A Retrospective Review of the Relation of Ovarian Volume Measured by Transvaginal Ultrasound and Perimenopausal Transition to Menopause*

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Objective: To determine the relation of ovarian volume measured by transvaginal ultrasound and perimenopausal transition to menopause.

Methodology: This was a retrospective cohort review of all transvaginal ultrasound measurements of ovarian volume among reproductive-aged non-pregnant women. Details on their age and menstrual cycle were obtained and menstrual pattern categorized as regular, irregular, less than 1 year of menopause or menopause.

Results: A total of 301 patients were included with a mean age of 45.1 ± 8.84 years. There was a significant decrease in mean volume of either ovary as age of the patient advanced. The ovarian volume dropped significantly between the age category of 30s and 50s (P<0.001). The mean age at onset of irregular menstrual pattern was 45.2 ± 3.71 years with mean ovarian volume of 2.6 ± 1.8cm³ and 2.8 ± 2.06cm³. The mean age of menopause was 55.0 ± 2.59 years with a mean ovarian volume of 1.1 ± 0.9cm³ and 1.1 ± 0.93cm³. At onset of irregular menstrual pattern, the ovarian volume dropped significantly (P<0.001). After the onset of irregular menstrual pattern, there was no further evidence to indicate significant decrease in ovarian volume in the succeeding changes in menstrual pattern.

Conclusion: There was significant inverse correlation between advancing age and declining ovarian volume measured by transvaginal ultrasound. At onset of irregular menstrual pattern, the ovarian volume dropped significantly.

Key words: ovarian volume, transvaginal ultrasound, perimenopausal transition

Menopause is a normal, nature event-defined as the final menstrual period and usually confirmed when a woman has missed her periods for 12 consecutive months in the absence of other obvious causes.1,2 The adult ovary measures 2.5cm to 5.0cm long, 1.5cm to 3.0cm wide and 0.6cm to 1.5cm thick with volumes ranging from 1.2cm to 11.8cm³. The ovarian size peaks in the third decade and declines slowly through menoapuse.1 After the menopause, the ovaries shrink to a size approximately one-half of that seen in the reproductive age. They weigh 3 to 4 grams.3 Most postmenopausal ovaries have a shrunken gyriform external appearance. They are firm and have predominantly solid, pale cut surface.

Andolf, et al., showed that ovarian size decreases in menstruating women over 40 years of age and that this trend is not related to parity.4 Merz, et al. investigated 155 premenopausal women and did not find any parity-related changes in ovarian volume.5 However, postmenopausal women had significantly smaller ovaries and women who were > 5 years into their menopause had smaller ovaries than women < 5 years from the menopause. Higgins, et al. also found a dramatic drop in ovarian volume at the menopause, with the average upper limit of normal falling from 18.3cm³ in premenopausal women to 8cm³ in postmenopausal women.6 Tepper, et al. suggested an ovarian size normogram for postmenopausal women based on transvaginal examinations in 311 healthy women. They found a linear relationship between menopause age and ovarian volume. The mean ovarian volume dropped from 8.6cm³ a year after the menopause to 2.2cm³ 15 years into the menopause.7

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The human ovary contains a fixed pool of primordial follicles, maximal at 5 months of gestational age, which declines with increasing age in bi-exponential fashion, culminating in the menopause at an average age of 50-51 years. The rate of follicle decline represents an instantaneous rate of temporal change based upon the remaining population pool which increases around age 37 years when ~ 25,000 follicles remain and precedes the menopause by 12-14 years. Reproductive aging in women is due to ovarian depletion, with ~ 1000 follicles remaining at menopause.

The precise number of primordial follicles remaining at menopause is unclear, but is of the order of 1000. Ovarian follicles were counted in the ovaries of 43 females aged 6-44 years, following accidental death, and using linear extrapolation, the number of follicles present at menopause was predicted to be 2000. This is now considered an overestimate, as further studies of follicle number present in the ovaries of pre-, peri- and post-menopausal women have demonstrated that <1000 ovarian follicles remain in peri-menopausal women, indicating that follicle decline accelerates in the decade preceding menopause.

Menarche is followed by approximately 5-7 years of relatively long cycles at first, and then there is increasing regularity as cycles shorten to reach the usual reproductive age pattern. In the 40s, cycles begin to lengthen again. At age 25, 40% of cycles are between 25 and 28 days in length; from age 25 to 35, over 60% are between 25 and 28 days. When women are in their 40s, anovulation becomes more prevalent, and prior to anovulation, menstrual cycle length increases, beginning 2 to 8 years before menopause. In an Australian longitudinal study, when cycle length exceeded 42 days, menopause predictably followed within 1 or 2 years. This menstrual cycle change prior to menopause marked by elevated follicle-stimulating hormone (FSH) level and decreased levels of inhibin, but normal levels of luteinizing hormone (LH) and slightly elevated levels of estradiol.

Thus, the alteration of ovarian steroids secretion as a consequence of oocyte and follicle depletion results in modifications of the menstrual cycle. However, these modifications are also aging-related and due to pathologic related changes in the uterus. Twenty percent of perimenopausal women experience menorrhagia or metrorrhagia. From these, 9% have a genital tract malignancy, 14% have endometrial hyperplasia, 70% experience oligomenorrhea and 12% have regular cycles to the onset of postmenopause amenorrhea.

Objectives

General Objective

To determine the relation of ovarian volume measured by transvaginal ultrasound and perimenopausal transition to menopause.

Specific Objectives

To describe the ovarian volume measured by transvaginal ultrasound across the decades of life among Filipino women.

To correlate the ovarian volume measured by transvaginal ultrasound and the menstrual cycle change during perimenopausal transition to menopause.

Definition of Terms

Ovarian Volume

The ovarian dimensions are measured in three planes and ovarian volume is calculated using the prolate ellipsoid formula (Volume = D1 x D2 x D3 x 0.523). D1, D2 and D3 are the three maximal longitudinal, antero-posterior and transverse diameters, respectively. Similarly, the ovarian volume in ml is calculated using the formula (Volume = L x D x W/2).

Perimenopausal Transition

Menstrual irregularity is the only marker that can be used to objectively define and establish what is called the “perimenopausal transition.” The years prior to menopause that encompass the change from normal ovulatory cycles to cessation of menses are known as perimenopausal transitional years, marked by irregularity of menstrual cycles. According to WHO, the term perimenopause should include the period immediately before the menopause (when the endocrinological, biological and clinical features of approaching menopause commence) and the first year after menopause. The term “menopause transition” is used by the WHO to include only the portion of the perimenopause before the final menstrual period.

Menopause

The menopause the time when permanent cessation of menstruation occurs following the loss
of ovarian activity. It is also defined as the final menstrual period and usually confirmed when a woman has missed her periods of 12 consecutive months (in the absence of other obvious causes). According to WHO, it is the “final menstrual period” (retrospectively defined as 1 year without flow).12,13

**MATERIALS AND METHODS**

This was a retrospective cohort review of all transvaginal ultrasound results of reproductive-aged non-pregnant women at the Ultrasound Section from January 2003 to December 2008. Period of study covered all the years when the gynecology ultrasound reports issued by certified OB-Gyne sonologists were compiled and kept in file.

Included in this study were patients with gynecologic transvaginal ultrasound reports with age at time of scan of 30 to 69 years using the 5MHz transvaginal probe of the GE Logic 200 ultrasound machine conducted by board certified OB-Gyne sonologist-consultants of the Section of Ultrasound. For patients with regular menstrual cycles the scan should have been done during the early follicular phase (Day 1 to 5 of cycle). Excluded were women who were pregnant; women with ovarian and/or endometrial pathology, previous ovarian surgery (oophorocystectomy and oophorectomy), previous hysterectomy or endometrial ablation; women on oral contraceptives or any other hormonal treatment; women with intra-uterine device; women with anovulatory status, cases of polycystic ovaries and those who are smokers and obese (BMI > 30kg/m²). All patients included in the study had accessible clinical histories at their respective physicians' offices for review of pertinent demographical data and gynaecologic history. Patients with incomplete records were likewise excluded from the study.

A data collection sheet was filled up for each patient included in the study. Pertinent data obtained from their out-patient records included age, weight and height, medical, personal, social and gynecologic histories. Details on the menstrual cycle were obtained and categorized as gynecologic histories. Details on the menstrual cycle were obtained and categorized as regular, irregular, less than 1 year of menopause or menopause. The date of first day of last normal menses (if applicable) was double checked and correlated with the date of the transvaginal scan. Based on ultrasound reports, measurements of both ovaries, length, width and height were recorded and their respective volumes (cm³) were computed and recorded.

There were a total of 301 patients with complete data included in this study. All data were entered and recorded in MS EXCEL format and computations and analysis were carried out using SSPS version 13.

**RESULTS**

A total of 301 patients were included in this investigative study over the period January 2003 to December 2008. Age of patients included in this study ranged from 30 to 69 years.

Table 1 shows that the mean age of the patients included in the study was 45.1 ± 8.84 years. Mean lengths, widths and heights of both right and left ovaries were similar. The mean of the computed volumes of the right and left ovaries were likewise similar at about 2.9 ± 2.46 cm³ for the right ovary and 2.8 ± 2.56 cm³ for the left ovary.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45.1 ± 8.84 years</td>
</tr>
<tr>
<td>Right Ovary</td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>2.0 ± 0.61 cm</td>
</tr>
<tr>
<td>Width</td>
<td>1.6 ± 0.59 cm</td>
</tr>
<tr>
<td>Height</td>
<td>1.4 ± 0.46 cm</td>
</tr>
<tr>
<td>Volume</td>
<td>2.9 ± 2.46 cm³</td>
</tr>
<tr>
<td>Left Ovary</td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>2.0 ± 0.58 cm</td>
</tr>
<tr>
<td>Width</td>
<td>1.6 ± 0.60 cm</td>
</tr>
<tr>
<td>Height</td>
<td>1.4 ± 0.48 cm</td>
</tr>
<tr>
<td>Volume</td>
<td>2.8 ± 2.56 cm³</td>
</tr>
</tbody>
</table>

There were 107 patients who were in their 30s, 114 women in their 40s, 127 women in their 50s and only 3 women in their 60s. Among the women in their 30s, the mean ovarian volume was 4.8 ± 2.79cm³ for the right ovary and 4.6 ± 2.63cm³ for the left ovary. Among the women in their 40s, the mean ovarian volume was 2.9 ± 1.94cm³ for the right ovary and 3.0 ± 2.62cm³ for the left ovary. Among the women in their 50s, the mean ovarian volume was 1.2 ± 1.00cm³ for the right ovary and 1.2 ± 0.95cm³ for the left ovary. Among the women in their 60s, the mean ovarian volume was 0.7 ± 0.34cm³ for the right ovary and 0.3 ± 0.19cm³ for the left ovary. There was a significant decrease in mean volume of either ovary as age of the patient advanced.
Table 2. Age according to decade category and mean volume of the right and left ovaries.

