demineralization of the cranial vault resulting to deformation of the skull after external compression. However, alkaline phosphate was normal in our case. Other differential diagnoses
include fibrochondrogeneses and achondrogenesis. Their common signs project skull demineralization. These two conditions are not compatible with life to say the least.

**Molecular and Biochemical Testing**

Molecular genetic testing utilizes fetal cells obtained by amniocentesis usually performed at about 15-18 weeks AOG or chorionic villus sampling (CVS) at 10-12 weeks AOG is possible if the disease-causing allele of an affected family member has been identified. On the other hand, biochemical analysis of collagen from fetal cells obtained by CVS at 10-12 weeks AOG has been reported. An abnormality of collagen from cultured cells of the proband must be identified before this technique can be used for prenatal testing. Biochemical analysis of collagen from amniocytes is not useful because amniocytes do not produce type I collagen. Both the collagen biopsy test and DNA test are thought to detect almost 90% of all collagen type I collagen mutations. Ideally, these tests should be performed on families with affected relatives. However, these are costly and not easily accessible.

**Management**

**Mode of Delivery**

Decision regarding the mode of delivery is still based on usual obstetric or fetal indications. Retrospective studies have failed to show a significant improvement in the outcomes of fetuses with skeletal dysplasia delivered by cesarean section. If vaginal delivery is chosen, instrumentation probably should be minimized with the most severely affected fetuses to avoid intracranial trauma. The risk of morbidity and mortality of cesarean delivery cannot be ignored especially when this intervention might not protect against fractures in infants with the mild forms of osteogenesis imperfecta.

**Treatment**

There is no cure yet for OI. Treatment is directed toward preventing or controlling the symptoms, maximizing independent mobility, and developing optimal bone mass and muscle strength. However, specific management goals and prognosis of each type is mainly influenced by the degree of bone fragility, whose severity increases in the order: type I, types IV, V, VI, type III, VII, VIII, type II as seen in table 2.

**Handling the Child with OI**

Special attention should be given even to the simple tasks such as changing diapers or lifting the neonate. Although instructions are given to the parents/caretakers, common sense is often the best guide when handling a child with OI.

**Bisphosphonate Therapy**

Bisphosphonates are structural analogues of inorganic pyrophosphate but are resistant to enzymatic and chemical breakdown. Bisphosphonates inhibit bone resorption by selective adsorption to mineral surfaces and subsequent internalization by bone-resorbing osteoclasts where they interfere with various biochemical processes. There are currently at least 10 bisphosphonates (etidronate, clodronate, tiludronate, pamidronate, alendronate, risedronate, zoledronate, and ibandronate and, to a limited extent, olpadronate and neridronate) that have been registered for various clinical applications in various countries. The simpler, non–nitrogen-containing bisphosphonates (eg, clodronate and etidronate) can be metabolically incorporated into non-hydrolysable analogues of adenosine triphosphate (ATP) that may inhibit ATP-dependent intracellular enzymes. In contrast, the more potent, nitrogen-containing bisphosphonates (eg, pamidronate, alendronate, risedronate, ibandronate, and zoledronate) inhibit a key enzyme, farnesyl pyrophosphate synthase, in the mevalonate pathway, thereby preventing the biosynthesis of isoprenoid compounds that are essential for the posttranslational modification of small guanosine triphosphate (GTP)-binding proteins (which are also GTPases) such as Rab, Rho, and Rac. The inhibition of protein prenylation and the disruption of the function of these key regulatory proteins explain the loss of osteoclast activity.

Although their method of action does not predict amelioration of collagen abnormalities found in OI, bisphosphonates has been used in people with OI to increase bone density, mass and strength, reduce fractures and bone deformity, and improve function in children and adults with OI.