<table>
<thead>
<tr>
<th>Age (decade category)</th>
<th>No. of Cases</th>
<th>Volume of Right Ovary Mean + SD (cm³)</th>
<th>Volume of Left Ovary Mean + SD (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 to 39 years (30s)</td>
<td>107</td>
<td>4.8 + 2.79</td>
<td>4.6 + 2.63</td>
</tr>
<tr>
<td>40 to 49 years (40s)</td>
<td>114</td>
<td>2.9 + 1.94</td>
<td>3.0 + 2.62</td>
</tr>
<tr>
<td>50 to 59 years (50s)</td>
<td>127</td>
<td>1.2 + 1.00</td>
<td>1.2 + 0.95</td>
</tr>
<tr>
<td>60 to 69 years (60s)</td>
<td>3</td>
<td>0.7 + 0.34</td>
<td>0.3 + 0.19</td>
</tr>
</tbody>
</table>

In Table 3, the one-way analysis of variance was used to determine whether a significant difference exists in at least one pair of age category (in decades). There were significant differences in at least one pair of age category (in decades) (P < 0.001). Post-hoc tests determined at which decade of age category the ovarian volumes of women changed significantly. Post-hoc test showed that on the average, the ovarian volume dropped significantly between the age category of 30s and 50s (P < 0.001). The correlation proves further evidence of this relationship with significant negative coefficients, indicating a moderate inverse relationship - as age increases, the ovarian volumes tend to decrease.

Table 3. One-way analysis of variance (ANOVA), age (in decades) and mean volume of right and left ovaries.

<table>
<thead>
<tr>
<th></th>
<th>Volume of Right Ovary</th>
<th>Volume of Left Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One-way</td>
<td>Post-hoc Test</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>30s vs 40s</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>40s vs 50s</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>50s vs 60s</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Correlation</td>
<td>Coefficient</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.635</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4. Menstrual pattern, mean age and mean volume of right and left ovaries.

<table>
<thead>
<tr>
<th>Menstrual Pattern</th>
<th>Age Mean + SD</th>
<th>Volume of Right Ovary Mean + SD (cm³)</th>
<th>Volume of Left Ovary Mean + SD (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td>36.9 + 5.45</td>
<td>4.4 + 2.71</td>
<td>4.2 + 2.92</td>
</tr>
<tr>
<td>Irregular</td>
<td>45.2 + 3.71</td>
<td>2.6 + 1.8</td>
<td>2.8 + 2.06</td>
</tr>
<tr>
<td>&lt;1-yr Menopause</td>
<td>50.7 + 1.02</td>
<td>2.0 + 1.18</td>
<td>1.8 + 0.95</td>
</tr>
<tr>
<td>Menopause</td>
<td>55.0 + 2.59</td>
<td>1.1 + 0.9</td>
<td>1.1 + 0.93</td>
</tr>
</tbody>
</table>

Table 4 shows that the mean age of women with regular menstrual pattern is 36.9 + 5.45 years with a mean ovarian volume of 4.4 + 2.71cm³ and 4.2 + 2.92cm³. Among those women with irregular menstrual pattern, the mean age was 45.2 + 3.71 years with a mean ovarian volume of 2.6 + 1.8cm³ and 2.8 + 2.06cm³ while those who were about < 1 year into menopause the mean age was 50.7 + 1.02 years with a mean ovarian volume of 2.0 + 1.1cm³ and 1.8 + 0.95cm³. An average ovarian volume less than 3 cm³ was associated with irregular menstrual pattern and amenorrhea of less than 1 year. By menopause status, the mean ovarian volume was about 1 cm³.

In Table 5, the one-way analysis of variance was used to determine whether a significant difference exists between at least one pair of menstrual pattern. There was significant difference in at least one pair of menstrual pattern (P<0.001). Post-hoc tests determined which menstrual pattern showed significant change in ovarian volume. Post-hoc test showed that at onset of irregular menstrual pattern, the ovarian volume dropped significantly (P<0.001). After the onset of irregular menstrual pattern, there is no further evidence to indicate significant decrease in ovarian volume in the succeeding changes in menstrual pattern.
Table 5. One-way analysis of variance (ANOVA), menstrual patterns and mean volume of right and left ovaries.

<table>
<thead>
<tr>
<th>Analysis of Variance</th>
<th>Volume of Right Ovary</th>
<th>Volume of Left Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>one-way</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post-hoc Tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular vs. Irregular</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Irregular vs. &lt;1-yr Menopause</td>
<td>&lt;0.671</td>
<td>&lt;0.220</td>
</tr>
<tr>
<td>&lt;1-yr Menopause vs. Menopause</td>
<td>&lt;0.247</td>
<td>&lt;0.604</td>
</tr>
</tbody>
</table>

DISCUSSION

Measuring the ovarian volume and estimating its size are not common practice, nor is the relevance of ovarian size and its clinical implications in normal and pathological conditions clear. Currently, there are very few publications on ovarian volume in normal healthy fertile women in their reproductive age.

The human ovary contains a fixed number of primordial follicles that decrease biexponential with age, culminating in menopause at an average age of 50-51 years. There currently is no reliable test of ovarian reserve for individual women that will accurately predict their remaining reproductive lifespan. The rate of follicle decline represents an instantaneous rate of temporal change based upon the remaining population pool which increases around age 37 years when ~ 25,000 follicles remain precedes the menopause by 12-14 years. Reproductive aging in women is due to ovarian depletion, with ~ 1000 follicles remaining at menopause.

Using published data on age-related ovarian volume as measured by transvaginal sonography, there is a highly significant correlation between primordial follicle population and ovarian volume. Results showed that ovarian volume in women aged 25-51 years accurately reflects the number of primordial follicles remaining, and describe how measurement of ovarian volume by transvaginal ultrasonography may determine ovarian reserve and reproductive age. Only measurements of ovaries not containing cysts or large follicles will achieve an accurate net ovarian volume. Therefore in most of these studies, only ovaries with follicles of <10-15mm were included. However, the maximum follicular size for ovarian volume measurement without skewing the net results is not clear.

For each chronological age, from 25 to 51 years, Wallace and Kelsey (2004) determined the primordial follicle population from the Faddy-Gosden equation. They have demonstrated a significant correlation between primordial follicle population (for both average and late menopausal women) and ovarian volume. This provides good evidence that ovarian volume in women aged 25-51 years is strongly associated with the remaining primordial follicle population. They have shown that ovarian reserve and reproductive age in healthy pre-menopausal women, who are not using hormonal contraception, can be determined from the measurement of ovarian volume by transvaginal sonography.

Andolf, et al. showed that ovarian size decreases in menstruating women over 40 years of age and that this trend is not related to parity. Merz, et al. investigated 155 premenopausal women and did not find any parity-related changes in ovarian volume. Christensen, et al. (1997) measured the ovarian volume of 428 healthy women aged 14-45 who attended a family planning clinic. They found that the ovarian volume was not correlated to age, height, weight and parity. While the smaller ovary had the same volume throughout the cycle, the larger ovary increased in size from the beginning of cycle to day 19 and decreased thereafter due to the development of the preovulatory follicle in that ovary. The ovarian volumes in women with intrauterine devices were shown to be larger than in women on the contraceptive pills. However, cycle variations in volume were not observed in the latter.

There is good evidence that adult ovarian volume decreases with increasing age as the remaining pool of primordial follicles becomes exhausted. As part of the University of Kentucky Ovarian Cancer Screening program, 13,963 women between 25 and 91 years of age underwent annual transvaginal sonography. From 58,673 observations of ovarian volume, a statistically significant decrease in ovarian volume was shown with each decade of life from age 30 to 70 years. Mean ovarian volume was 6.6 ± 0.19cm³ in women <30 years old, 6.1 ± 0.06cm³ in women 30 to 39 years, 4.8 ± 0.03cm³ in women aged 40 to 49 years, 2.6 ± 0.01cm³ in women 50 to 59 years old and 2.1 ± 0.01cm³ in women aged 60 to 69 years. Mean ovarian volume was 4.9cm³ in pre-menopausal women and 2.2cm³ in post menopausal women.

In this investigative study, a total of 301 patients were included with age range from 30 to 69 years. The mean age of the patients included in the study was 45.1 ± 8.84 years. Mean lengths, widths and
heights of both right and left ovaries were similar. The mean of the computed volumes of the right and left ovaries were likewise similar at about $2.9 + 2.46$ cm$^3$ for the right ovary and $2.8 + 2.56$ cm$^3$ for the left ovary.

As compared to the measurement finding of Pavlik, et al. (2000) among patients from the University of Kentucky, volume measurements by age group in our study population was consistently smaller. Among the women in their 30s, the mean ovarian volume was $4.8 + 2.79$ cm$^3$ for the right ovary and $4.6 + 2.63$ cm$^3$ for the left ovary. Among the women in their 40s, the mean ovarian volume was $2.9 + 1.94$ cm$^3$ for the right ovary and $3.0 + 2.62$ cm$^3$ for the left ovary. Among the women in their 50s, the mean ovarian volume was $1.2 + 1.00$ cm$^3$ for the right ovary and $1.2 + 0.95$ cm$^3$ for the left ovary. Among the women in their 60s, the mean ovarian volume was $0.7 + 0.34$ cm$^3$ for the right ovary and $0.3 + 0.19$ cm$^3$ for the left ovary. However, there was likewise a significant decrease in mean volume of either ovary as age of the patient advanced.

Similar to previous reports, results of this study showed that there was significant and inverse correlation between advancing age and declining individual ovarian dimensions. This relationship was statistically significant for each of the ovarian dimensions. A decrease in the ovarian size parameters (length, width, height and volume) accompanied advancing age. In the one-way analysis of variance to determine whether a significant difference exists between at least one pair of age category (in decades), there was significant difference in at least one pair of age category (in decades) ($P<0.001$). Post-hoc test showed that on the average, the ovarian volume dropped significantly between the age category of 30s and 50s ($P<0.001$). The correlation proves further evidence of this relationship with significant negative coefficients, indicating a moderate inverse relationship - as age increases, the ovarian volumes tend to decrease.

When women are in their 40s, anovulation becomes more prevalent, and prior to anovulation, menstrual cycle length increases, beginning 2 to 8 years before menopause. In an Australian longitudinal study, when cycle length exceeded 42 days, menopause predictably followed within 1 or 2 years. This menstrual cycle change prior to menopause marked by elevated follicle-stimulating hormone (FSH) level and decreased levels of inhibin, but normal levels of luteinizing hormone (LH) and slightly elevated levels of estradiol.  

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Postmenopausal women had significantly smaller ovaries and women who were > 5 years into their menopause had smaller ovaries than women < 5 years from the menopause. Higgins, et al. also found a dramatic drop in ovarian volume at the menopause, with the average upper limit of normal falling from 18.3 cm$^3$ in premenopausal women to 8 cm$^3$ in postmenopausal women.  

The mean ovarian volume dropped from 8.6 cm$^3$ a year after the menopause to 2.2 cm$^3$ 15 years into the menopause.  

Webba, et al. compared 98 postmenopausal women to 40 with regular periods and showed a decrease in ovarian volume after the
first year of menopause followed by slow and gradual shrinkage thereafter and more significantly after 4 years in the menopause.\textsuperscript{18}

In this study, the mean ovarian volume of women with regular menstrual pattern was $4.4 + 2.71 \text{ cm}^3$ and $4.2 + 2.92 \text{ cm}^3$ while among those women with irregular menstrual pattern, possibly perimenopausal, the mean ovarian volume was $2.6 + 1.8 \text{ cm}^3$ and $2.8 + 2.06 \text{ cm}^3$. Compared to the previous reports, in this study, those who were about $< 1$ year into menopause, the mean ovarian volume was $2.0 + 1.18 \text{ cm}^3$ and $1.8 + 0.95 \text{ cm}^3$. The mean age of menopause status in the study population was $55.0 + 2.59$ years with a mean ovarian volume of $1.1 + 0.9 \text{ cm}^3$ and $1.1 + 0.93 \text{ cm}^3$, a mean volume smaller than the previous reports. In our study population, an average ovarian volume less than 3 cm\textsuperscript{3} was associated with irregular menstrual pattern and amenorrhea of less than 1 year. By menopause status, the mean ovarian volume was about 1 cm\textsuperscript{3}.

Results showed that at onset of irregular menstrual pattern, possibly perimenopausal, the ovarian volume dropped significantly (P<0.001). After the onset of irregular menstrual pattern, there was no further evidence to indicate significant decrease in ovarian volume in the succeeding changes in menstrual pattern.

There is wide variation in age of menopause. A prospective study of 529 Western women gives a mean age of 50.4 years with an SD of 3.9 years. This gives a 95\% confidence interval (CI) for cessation of menses of 42.8 - 58.0 years.\textsuperscript{19} A more recent study of 4,686 women is in close agreement with a mean age of 50.16 years and an SD of 4.15 years.\textsuperscript{20} The wide variation in age at menopause of otherwise healthy Western women must be due to either variation in the rate of primordial follicles present at birth, for which there is good histological evidence.\textsuperscript{19,20,21} It has been accepted for some time that it is a critical number of primordial follicles rather than a critical age that determines the timing of menopause.\textsuperscript{22}

The precise number of primordial follicles remaining at menopause is unclear, but is of the order of 1000. Ovarian follicles were counted in the ovaries of 43 females aged 6-44 years, following accidental death, and, using linear extrapolation, the number of follicles present at menopause was predicted to be 2000.\textsuperscript{10} This is now considered an overestimate, as further studies of follicle numbers present in the ovaries of pre, peri- and post-menopausal women have demonstrated that <1000 ovarian follicles remain in peri-menopausal women, indicating that follicle decline accelerates in the decade preceding menopause.\textsuperscript{8}

There are no reliable markers or clinical methods to assess ovarian reserve accurately in the normally menstruating pre-menopausal woman. Follicular density measured in ovarian biopsies from infertile women show a significant negative correlation with increasing age. Women > 35 years of age have mean ovarian volume significant smaller than in women < 35 years and have been shown to have only a third of the follicles of younger women.\textsuperscript{23}

**CONCLUSION**

In this investigative study, a total of 301 patients were included. Their ages ranged from 30 to 69 years. (Mean age of 45.1 + 8.84 years). Mean lengths, widths and heights of both right and left ovaries were similar. The mean of the computed volumes of the right and left ovaries were likewise similar at about $2.9 + 2.46 \text{ cm}^3$ for the right ovary and $2.8 + 2.56 \text{ cm}^3$ for the left ovary. Volume measurements by age group in our study population were consistently smaller as compared to published reports. However, as in published reports, there was a significant decrease in mean volume of either ovary as age of the patient advanced. On the average, the ovarian volume dropped significantly between the age category of 30s and 50s (P<0.001).

Similarly, in this study, the mean age at onset of irregular menstrual pattern, probably perimenopausal, was 45.2 + 3.71 years. The mean age of menopause status in the study population was $55.0 + 2.59$ years with a mean ovarian volume of $1.1 + 0.9 \text{ cm}^3$ and $1.1 + 0.93 \text{ cm}^3$, a mean volume smaller than the previous reports. Results showed that at onset of irregular menstrual pattern, possibly perimenopausal, the ovarian volume dropped significantly (P<0.001). After the onset of irregular menstrual pattern, there was no further evidence to indicate significant decrease in ovarian volume in the succeeding changes in menstrual pattern.

**RECOMMENDATIONS**

In recent years, there has been a rapid increase in the use of transvaginal sonography in gynecology and reproductive medicine and measurement of ovarian volume is emerging as an important tool in the screening, diagnosis and monitoring of treatment of conditions such as infertility, ovarian stimulation, ovarian cancer and abnormalities of adolescence. In reproductive medicine, it would appear that ovarian
volume has a role in the assessment of ovarian reserve and perimenopausal transition. However, more studies are still required to explore the full potential benefit of this simple, safe and cost-effective technique. We recommend that a local, multicenter prospective construction of ovarian volume normogram be done together with validation of correlation with perimenopausal transition, both with clinical and biochemical manifestations.

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Effects of Oral Reduced Glutathione Treatment on Semen Characteristics of Male Infertility Patients with Oligozoospermia: A Randomized, Placebo-Controlled Trial

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Objective: To determine the effects of oral reduced glutathione treatment on the semen quality of male infertility patients diagnosed with oligozoospermia.  
Design: Experimental; randomized, placebo-controlled trial.  
Setting: Tertiary referral center.  
Study Population: Male infertility patients aged 21 to 40 years; diagnosed of having oligozoospermia on semen analysis; with at least one year of attempting to achieve pregnancy; randomized to either glutathione or identical placebo capsules to be taken once daily. Repeat semen analyses were done at the end of the 1st, 2nd and 3rd month of treatment.  
Outcome measures: Semen parameters such as semen volume, total sperm count, sperm motility, sperm morphology and leucocyte count per month were determined and analyzed for statistical significance.  
Results: Oral reduced glutathione therapy demonstrated a statistically significant positive effect on total sperm count and absolute total motile sperms, but no significant effect on percent motility and morphology.  
Conclusion: The findings of this study indicate that oral glutathione therapy could be a potential treatment for oligozoospermia.  

Key words: reduced glutathione, antioxidants, oligozoospermia

Worldwide, infertility affects approximately 10-15 percent of couples trying to conceive. Male factor infertility, specifically oligozoospermia, is implicated as the sole or a contributory factor in nearly half of these cases. Assisted reproductive technologies remain an effective option for these infertile couples. However, these procedures may be costly and not without inherent risk. Accordingly, the identification of a treatment for the underlying cause of oligozoospermia will therefore help allow couples to regain fertility and conceive naturally on their own.  

The goal of specific medical management of infertility is to diagnose reversible causes of infertility and treat them with appropriate medications to achieve seminal improvement and ultimately, pregnancy. A variety of empiric medical therapies have been recommended to treat these patients. However, with few exceptions, none of these therapies have been shown to be effective in repeated controlled randomized studies. Effective treatments are available; however, they are expensive and are virtually inaccessible to most patients. Hence, further development of effective, more affordable treatment methods is highly desirable. Such approaches could include improvements in sperm quality and identification of more treatment-responsive subgroups of infertile men.  

Numerous factors may influence male infertility. Of which, oxidative stress has generated interest among researchers in recent period. Oxidative stress occurs when the delicate balance between the production of reactive oxygen species (ROS) and the inherent antioxidant capacity of any system is distorted.  

ROS are highly reactive oxidizing agents belonging to the class of free radicals containing one or more unpaired electrons which are continuously being generated through various metabolic and pathophysiologic processes in our system. At different
levels, like at the cell membrane; the cellular proteins; and the nuclear DNA, the excess of ROS can affect the living cell leading to different types of damage. Human spermatozoa are especially susceptible as their plasma membrane contains abundant polyunsaturated fatty acid (PUFA) which can undergo lipid peroxidation in the presence of ROS, grossly damaging them. Herein lays the potential therapeutic role of antioxidants for cases of dyspermia. In order to verify these findings, it is the objective of this experimental study to investigate the effects of a powerful antioxidant in the form of oral glutathione on the semen quality of male infertility patients diagnosed with oligozoospermia - especially, in terms of total sperm count, motility, morphology and volume.

Through this research paper, we aim to explore the prospect of oral reduced glutathione as a cost-effective treatment for male infertility with oligozoospermia. We further determine the effectiveness of the single daily dose of 600 mg oral reduced glutathione in improving semen parameters.

**MATERIALS AND METHODS**

**Study Protocol**

The investigation was a single-center, double-blind, placebo-controlled, randomized study. The protocol was approved by the Ethics Review Board of the hospital where the study was conducted. Before enrollment in the study, each subject's written informed consent was obtained in response to a fully written and verbal explanation of the nature of the study. Patient information for this study remained confidential and within the institution.

**Study Subjects**

Male partners of patients seen at the outpatient department consulting for infertility and diagnosed to have oligozoospermia on conventional semen analysis were enrolled. The following were the inclusion and exclusion criteria:

a. Inclusion Criteria:

- i. Seminological inclusion criteria:
  - total sperm count > 1 x 10^6 and <40 x 10^6
  - leucocyte concentration < 20 hpf
- ii. Age range 21 - 40 years old
- iii. At least 1 year of unprotected coitus attempting to achieve pregnancy

b. Exclusion Criteria:

- i. patients with clinical history of chronic systemic diseases, hormonal pathologies, cryptorchidism and uni- or bilateral hypotrophy of the testes
- ii. patients with undescended testes, or with uncorrected defect of the genitourinary tract
- iii. patients who underwent or are undergoing cancer chemotherapy, colchicines or hormonal preparation which directly suppress spermatogenesis

c. Withdrawal and Dropouts:

- i. patients who will withdraw their consent for inclusion in the study
- ii. patients who will fail to follow-up as scheduled

The patients were randomly and blindly assigned either to the glutathione treatment or the placebo group. Medical histories of patients and their female partners were recorded using a standardized patient database form. The patients underwent baseline semen analysis using the WHO criteria. All semen analyses were carried out by the same examiner (a licensed medical technologist) in a blind protocol. For proper collection of semen, subjects were advised 2 days of sexual abstinence prior to semen collection. Semen samples were obtained by masturbation with collection of the semen sample into a clean cup in the privacy of a designated collection room in the andrology laboratory.