A regimen developed by Glorieux, et al. of cyclic IV pamidronate (given in 3-day cycles every 2 to 4
Table 2. Problems and objectives of treatment in three typical situations of osteogenesis imperfecta (OI)\(^2\)

<table>
<thead>
<tr>
<th>MILD FORMS</th>
<th>GOALS</th>
<th>POSSIBLE SOLUTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children</strong></td>
<td><strong>GOALS</strong></td>
<td><strong>POSSIBLE SOLUTIONS</strong></td>
</tr>
<tr>
<td>Decreased bone strength and high fracture rate</td>
<td>Increase bone strength and decrease fracture frequency</td>
<td>Rehabilitation for correct motor and psychological development and after fractures (intramedullary rods in some patients) Bisphosphonates treatment Growth hormone or other growth factors</td>
</tr>
<tr>
<td>Short stature</td>
<td>Increase BMD or collagen synthesis Attain “normal life” Increase stature</td>
<td>Correct job choice Avoid traumatic activities Growth hormone or other growth factors</td>
</tr>
<tr>
<td><strong>Adults</strong></td>
<td><strong>GOALS</strong></td>
<td><strong>POSSIBLE SOLUTIONS</strong></td>
</tr>
<tr>
<td>Hearing loss</td>
<td>Reduce hearing loss or normalize acoustic function</td>
<td>Hearing aids Stapes surgery Cochlear implantation</td>
</tr>
<tr>
<td>Decreased bone strength and high fracture frequency</td>
<td>Increase or avoid decrease of BMD</td>
<td>Bisphosphonate treatment</td>
</tr>
<tr>
<td><strong>OI SEVERE-MODERATE FORMS (TYPES III-IX)</strong></td>
<td><strong>GOALS</strong></td>
<td><strong>POSSIBLE SOLUTIONS</strong></td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td><strong>GOALS</strong></td>
<td><strong>POSSIBLE SOLUTIONS</strong></td>
</tr>
<tr>
<td>Decreased bone strength, high fracture rate, bone deformities</td>
<td>Increase bone strength and decrease fracture frequency Increase BMD</td>
<td>Intensive rehabilitation program Intramedullary rodding in majority of patients Bisphosphonates treatment</td>
</tr>
<tr>
<td>Absence of autonomy</td>
<td>Reach the maximum of autonomy</td>
<td>Assure adequate aids at home and the possibility of autonomy outside (motorized wheelchairs)</td>
</tr>
<tr>
<td>Progressive kyphoscoliosis and reduced ventilatory capacity</td>
<td>Reduce or stop the progression of kyphoscoliosis. Ameliorate respiratory function</td>
<td>Surgical intervention Monitoring oxygen saturation, possible oxygen therapy at home</td>
</tr>
<tr>
<td>Short stature</td>
<td>Increase stature</td>
<td>Cautious use of growth hormone</td>
</tr>
<tr>
<td><strong>Adults</strong></td>
<td><strong>GOALS</strong></td>
<td><strong>POSSIBLE SOLUTIONS</strong></td>
</tr>
<tr>
<td>Hearing loss</td>
<td>Reduce hearing loss or normalize acoustic function</td>
<td>Hearing aids Stapes surgery Cochlear implantation</td>
</tr>
<tr>
<td>Adulthood osteoporosis</td>
<td>Increase BMD</td>
<td>Bisphosphonates therapy</td>
</tr>
</tbody>
</table>

**OI LETHAL PERINATAL (TYPE II)**

<table>
<thead>
<tr>
<th><strong>GOALS</strong></th>
<th><strong>POSSIBLE SOLUTIONS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre- and perinatal fractures</td>
<td>Reduce number of fractures</td>
</tr>
<tr>
<td>Respiratory insufficiency</td>
<td>Assure supportive therapy Treat respiratory infections</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>Adequate caloric intake</td>
</tr>
</tbody>
</table>

months at an annual dose of 9 mg/kg) has been used most successfully, leading to an 88% increase in cortical thickness, a 46% increase in trabecular bone volume, and substantial improvement in functional status. More recently, several studies have demonstrated that daily oral alendronate therapy is
safe and effective in improving QOL in children with OI.\textsuperscript{34}