Subjects were given 600mg reduced glutathione capsules or identical capsules (placebo) to be taken once daily.

Repeat semen analyses were done after the 1st month, after the 2nd month and after the 3rd month of treatment. Semen parameters such as semen volume, motility, total sperm count, sperm morphology, and leucocyte count were determined.

**Statistical Analysis**

All variables were checked for normal distribution by applying the one-sample t-test for goodness of fit. Means and standard deviations were calculated on the variables measured in the two groups at different times. To offset the possibility of a skewed distribution due to the relatively small
population, bootstrapping or resampling was done to arrive at more accurate measurements. Bootstrapping provides a way of accounting for the distortion caused by the specific sample that may not be fully representative of the population. Factorial analysis of variance (ANOVA) for repeated measurements was applied for testing significant differences between the study groups. Differences with P-values < 0.05 were considered significant. Computations were performed using the statistical software package SPSS v9.1.

RESULTS

Of the 27 patients who initially signed up for the study, 13 received glutathione treatment, while 14 received placebo capsules. Results from 7 patients were rejected from analysis, 3 from the glutathione group owing to poor compliance, and 4 from the placebo group due to failure to follow-up.

A total of 20 patients completed the trial (10 for glutathione group, 10 for placebo group). None of the patients showed side effects. The oral regimen was well-tolerated.

Baseline (pre-treatment) clinical parameters of both groups are presented in Table 1. Patient age for both groups had mean values of 35-37 years old, with a mean of 4-5 years of infertility. All P-values for the baseline parameters, including semen characteristics, are greater than 0.05, indicating no significant differences in the distribution of patients per group.

The semen parameters in the pre-treatment phase and after 1 month, 2 months and 3 months of treatment are presented in Table 2 (glutathione group vs placebo group, for each month of treatment). Only the total sperm count clearly and consistently manifested significant changes/improvement after glutathione treatment on all 3 months of treatment period (month 1 P-value = 0.024, month 2, P-value = 0.007, month 3 P = 0.004). This is an effect which was observed even after the first month of treatment (Table 3 P = 0.034). However, no significant changes were observed in terms of percent motility and morphology (all P-values > 0.05). Figures 1, 2 and 3 are boxplot representations of means and distribution placebo group and glutathione group, each for month 1 (figure 1, P = 0.034), month 2 (figure 2, P = 0.013) and month 3 (figure 3, P = 0.022). Another graphic representation of the outstanding improvement in total sperm count across the treatment months is depicted in figure 3. Figure 5 is a scatter plot depicting the final total sperm count for all 10 subjects, after 3 months of treatment, with 7 out of the 10 actually achieving normal sperm count values at the end of treatment. Figure 6 is a linear graph showing the increasing trend in the total sperm count across all treatment months, compared with placebo, with greatest increase noted after only 1 month of treatment.

Table 3 examines the glutathione group and compares the differences in semen parameters between each treatment month and baseline value. Table 4, on the other hand, employed the one-way ANOVA test for repeated measurements to analyze the significant differences between pre-treatment values and treatment values. Both tables depict the same results: only total sperm count showed significant, consistent improvement, among the semen parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo group</th>
<th>Glutathione group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (years)</td>
<td>37.1 ± 2.5</td>
<td>35.1 ± 4.2</td>
<td>0.214</td>
</tr>
<tr>
<td>Age of female partner (years)</td>
<td>30 ± 3.1</td>
<td>32 ± 2.3</td>
<td>0.108</td>
</tr>
<tr>
<td>Number of years of infertility</td>
<td>5.4 ± 3.4</td>
<td>4.6 ± 2.4</td>
<td>0.55</td>
</tr>
<tr>
<td>Total sperm count (millions)</td>
<td>21.5 ± 13.2</td>
<td>13.4 ± 8.5</td>
<td>0.169</td>
</tr>
<tr>
<td>Sperm morphology (%)</td>
<td>77 ± 30.9</td>
<td>72 ± 17.7</td>
<td>0.625</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>38 ± 21</td>
<td>60 ± 28.4</td>
<td>0.077</td>
</tr>
<tr>
<td>Semen volume</td>
<td>2.1 ± 0.8</td>
<td>2.5 ± 1.08</td>
<td>0.36</td>
</tr>
<tr>
<td>Leucocyte count</td>
<td>5.8 ± 4.01</td>
<td>3.5 ± 2.01</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Table 2. Total sperm count, motility, morphology and volume: means and standard deviation at various times for placebo group vs glutathione group with independent t-tests and bootstrapping between groups.

<table>
<thead>
<tr>
<th>Semen parameter</th>
<th>t-ratio</th>
<th>p-value</th>
<th>Decision</th>
<th>P-value</th>
<th>Bootstrap Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Count</td>
<td>-1.37</td>
<td>0.187</td>
<td>NS</td>
<td>0.169</td>
<td>NS</td>
</tr>
<tr>
<td>Motility</td>
<td>1.82</td>
<td>0.085</td>
<td>NS</td>
<td>0.077</td>
<td>NS</td>
</tr>
<tr>
<td>Morphology</td>
<td>0.46</td>
<td>0.649</td>
<td>NS</td>
<td>0.625</td>
<td>NS</td>
</tr>
<tr>
<td>Volume</td>
<td>0.541</td>
<td>0.551</td>
<td>NS</td>
<td>0.071</td>
<td>NS</td>
</tr>
<tr>
<td>Leucocyte ct</td>
<td>0.06</td>
<td>NS</td>
<td>NS</td>
<td>0.017</td>
<td>NS</td>
</tr>
<tr>
<td>Month 1 Count</td>
<td>2.47</td>
<td>0.024</td>
<td>S</td>
<td>0.023</td>
<td>S</td>
</tr>
<tr>
<td>Motility</td>
<td>-0.34</td>
<td>0.735</td>
<td>NS</td>
<td>0.725</td>
<td>NS</td>
</tr>
<tr>
<td>Morphology</td>
<td>0.05</td>
<td>0.964</td>
<td>NS</td>
<td>0.967</td>
<td>NS</td>
</tr>
<tr>
<td>Volume</td>
<td>0.435</td>
<td>NS</td>
<td>NS</td>
<td>0.467</td>
<td>NS</td>
</tr>
<tr>
<td>Leucocyte ct</td>
<td>0.334</td>
<td>NS</td>
<td>NS</td>
<td>0.43</td>
<td>NS</td>
</tr>
<tr>
<td>Month 2 Count</td>
<td>3.08</td>
<td>0.007</td>
<td>S</td>
<td>0.010</td>
<td>S</td>
</tr>
<tr>
<td>Motility</td>
<td>0.12</td>
<td>0.905</td>
<td>NS</td>
<td>0.894</td>
<td>NS</td>
</tr>
<tr>
<td>Morphology</td>
<td>-0.54</td>
<td>0.599</td>
<td>NS</td>
<td>0.580</td>
<td>NS</td>
</tr>
<tr>
<td>Volume</td>
<td>0.867</td>
<td>NS</td>
<td>NS</td>
<td>0.811</td>
<td>NS</td>
</tr>
<tr>
<td>Leucocyte ct</td>
<td>0.438</td>
<td>NS</td>
<td>NS</td>
<td>0.514</td>
<td>NS</td>
</tr>
<tr>
<td>Month 3 Count</td>
<td>3.32</td>
<td>0.004</td>
<td>S</td>
<td>0.005</td>
<td>S</td>
</tr>
<tr>
<td>Motility</td>
<td>1.65</td>
<td>0.116</td>
<td>NS</td>
<td>0.106</td>
<td>NS</td>
</tr>
<tr>
<td>Morphology</td>
<td>-0.07</td>
<td>0.948</td>
<td>NS</td>
<td>0.953</td>
<td>NS</td>
</tr>
<tr>
<td>Volume</td>
<td>0.678</td>
<td>NS</td>
<td>NS</td>
<td>0.698</td>
<td>NS</td>
</tr>
<tr>
<td>Leucocyte ct</td>
<td>0.347</td>
<td>NS</td>
<td>NS</td>
<td>0.356</td>
<td>NS</td>
</tr>
</tbody>
</table>

S - significant; NS - not significant

Table 3. Total count, motility, morphology and volume of glutathione group: determining significant changes between baseline values and post-treatment at different months.

<table>
<thead>
<tr>
<th>Glutathione Group (P-value)</th>
<th>Total Count</th>
<th>Motility</th>
<th>Morphology</th>
<th>Volume</th>
<th>WBC ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline vs post-treatment month 1</td>
<td>0.034 (S)</td>
<td>0.058 (S)</td>
<td>0.032 (S)</td>
<td>0.43 (NS)</td>
<td>0.06 (NS)</td>
</tr>
<tr>
<td>Baseline vs post-treatment month 2</td>
<td>0.013 (S)</td>
<td>0.124 (NS)</td>
<td>0.20 (NS)</td>
<td>0.98 (NS)</td>
<td>0.12 (NS)</td>
</tr>
<tr>
<td>Baseline vs post-treatment month 3</td>
<td>0.022 (S)</td>
<td>0.043 (S)</td>
<td>0.134 (NS)</td>
<td>0.29 (NS)</td>
<td>0.134 (NS)</td>
</tr>
</tbody>
</table>

S - significant NS - not significant

Figure 1. Boxplot representation of total sperm count 1 month post-treatment showing significant difference of the mean in glutathione group as opposed to placebo group.

Figure 2. Boxplot representation of total sperm 2 months post-treatment showing significant difference of the mean in glutathione group as opposed to placebo group. Placebo group showed one outlier.
Figure 3. Boxplot representation of total sperm count 3 months post-treatment showing significant difference of the mean in glutathione group as opposed to placebo group.

Figure 4. Boxplot representation of total sperm count values from pre-treatment to 3 months of treatment. Means of each treatment period are shown.

Figure 5. Scatter plot post-treatment total sperm count values (after 3rd month of treatment). Note that 7 out of 10 subjects actually achieved normal total sperm count.

Figure 6. Line graph representation of improvement of sperm count from pre-treatment to 3 months post-treatment.

Table 4. One-way ANOVA for repeated measurements to determine significant differences between the treatment period and the baseline values.

<table>
<thead>
<tr>
<th>Semen Parameter</th>
<th>f-ratio</th>
<th>p-value</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>3.58</td>
<td>0.023</td>
<td>S</td>
</tr>
<tr>
<td>Motility</td>
<td>1.75</td>
<td>0.174</td>
<td>NS</td>
</tr>
<tr>
<td>Morphology</td>
<td>2.51</td>
<td>0.075</td>
<td>NS</td>
</tr>
<tr>
<td>Volume</td>
<td>1.25</td>
<td>0.145</td>
<td>NS</td>
</tr>
</tbody>
</table>

S - significant
NS - not significant

Improvement in percent motility manifested only after the 3rd month of treatment (P = 0.043). Figures 7 is a boxplot representation of the means and distribution of values for motility in percent for all treatment months. Although a significant difference is noted, the increasing trend in motility is quite apparent. Remarkably though, when we converted the percent motility across all treatment months into absolute values, we noted a dramatic improvement in the actual number of sperms with good motility (Table 5). Figure 8 is a linear graph representation of the increasing trend of the absolute motility count from baseline values up to the end of treatment.

A significant difference in terms of percent morphology was observed only on the first month of treatment (P = 0.032). Figure 9 is a boxplot representation of the mean and distribution of values for morphology in percent for all treatment months. No clear trend or pattern is evident in terms of morphology.
Table 5. Number of motile sperms converted to absolute count: determining significant changes between baseline values and post-treatment at different months.

<table>
<thead>
<tr>
<th>Glutathione Group</th>
<th>Absolute Count of Motile Sperms</th>
<th>P value</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline vs post-treatment Month 1</td>
<td>0.036</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Baseline vs post-treatment Month 2</td>
<td>0.019</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Baseline vs post-treatment Month 3</td>
<td>0.013</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 shows no significant difference in the number of leucocytes found across all treatment months.

Table 6. Agents used for treatment of idiopathic oligozoospermia.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Main Action Site</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-estrogens</td>
<td>Hypothalamus</td>
<td>Clomiphene; tamoxifen</td>
</tr>
<tr>
<td>GnRH</td>
<td>Anterior pituitary</td>
<td>GnRH preparations</td>
</tr>
<tr>
<td>hMG/hCG</td>
<td>Testicular action</td>
<td>hMG, hCG</td>
</tr>
<tr>
<td>FSH</td>
<td>Testicular action</td>
<td>u-FSH, hp-FSH, rec-FSH</td>
</tr>
<tr>
<td>Weak androgens</td>
<td>Accessory glands</td>
<td>Mesterolene, testosterone</td>
</tr>
</tbody>
</table>

No pregnancies have yet been reported during the study period in either placebo or glutathione groups.

DISCUSSION

Many therapies for male infertility have been tested using drugs that theoretically could improve semen parameters by acting directly on sperm production or epididymal maturation. Spermatogenesis occurs in the male testes and...
epididymis in a stepwise fashion, and for humans, it takes approximately 70-90 days. Spermatogenesis is highly dependent upon optimal conditions for the process to occur correctly. It starts at puberty and usually continues uninterrupted until death (figure 10). Glutathione and other antioxidants play key roles in building and maintaining the sperm integrity almost throughout the whole process of spermatogenesis (figure 11). The length of time for spermatogenesis to complete is the basis of the duration of treatment (3 months) employed in this study.

Figure 10. Spermatogenesis cycle. Spermatogenesis takes approximately 70 days. Spermatogenesis is under the control of 2 somatic cells: the Leydig cells which have LH receptors and Sertoli cells which have FSH receptors. LH stimulates Leydig cell synthesis of testosterone, which acts on Sertoli cells together with FSH to support all of the phase of spermatogenesis. Testosterone exerts a negative feedback control over LH secretion, while inhibin secreted by Sertoli cells, exerts a negative feedback control over FSH secretion. All aspects of Sertoli cell function seem to depend on adequate stimulation by FSH, which plays a central role on the control of testicular function. (adapted from Best Practice and Research Clinical Obstetrics and Gynecology, 2003; 17 (2)).

Glutathione seems to be the drug most likely to treat conditions such as oligozoospermia, as it has already been tested in other degenerative pathologies (cirrhosis, neoplasia, consequences of anti-neoplastic therapies). Our experimental research with orally administered glutathione, is intended to be a corollary to the landmark randomized, double-blind, placebo-controlled study by Lenzi, et al. which investigated on the effects of parenteral glutathione on semen parameters. In that study, glutathione therapy demonstrated a statistically significant positive effect on sperm motility and sperm morphology, however, with no significant effect on the total sperm count and concentration.

Surprisingly however, our findings are not quite similar to that of Lenzi and colleagues. Instead, we observed a statistically significant increase in total sperm count and no significant difference was noted in terms of percent motility, percent morphology and volume. Nevertheless, the remarkable improvement in total sperm count is a confirmation of the pharmacological effect of glutathione. Moreover, the pharmacologic action of glutathione has to be a post-testicular effect since it did not need a complete cycle of spermatogenesis (90 days) to show changes. The improvement manifested as early as the first month (30 days) of treatment.

Based on the established physiologic role of glutathione as an antioxidant, results of this study admittedly deviates from the expected outcome. Improvement of sperm motility in percent was the intended outcome. This is the limitation we encountered doing this study with the conventional WHO semen analysis parameters which reports motility in percentages rather than in absolute numbers. If we correlate this with the boxplot graph...
shown in figure 6, we note that the means of the values from pre-treatment up to 3 months of treatment are relatively close and therefore registered “no significant” on independent t-test (P >0.05). However, the fact that the total sperm count remarkably increased with no significant change in percentage motility, we could deduce that the absolute number of motile sperm also increased proportionately. After converting the percent motility into absolute number of motile sperms, we found a dramatic increase from baseline values all throughout the 3 months of treatment (Table 5).

**Reactive Oxygen Species on Impaired Sperm Function, and the Role of Glutathione**

Impaired sperm function is an obvious and general cause of male infertility. The controlled generation of reactive oxygen species (ROS) has a physiologic role in spermatozoa functions such as hyperactivation, capacitation and acrosome reaction. Uncontrolled and excessive production of ROS, however, seems to have a significant role as one of the major factors leading to subfertility or even infertility. Excessive ROS production causes oxidative stress, resulting in decreased sperm motility, viability, and increased midpiece sperm defects that impair sperm capacitation and acrosome reaction. Human spermatozoas are known to be susceptible to loss of motility in the presence of exogenous H$_2$O$_2$, as a consequence of lipid peroxidation. This susceptibility of human spermatozoa to oxidative stress is a consequence of the abundance of polyunsaturated fatty acids (PUFA) in the sperm plasma membrane that gives this structure the fluidity it needs to engage in the membrane fusion events associated with fertilization. Unfortunately, the presence of double bonds in the molecules make them vulnerable to free radical attack and the initiation of a lipid peroxidation cascade.

In order to counteract the harmful effects of ROS, sperms and seminal plasma possess a number of antioxidant systems that scavenge ROS and prevent internal cellular damage. These antioxidants compensate for the loss of cytoplasmic enzymes as the cytoplasm is extruded during spermatogenesis (figure 8), which in turn, diminishes endogenous repair mechanisms and enzymatic defense. Due to the scant cytoplasm in the midpiece of the spermatozoa, the ability to scavenge oxidants is very much limited. Indeed, the relative lack of cytoplasmic space is a striking feature of human spermatozoa, and contributes greatly to their vulnerability to oxidative stress. Fortunately, seminal plasma and epididymal fluid are potent sources of antioxidants and act as nutritive-protective medium for sperm cells during maturation. At the same time however, it could also be a hostile environment when andrological pathologies alter its anti/pro-oxidant equilibrium especially when the sperm membrane is more fragile. The higher epididymal concentration of glutathione obtained by therapy can thus play a protective role. Pretreatment with antioxidants the dispose, scavenge, and suppress the formation of ROS can also reduce DNA damage.

Several studies have been made on the existence of enzymatic defenses in spermatozoa and seminal plasma, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). Cellular glutathione plays an important role in many biological processes including the protection against damage produced by oxidants, electrophiles, and free radicals. This is mainly due to its ability to react directly with hydrogen peroxide and superoxide-anion, hydroxyl, and alkoyl radicals by its free sulphhydryl groups.

In human reproductive tissues like the seminal plasma and spermatozoa, the glutathione cycle has been demonstrated to be present in different concentrations in different localizations. The glutathione content of mammalian epididymis is about 10% that of the testes. The glutathione content during sperm maturation decreases to about 75% from caput epididymis spermatozoa to ejaculated spermatozoa. The reduction in the glutathione concentration during spermatogenesis particularly rendered late spermatids susceptible to mutagenesis by free radicals or other compounds. The late spermatids then become highly prone to chemically induced DNA damage, dominant-lethal mutations, and oxidative stress. These data show that intracellular glutathione with its essential protective properties is present in mammalian spermatogenic tissues with decreasing concentrations through the process of spermatogenesis. This particular fact about spermatogenesis may explain the remarkable effect of exogenous glutathione on total sperm count after just the first month of treatment. Early in the therapy, the augmented seminal glutathione titers could have already “rescued” the late-stage spermatids from the ROS induced oxidative stress.

**Selecting Glutathione as a Therapy**

One of the most important reasons for selecting glutathione as a therapy is its significant
physiological presence in seminal plasma. Even though it cannot cross cell membranes, the concentration of this antioxidant can be increased in biological fluids by systemic administration. It is able to reach the seminal plasma and concentrate there, thus exerting its physiological and therapeutic effect from the sperms environment. Glutathione’s thiolic group takes care of the harmful hydrogen peroxide, superoxide anion and hydroxyl radicals while its sulphhydrl group neutralizes the damaging alkoxyl radicals and hydroxyperoxides. Moreover, glutathione is the substrate of the selenium-containing enzyme glutathione peroxidase (the main enzyme involved in removing hydrogen peroxides) and of glutathione transferase (an enzyme which catalyzes covalent reactions of glutathione with electrophile substances such as quinones).

Glutathione has a protective effect on the lipid components of the cell membrane. Glutathione modifies the biochemical lipid membrane constitution by improving the epididymal microenvironment subsequently causing a higher unsaturation of the PUFA of the sperm plasma membrane. Glutathione also acts as a free radical scavenger in the epididymis, thus reducing the lipoperoxidative process generated by vascular and inflammatory pathologies.

Glutathione is involved in a protective mechanism that involves inactivation of ROS, including peroxidase formed in cellular oxygen metabolism. These toxic oxygen species may be detoxified via reduction by glutathione peroxidase (GPx), which is converted to oxidized glutathione (GSSG) in the process. In turn, oxidized glutathione is reduced by glutathione reductase (GRD). In addition, other electrophilic foreign compounds (xenobiotics) may be detoxified in a reaction catalyzed by a group of enzymes named glutathione S-transferases, by which they are conjugated with the reduced glutathione (GSH).

Therapeutic Oral Dose of Glutathione

A study by Demopolos, et al. in 2002, showed that the therapeutic oral dose of reduced glutathione (GSH) as an antioxidant is at 10-15mg/kg (skin whitening dose is at 20-40mg/kg per day). This raised the plasma glutathione levels in humans 1.5-10 folds over the basal concentration in the subjects tested. A mean value three times that of normal plasma GSH levels. The average Filipino male weighs around 55 to 65kg. Therefore, if we multiply this to the recommended antioxidant dose of 10mg/kg/day, we get an oral dosage of 600mg/day, the dose used in our clinical trial.