Antoniazzi, et al. conducted a RCT of 10 children (6 females) with OI type III, 5 (group A) started treatment (2 mg/kg neridronate administered intravenously for 2 consecutive days, every 3 months) just after diagnosis at birth and 5 (group B) after 6 months. Ten untreated children, matched for sex, age, and clinical severity of OI, constituted a historical control group (group C). Group A had better growth and a lower incidence of fractures than groups B and C in the first 6 months of treatment. In the second 6 months, both groups A and B had lower fracture rates than group C. Vertebral body area and the structure of vertebral bodies improved in all treated patients, but especially in group A.

Bisphosphonates have long skeletal half-life. There is evidence that pamidronate can be found in urine specimens up to 8 years after administration. At present, only limited, anecdotal data have assessed the safety of long term pamidronate or other bisphosphonate treatment during fetal development.\textsuperscript{10}

Cochrane review of bisphosphonate use for OI using eight RCTs (403 participants), six pediatric and two adult studies. Authors concluded that both oral and intravenous bisphosphonates increase BMD in children and adults with OI. However, it is unclear whether either treatment decreases fractures. Additional studies may determine whether bisphosphonates improve clinical status (reduce fractures and pain; improve growth and functional mobility) in this population.

Optimal method, dose, initiation, duration of therapy and long-term safety of bisphosphonate therapy requires further investigation.

Reported complications include osteonecrosis of the jaw, atrial fibrillation, oversuppression of bone turnover, hypocalcemia, acute inflammatory response, severe musculoskeletal pain, and esophageal irritation and erosion.

**Surgery**

Indications for surgical realignment and intramedullary rodding are recurrent fractures and severe bowing deformities in children with severe osteogenesis imperfecta who are attempting to stand. The lower extremities are typically involved to a greater extent than the upper extremities functionally. In addition to medical treatment, surgery can decrease pain and reduce the incidence of fracture and consequently, enhance the child’s overall function, comfort, development, and ability to stand and walk. There rarely are indications for operative intervention prior to attempts at standing, but, based on recent studies, there is no advantage to delaying surgery until some arbitrary older age if the child has bone with adequate sized medullary canals to accommodate available rods.\textsuperscript{11}

**Stem Cell Therapy**

Mesenchymal stem cells (MSC) are progenitors of mesenchymal tissues such as bone, cartilage, and adipose. Guillot, et al. have done a successful intrauterine transplantation of fetal MSCs in a mouse model of the intermediate severity type III resulting to markedly reduced fracture rates and skeletal abnormalities. Le Blanc and colleagues were able to transplant allogeneic HLA-mismatched male fetal MSC in utero in a female fetus with confirmed severe OI during 32 weeks AOG, who presented with multiple fractures. From the time of transplant until bone biopsy, the fetus grew appreciably. Complementary bisphosphonate treatment was begun at 4 months. During the first 2 years of life, three fractures were noted. At 2 years of corrected age, psychomotor development was normal and growth followed the same channel, -5 SD. This report showed that the fetal liver MSCs were capable of engrafting and differentiating into bone in the human fetus, even when the recipient was immunocompetent and HLA incompatible, without provoking any graft-vs-host disease in the absence of immunosuppressive therapy.

Further studies are to be done to determine the appropriate stem cell and optimal timing for prenatal transplantation.

**Prognosis**

The prognosis may vary according to the type and severity of symptoms. Frequently encountered problems are respiratory failure, new onset fractures and restricted mobility.

**CONCLUSION**

Osteogenesis imperfecta can be diagnosed prenatally through sonographic detection of persistently short femur and fractures in utero. Early and accurate diagnosis can pave the way for proper genetic counseling and optimizing management of cases of OI through coordination with neonatologists and orthopedic surgeons.
REFERENCES