Plasma GSH values become maximal one hour after oral intake and drops down to approximately half the maximal value after 3 hours. In order to provide high bioavailability for oral administration, it has been found ideal to provide a relatively high concentration of GSH at a time when absorption by the duodenum is at its greatest. Thus, GSH is preferably taken on an empty stomach. The preferred dosage is between 100 to 1,000mg glutathione, and more preferably between about 250-3,000 mg glutathione.

Contrary to earlier reports by other scientists, it has been found that glutathione is not largely degraded in the stomach. A study by Hagen, et al. in 1990 showed that dietary GSH can be absorbed intact and results in a substantial increase in blood plasma GSH. This indicates that oral supplementation may be a useful and practical way of enhancing tissue availability of GSH.

Oral doses of up to 600 milligrams daily are well tolerated. There are no reports of adverse reactions. There have been no reports of glutathione overdosage or toxic effects in the literature. Oral supplementation may be a useful and practical way of enhancing tissue availability of GSH.

Present Therapeutic Strategies for Idiopathic Oligozoospermia: Standard of Care?

The types of pharmaceutical preparations commonly used for treatment of idiopathic oligozoospermia include a number of agents, which exert a direct and/or an indirect endocrine effect at one or more levels of the male reproductive system (table 6). However, there is no evidence that empiric hormonal therapies, such as human menopausal gonadotrophin (hmG)/human chorionic gonadotrophin (hcG), androgen, anti-estrogens (clomiphene and tamoxifen), prolactin inhibitors (bromocriptine) and steroids, improve pregnancy rates by men with idiopathic oligozoospermia. Hormones have been used with the presumption that gonadotrophins should stimulate spermatogenesis, but pituitary and urinary gonadotrophins should stimulate spermatogenesis, but pituitary and urinary gonadotrophins, anti-estrogens and the rebound rise of gonadotrophins after suppression with testosterone
have no clear benefit. Low-dose androgens and human chorionic gonadotrophin have been used particularly for motility disorders supposedly to stimulate epididymal function, but as might be expected, sperm concentration was also reduced.\textsuperscript{19}

Therefore, for many of these infertile men, no specific therapies are available to improve their fertility potential short of doing intrauterine insemination (IUI) and in-vitro fertilization (IVF).\textsuperscript{18,19} Both of which provides only a helpful ‘bypass’ to the problem rather than treatment.\textsuperscript{20}

Although there have been major advances in the treatment of male infertility through assisted reproductive technology, less than 10-20% are able to avail of these in our country. Thus the search for effective and more affordable infertility therapies remains. Additionally, the majority of patients would still prefer to produce pregnancies naturally. Understanding the mechanisms of defective sperm production and function remains a major concern so that logical treatment and preventive strategies addressing the problem can be developed and tested.

**CONCLUSION**

This study indicates that oral reduced glutathione therapy improves total sperm count in patients with oligospermia. Although it showed no significant effect on percent motility and morphology, it showed significant increase in absolute number of total motile sperms. The findings of this study indicate that oral glutathione therapy could possibly represent an effective and more affordable therapeutic alternative for male infertility patients with oligozoospermia.

**RECOMMENDATIONS**

The investigators of this study recommend further continuing research on the potential of oral glutathione for male infertility. Although various mathematical interventions have been utilized in this study to offset a possible skewed data derived from a small population, a bigger population is still highly recommended. It is also recommended further research using higher doses of oral glutathione which could possibly bring about different results in terms of percent motility. A crossover trial to observe if the improvement in semen parameters will last longer than the therapy period is another suggestion. It would also be interesting to quantify actual seminal glutathione concentration and its correlation with semen concentration of reactive oxygen species and clinical semen parameters.

**REFERENCES**

The Effect of Topical Mupirocin Ointment Versus Aloe Vera with Vitamin E Cream on Post Surgical Wound Healing: A Randomized Double Blind Placebo Controlled Trial

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Objective: A randomized double blind placebo controlled trial was undertaken to compare the effect of topical Mupirocin ointment and Aloe vera with Vitamin E cream versus a placebo on post surgical wound healing.

Methods: A total of 101 patients with primary elective vertical infraumbilical incision were enrolled in the investigative study. Mupirocin ointment was used in 34 patients, Aloe vera with vitamin E cream in 33 patients and placebo in 34 patients. First application was immediately post operation prior to occlusive dressing followed by application on the third day post operation. The assigned topical agent was used for daily wound care. Wound healing was assessed on the third postoperative day, first, second and third week post surgery. Wound infection, contact allergic dermatitis and wound healing characteristics specially redness, edema, presence of wound discharge and wound dehiscence were evaluated.

Results: There were no recorded cases of wound infection and allergic contact dermatitis in the patients enrolled in the different study groups at any time during the post surgery evaluation. Comparing the occurrence of the different wound healing characteristics, there were no significant differences during the first post operative day up to first to third week post operation evaluations.

Conclusion: Topical application of these agents before occlusive dressing immediately post operation and its use in daily wound care up to three weeks post operation did not show significant benefit for the patient.

Key words: mupirocin, aloe vera, vitamin E, placebo, topical agent, wound healing

Wound healing is a complex process, whether caused by surgical intervention or accidental injury. The healing involves a complex series of interactions between cell types, cytokine mediators and the extracellular matrices. Irrespective of the cause of the damage, in the attempt to heal, proliferation and division of cells occur, followed by the release of growth factors, formation of new blood vessels and collagen matrix and repair. Wound repair must occur in physiologic environment conducive to tissue repair and regeneration.

Tissue injury initiates a response that first clear the wound of devitalized tissue and foreign material, setting the stage for the subsequent tissue regeneration. The initial vascular response involves a brief and transient period of vasoconstriction and hemostasis. A 5-10 minute period of intense vasoconstriction is followed by active vasodilatation accompanied by an increase in capillary permeability. Platelets aggregated within a fibrin clot secrete a variety of growth factors and cytokines that set the stage for an orderly series of events leading to tissue repair.

The phase of wound healing, or the inflammatory phase, presents itself as erythema, swelling, and warmth, and is associated with pain. The inflammatory response increases vascular permeability, resulting in migration of neutrophils and monocytes into the surrounding tissue. The neutrophils engulf debris and microorganisms, providing the first line of defense against infection. Neutrophil migration ceases after the first few days post-injury if the wound is not contaminated. If this acute inflammatory phase persists, due to hypoxia,
infection, nutritional deficiencies, medication use, or other factors related to the patient's immune response, it can interfere with the late inflammatory phase.\(^3\)

In the late inflammatory phase, monocytes convert to the tissue to macrophages, which digest and kill bacteria pathogens, scavenge tissue debris and destroy remaining neutrophils. Macrophages begin the transition from wound inflammation to wound repair by secreting a variety of chemotactic and growth factors that stimulate cell migration, proliferation, and formation of the tissue matrix.\(^1\)

The subsequent proliferative phase, sometimes called the fibroblastic or collagen phase, is dominated by the formation of granulation tissue and epithelialization. Its duration is as early as 48 hours following the injury and may be completed after 21 days, although it depends on the size of the wound. Two processes run in parallel at this time, dermal repair and epidermal regeneration, and involve epithelialization, granulation and angiogenesis. Formation of granulation tissue, consisting of inflammatory cells, fibroblasts and vascular tissue, in a matrix of collagen, fibronectin, glycosaminoglycans and proteoglycans, is a central event during this proliferative phase. The restoration of surface epithelium is precisely synchronized with the repair of the dermal layer. Epithelial cells can only migrate over moist, warm tissue, not through the dry scabs where it takes at least twice as long for epithelialization to occur. Once the cells meet, they stop migration but continue to divide to restore normal epidermal tissue thickness. This new tissue is fragile and easily damaged by infection, abrasion or drying. Re-epithelialization continues until the keratinocytes have covered the dermis and stratified, and a new basement membrane is formed. The basement membrane plays an essential role in wound healing by providing molecular signals to synchronize repair between the epidermal and dermal layers. Fibroblasts enlarge and divide to produce a collagen network surrounding and strengthening the newly formed capillaries. The granulation tissue that is seen in wounds results from capillary and fibroblast proliferation. As collagen content increases, the wound site is strengthened, a process that continues over several weeks. The restoration of dermal architecture by collagen fibers eventually enables wounds to have strength that is similar to that of unaffected skin.\(^2\)

The longest phase, the maturation or wound remodeling phase, may continue up to two years, achieving 40-70% of the strength of undamaged tissue at fourth week.\(^4\) Vascularization lessens with excessive blood vessels being destroyed by enzymes, tight collagen cross-links are formed and collagen bundles realign along the direction of maximal stress to increase the tensile strength of the scar. Normal progressive collagen replacement yields a softer, less conspicuous scar, but excessive amounts of collagen leading to hypertrophic or keloid scarring.\(^2\)

Topical ointments play an important role in wound healing. An optimal wound environment lessens the duration of the inflammatory and proliferative phases. Proliferation of epidermal cells, fibroblasts and macrophage activity occurs most effectively in a slightly moist environment with minimal bacterial colonization. Applying a topical agent to a dressing can provide a moist environment to promote this healing process.\(^5\)

Topical antibiotics offer a useful alternative to oral and parenteral agents in certain conditions and have some advantages such as easy to use, lower side effects, higher drug concentrations in the infected area, lower risk of developing bacterial resistance and being economical. The goals of topical antimicrobial therapy are to control microbial colonization, thus preventing development of invasive infections, prophylaxis and treatment of wound infection, pyoderma, burn infections, and eradication of \textit{S. aureus}. Topical antibiotics of choice include bacitracin, neomycin, polymyxin B, mupirocin, nitrofurazone and fusidic acid.\(^6\)

Numerous studies support the prophylactic application of topical antibiotics to wounds that are clean. Topical bacitracin zinc (Bacitracin), a triple ointment of neomycin sulfate, bacitracin zinc, and polymyxin B sulfate (Neosporin), and silver sulfadiazine (Silvadene) were compared with petrolatum as a control in a well-conducted randomized controlled trial of 426 patients with uncomplicated wounds seen at a military community hospital. Wound infection rates were 17.6\% for petrolatum, 5.5\% for bacitracin (number needed to treat, NNT=8), 4.5\% for Neosporin (NNT=8), and for Silvadene (NNT=18). Most (60\%) of the infections were “stitch abscesses” and were treated with local care only. There was no difference in rates of more serious infections between groups. One patient (0.9\%) developed a hypersensitivity reaction to Neosporin.\(^7\)

Another randomized controlled trial of 933 outpatients – with a total of 1249 wounds from sterile dermatologic surgeries – compared white petrolatum with bacitracin zinc ointment prophylaxis. The study found no statistically significant differences in post-procedure infection rates, though only 13 patients
developed an infection (2% in petrolatum group vs 10.9% in bacitracin group; 95% CI – 0.4 to 2.7).8

A clinical trial compared the efficacy of a cetrimide, bacitracin zinc, and polymyxin B sulfate gel with placebo and povidone-iodine cream in preventing infections in 177 minor wounds among children. The antibiotic gel was found to be superior to placebo and equivalent to povidine-iodine, in that it reduced clinical infections from 12.5% to 1.6% (absolute risk reduction, ARR=0.109, 95% CI 0.011-0.207; NNT=11).9

A small randomized prospective trial of 99 patients, who self-reported compliance with wound care and dressing changes, compared Neosporin with Mupirocin (Bactroban) in preventing infections in uncomplicated soft tissue wounds. The study found no statistical difference in infection rates, and the authors recommend the more cost-effective Neosporin, as well as a larger trial to confirm the results.10

A blinded randomized clinical trial was undertaken to evaluate the effect of applying ointment (Mupirocin) to a wound before occlusive dressings, in comparison with no ointment or sterile paraffin. Some 778 patients with 1801 surgical wounds following excision of skin lesions were enrolled in the trial. Wound infection, scar, hemorrhage, dehiscence and other complications were assessed at suture removal. There were no significant differences in outcome for all endpoints evaluated. The infection rate was 1.4% with no ointment, 1.6% for paraffin and 2.3% for mupirocin (P=0.490). Total complication rates were 3.5%, 4.7% and 4.8% for no ointment, paraffin and mupirocin respectively (P=0.590). There was no difference in post operative pain, degree of inconvenience or overall level of satisfaction with treatment. Putting ointment on surgical wound before occlusive dressing did not benefit the patient. In view of antibiotic resistance, mupirocin ointment was indicated for clean surgical wounds.11

Based on RCTs, the use of topical triple-antibiotic ointments significantly decrease infection rates in minor contaminated wounds compared with a petrolatum control while plain petrolatum ointment is equivalent to triple-antibiotic ointments for sterile wounds as a post-procedure wound dressing.12

Guidelines for antibiotic prophylaxis of surgical wounds uniformly recommend prophylaxis for all clean-contaminated, contaminated, and dirty procedures. Prophylaxis is considered optional for most clean procedures, although it may be indicated for certain at-risk patients and for clean procedures that fulfill specific risk criteria.13

The Infectious Diseases Society of America recommends mupirocin as the best topical agent for treatment and prevention of S aureus and S pyogens infections, followed by bacitracin zinc and neomycin, although resistance is emerging.14

Mupirocin, an antibiotic produced by Pseudomonas fluorescens, is effective in the topical treatment of skin infections caused by a wide range of gram-positive and some gram-negative bacteria. Mupirocin is thought to be bactericidal at the 2% concentration used in the clinical ointment. Mupirocin acts by a different mechanism than other available antibiotics because it reversely binds to bacterial isoleucul transfer-RNA synthetase. The use of mupirocin to treat secondary wound infection has a profile of high efficacy and does not impair the normal healing in traumatized skin. It has a low risk of systemic and topical complications.15-17

On the other hand, botanical remedies have long been used as topical preparations to enhance wound healing. These topical preparations may be administered in vehicles with high water content, such as moist compresses, hydrogels, and tinctures, or may be applied in formulations with high fat content such as lipophilic creams and ointments.18

In order to heal, a moist environment is needed and in wounds covered with occlusive dressings, epithelialization has been shown to occur twice as fast and with less pain.19 Aloe vera gel is a herbal medicine that is commonly incorporated in many cosmetic products for its wound-healing properties. Aloe vera gel, or mucilage, is a thin, clear, jellylike substance obtained from the parenchymal tissue making up the inner part of Aloe vera plant leaf.20 Component of Aloe vera gel has been shown to significantly enhance keratinocyte proliferation and migration in wounds with the cell proliferation-stimulating activity shortening healing time.21 Aloe vera contains several pharmacologically active ingredients, including a carboxypeptidase that inactivates bradykinin in vitro, salicylates, and substances that inhibit the local vasoconstrictive effect of thromboxane in vivo.22 Fresh aloe vera gel promotes the attachments and growth of normal human cells in vitro and enhances the healing of wounded monolayers of cells.23 Quality aloe vera gel products typically contain more than 95% pure aloe vera and have proven effective in preserving skin circulation following frostbite injury and in accelerating wound healing in patients who have undergone full-face dermabrasion.25 Aloe vera

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increased collagen activity and enhanced the breaking strength of scars resulting from excisional wounds.\textsuperscript{26} Aloe vera gel may aid in superficial wound healing (dermabrasion and minor surface wounds), although at least one study has indicated it may delay recovery in complex wound healing by secondary intention.\textsuperscript{27} The anti-inflammatory activity of aloe vera gel has been demonstrated in several studies making it particularly useful in wound care.\textsuperscript{28-29} Postoperative topical application of Aloe vera and Centenella asiatica extracts may facilitate the creation of a flexible, fine scar with high tensile strength at the wound site.\textsuperscript{1}

Vitamin E is popular among consumers for skin care and to prevent scar formation. It functions as the major lipophilic antioxidant, preventing peroxidation of lipids and resulting in more stable cell membranes. The antioxidant-membrane stabilizing effect of Vitamin E also includes stabilization of the lysosomal membrane, a function shared by glucocosteroids.\textsuperscript{30} The effect of vitamin E on wound healing is complex. It may have alternate effects in different types of wounds and in the presence of other nutrients, as well as different functions for water soluble versus lipid soluble preparations of vitamin E.\textsuperscript{1}

A randomized double blind study was conducted to determine whether topically applied vitamin E had any effect on the cosmetic appearance of scars. Results showed that topically applied Vitamin E does not help in improving the cosmetic appearance of scars and leads to a high incidence of contact dermatitis. In 90% of cases in this study, topical vitamin E either had no effect on, or actually worsened the cosmetic appearance of scars. Of the patients studied, 33% developed contact dermatitis to vitamin E. It was concluded that topical vitamin E on surgical wounds should be discouraged.\textsuperscript{31}

A single-blind prospective study was carried out to determine the effects of topical vitamin E on cosmetic results in children. Topical vitamin E was used in the intended incision site for at least 15 days, thrice daily, before surgery and for at least 30 days, twice daily, after surgery (group A). The control group received a topical petrolatum-based ointment (group B). No patients in group A developed keloids. A total of 96% of patients considered the cosmetic results very good. No patients had wound infection. In the control group, only 78% of patients considered the cosmetics results very good and 6.5% patients developed keloids after 6 months. There were no cases of wound infection. Topical vitamin E before and after surgery improved surgical wound healing and improved cosmetic results.\textsuperscript{32}

Most dressings used in the postoperative population involved the use of topical antimicrobial agents. However, it is important to remember that these frequently applied adjunctive medications are not without risks. One of the most common and easily observed adverse reactions is the development of an allergic contact dermatitis.

Allergic contact dermatitis associated with topical antimicrobial agents is an increasing problem in the postoperative wound care period. The topical antimicrobial agents most commonly used postoperatively in North America and Europe were reviewed and the incidence of allergic contact dermatitis from each agent were examined and guidelines were provided for the use of topical antimicrobials on closed and open wounds in postoperative period. Neomycin was the most common cause of allergic contact dermatitis (11%). Bacitracin was also a common culprit (8%). Polymyxin B and mupirocin were not significant allergens. It was concluded that for closed wounds, the use of topical neomycin postoperatively should be avoided. White petrolatum was an efficacious and cost-effective alternative for closed wounds.\textsuperscript{33}

Caring for wounds effectively is important because all wounds are exposed to bacteria which can cause bacterial infection. It is for this reason that in current times, there are clinicians and patients alike who believe that the answer is antibiotic ointment prior to occlusive dressing. Several postoperative wound care ointments and creams are advertised to prevent bacterial infections and likewise promote wound healing. It was the aim of this investigative study to evaluate the effect of a couple of commercially available topical ointment and cream being used in postoperative wound care.

**Objectives**

**General Objective**

To compare the effect of topical Mupirocin ointment and Aloe vera with Vitamin E cream on post surgical wound healing.

**Specific Objectives**

To assess the effect of topical Mupirocin ointment and Aloe vera with Vitamin E cream on the incidence of wound infection and allergic contact dermatitis and on wound healing characteristics.
To compare the effect of topical Mupirocin ointment and Aloe vera with Vitamin E cream versus a placebo on post surgical wound healing.

Definition of Outcome Measures

Wound infection or surgical site infection (SSI)

An infection is considered to be an SSI when it occurs at the site of the surgery within 30 days of an operation or within 1 year of an operation if a foreign body (e.g. an artificial heart valve or joint) is implanted as part of the surgery. Most SSIs (about 70%) are superficial infections involving the skin only. The remaining infections are more serious and can involve tissues under the skin, organs, or implanted material. The majority of SSIs do not become life-threatening. Signs and symptoms of an SSI can include fever and redness, swelling, heat, or pain, at the surgical wound site. Drainage of cloudy fluid or sudden opening of the surgical wound can also suggest an SSI. Presence of purulent suture site, suture abscess, cellulitis, infective necrosis, subcuticular abscess, regional lymphadenitis and septicemia all signify wound infection.

Allergic contact dermatitis

Allergic contact dermatitis is an itchy eczematous lesion confined to the site of contact with the allergen. In severe cases, it may extend outside the contact area or become generalized.

Wound healing characteristics

For this investigative study, measurement of healing characteristics was modified from the REEDA scoring. Redness is present if there is erythema not beyond 0.5 cm of the incision bilaterally. Edema is present if there is swelling not beyond 2 cm from the incision. Discharge is present if there is serous (non-purulent and non-foul-smelling) discharge. Purulent discharge which may or may not be foul-smelling is considered under wound infection or SSI. Dehiscence (loss of approximation) is present if there is at least 3 mm skin separation.

Materials and Methods

This was a prospective randomized double blind placebo controlled trial conducted at the Department of Obstetrics and Gynecology of our institution from January to December 2009. Permission to conduct the study was obtained from the Ethics Committee of the Department of Obstetrics and Gynecology. Included in the study were women admitted for an elective obstetric or gynecologic operative procedure. Inclusion criteria were patients aged 18 years or more, eligible to give informed consent and able to comply with treatment requirements who will undergo a primary elective vertical infraumbilical incision.

Patients were excluded if any of the following is present: obese (BMI>30kg/m²); underweight (BMI<18kg/m²); with any medical co-morbidity (e.g. hypertension, diabetes, anemia, kidney disease, connective tissue disease); immunocompromised status; a need to do blood transfusion perioperatively; presence of infection (e.g. leukocytosis, urinary tract infection, bacterial vaginosis); presence of any skin lesions or infections over the incision area and if the patient has a known allergy to occlusive dressing or one of the topical agent preparations. Patients will also be excluded if the pathology from the surgical procedure is malignant; if the operation extended for more than 3 hours; if the incision needs to be extended beyond the infraumbilical level; if there is excessive blood loss, more than accepted for the surgical procedure; and occurrence of any other peri-operative complications.

On admission, baseline demographic data were collected which included the age, height, weight and BMI. A detailed review of the past and present medical histories was conducted. Pre-operative laboratory examinations were reviewed including the complete blood count, hemoglobin count, urinalysis and most recent pap smear if available. A patient’s database form was filled up.

If the patient fulfills the inclusion criteria and none of the exclusion criteria preoperatively, the patient will be 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. It was reiterated to the patient that final inclusion in the study will also depend on the fulfillment of the intraoperative inclusion criteria and that she will be informed of her final inclusion upon alighting from postoperative anesthesia. An informed consent was subsequently obtained preoperatively.

All patients were preoperatively given 1g Cefazolin intravenous 1 hour prior to the elective procedure.
procedure and continued at 1g intravenously every 8 hours for three doses postoperatively.

Prior to the placement of initial dressing after incision closure, once the intraoperative inclusion criteria were fulfilled, patients were randomized using block randomization to one of three groups. Group A – Mupirocin ointment; Group B – Aloe vera with Vitamin E cream and Group C – placebo (KY gel). Allocation to the treatment group was undertaken by an independent senior resident (not involved in the investigation). All topical agents have been transferred into similar sterile plastic containers, labeled according to the letter of group assignment and packed in brown paper bags by an independent senior resident. Neither the surgeon nor the patient was aware of the randomization. All surgeons were oriented preoperatively on how to apply the topical agent. The primary investigator was blinded to the group assignments.

First application of the topical agent was prior to placement of initial dressing, after painting with one stroke of povidone-iodine form edge to edge of the incision. The assigned drug or topical agent, pean-size in amount was applied smoothly in one direction from top to bottom of the incision making a paper-thin-like application. The wound was then covered with sterile gauze until the third postoperative day when change of dressing prior to discharge was due.

Second application of the topical agent was on the third postoperative day. With the primary investigator present, after wound inspection, incision is thinly painted with one stroke of povidone-iodine and the topical agent assigned was again applied. Detailed instructions on subsequent once daily cleaning, application of topical agent and change of dressing were explained during this time. A detailed postoperative instruction sheet regarding wound management, warning signs and details of return appointments was also given. The supply of topical agent good up to next follow up (one week post op) was given in sterile plastic containers packed in their assigned brown paper bag.

All patients were sent home with the following oral home medications: Cefuroxime 500 mg/cap, 1 cap three times a day for 7 days and Tramadol 50 mg/cap, 1 cap every 6 hours as needed for pain.

Outcome measures were assessed by the primary investigator on the third postoperative day (prior to discharge) and on postoperative follow up on outpatient basis. Follow ups were due first, second and third week from operative date. Wound infection which was recorded according to presence of purulent suture site, suture abscess, cellulitis, infective necrosis, subcuticular abscess, regional lymphadenitis and septicemia which may signify wound infection. Contact allergic dermatitis was recorded according to presence of an itchy eczematous lesion confined to the site of contact with the topical agent which may extend outside the contact area. Wound healing characteristics were evaluated and recorded accordingly as follows: redness is present if there is erythema not beyond 0.5 cm of the incision bilaterally; edema is present if there is swelling not beyond 2 cm from the incision; discharge is present if there is at least 3 mm skin separation. Other clinical signs related to wound healing were likewise assessed such as presence of fever (temperature >38°C) and formation of granulation tissue described as light red or dark pink in color, soft to touch, moist and granular in appearance.

The patient received her one-week worth of subsequent topical agents supply on her follow up day, again, packed in similar sterile plastic containers labeled according to the letter of group assignment and packed in brown paper bags.

The patient database was completed for each patient 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 application of the topical agent was discontinued in the event of wound infection, allergic contact dermatitis or other postoperative complications. It was at this point that the blinding was opened to determine the assigned topical agent. The patient was dropped off from the completion of the study and was medically and/or surgically managed accordingly.

This study included 34 patients per group with a 95% level of confidence and 80% power of study. There were 34 patients assigned to Group A (Mupirocin ointment), 33 patients assigned to Group B (Aloe vera with Vitamin E cream) and 34 patients assigned to Group C (Placebo, KY gel).

All data were entered and recorded in MS EXCEL format. Analysis of variance was used to compare continuous variables among the 3 groups while Chi-square was used for categorical variables. For both tests, a 95% confidence level was considered significant. All statistical tests were analyzed using the SPSS software.

**RESULTS**

During the study period from January to December 2009, a total of 101 patients were enrolled in the investigative study. Patients were randomized
into the three different topical agents: Group A – Mupirocin ointment with 34 patients; Group B – Aloe vera with vitamin E cream with 33 patients; and Group C – Placebo with 34 patients. There were no drop outs during the study period.

Table 1 shows the profile of the subjects included in the study. There was some significant differences in their age (P=0.049) wherein the mean age in the Mupirocin ointment group was 41.44 + 9.21 years; 35.33 + 9.25 years in the Aloe vera with vitamin E cream group and 38.65 + 10.97 in the placebo group. There was significant difference (P=0.019) in their BMI with 21.31 + 1.53 kg/m² in the Mupirocin group; 22.41 + 1.73 kg/m² in the aloe vera with vitamin E cream group; and 22.01 + 1.47 kg/m² in the placebo group. There was no significant difference among the three different group as to their preoperative hemoglobin counts.

Table 2 shows that there were no recorded cases of wound infection among the patients enrolled in the different study groups at any time during the postoperative evaluaton.

Table 3 shows that there were no recorded cases of allergic contact dermatitis among the patients enrolled in the different study groups at any time during the postoperative evaluation.

Table 4 shows the different wound healing characteristics of the patients enrolled in the three different topical agent groups at different postoperative evaluation dates. As to redness or erythema, it was observed that there was decrease in occurrence in all groups from third day post op to the third week postoperative. Comparing the occurrence of redness among the three different topical agents, there was no significant difference during the third day post op and first week post op. There was only significant difference in the occurrence of redness erythema during the second week post op evaluation where there was still 21% (7/34) of patients in the Mupirocin ointment group.

### Table 1. Profile of subjects.

<table>
<thead>
<tr>
<th></th>
<th>Mupirocin ointment n = 34</th>
<th>Aloe vera with Vitamin E cream n = 33</th>
<th>Placebo n = 34</th>
<th>P value</th>
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<tbody>
<tr>
<td>Age, in year</td>
<td>41.44 ± 9.21</td>
<td>35.33 ± 9.25</td>
<td>38.65 ± 10.97</td>
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<td>BMI, in kg/m²</td>
<td>21.31 ± 1.53</td>
<td>22.41 ± 1.73</td>
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<tr>
<td>Preoperative Hemoglobin, in g/L</td>
<td>129.12 ± 7.06</td>
<td>128.91 ± 6.11</td>
<td>128.28 ± 6.91</td>
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### Table 2. Wound infection.

<table>
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<tr>
<th>Presence of wound infection</th>
<th>Mupirocin ointment n = 34</th>
<th>Aloe vera with Vitamin E cream n = 33</th>
<th>Placebo n = 34</th>
<th>P value</th>
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<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 1 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 2 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table 3. Allergic contact dermatitis.

<table>
<thead>
<tr>
<th>Presence of allergic contact dermatitis</th>
<th>Mupirocin ointment n = 34</th>
<th>Aloe vera with Vitamin E cream n = 33</th>
<th>Placebo n = 34</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 1 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 2 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>
with redness as compared to only 3% (1/33) in the Aloe vera with vitamin E cream group and 6% (2/34) in the placebo group. However, by the third week post op evaluation, there was already no significant difference in redness or erythema among these patients from the three different groups.

There was decrease in the occurrence of discharge or serous discharge in all groups from the third day post op to the third week postoperative. Comparing its occurrence among the three different topical agents, there was no significant difference during the third day postoperative and first to third weeks post op evaluation.

There were no recorded cases of wound dehiscence among the patients enrolled in the different study groups at all periods of evaluation postoperative.

Table 5 shows that there were no recorded cases of fever among the patients enrolled in the different study groups at all periods of postoperative evaluation. As to formation of granulation tissue described as light red or dark pink in color, soft to

<table>
<thead>
<tr>
<th>Wound healing characteristics</th>
<th>Mupirocin ointment n = 34</th>
<th>Aloe vera with Vitamin E cream n = 33</th>
<th>Placebo n = 34</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Redness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 post op</td>
<td>12</td>
<td>9</td>
<td>10</td>
<td>0.761</td>
</tr>
<tr>
<td>Week 1 post op</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>0.175</td>
</tr>
<tr>
<td>Week 2 post op</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>0.035</td>
</tr>
<tr>
<td>Week 3 post op</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0.168</td>
</tr>
<tr>
<td>2. Edema</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 post op</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>0.816</td>
</tr>
<tr>
<td>Week 1 post op</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>0.936</td>
</tr>
<tr>
<td>Week 2 post op</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0.999</td>
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<tr>
<td>Week 3 post op</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.780</td>
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<tr>
<td>3. Discharge</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 1 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 2 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>4. Dehiscence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 1 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 2 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 5. Other clinical sign of wound healing.

<table>
<thead>
<tr>
<th>Other clinical signs of wound healing</th>
<th>Mupirocin ointment n = 34</th>
<th>Aloe vera with Vitamin E cream n = 33</th>
<th>Placebo n = 34</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fever (T&gt;38ºC)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Day 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 1 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 2 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>2. Formation of granulation tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 1 post op</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Week 2 post op</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>0.612</td>
</tr>
<tr>
<td>Week 3 post op</td>
<td>16</td>
<td>10</td>
<td>15</td>
<td>0.331</td>
</tr>
</tbody>
</table>
touch, moist and granular in appearance, its formation was first recorded on the second week post op follow up in all three study groups. In all three study groups, there was increase in patients with formation of granulation tissue from second week post op to third week post op evaluation. Comparing its occurrence among the three different topical agents, there was no significant difference during the second to third weeks post op evaluation.

Table 6 shows the computations for the absolute risk reduction (ARR) and number needed to treat (NNT). As to redness, the placebo reduced the risk of redness by 5.9% more than Mupirocin ointment. Seventeen patients are needed to be treated with placebo to prevent one case of redness of the surgical wound. Aloe vera with vitamin E cream reduced the risk of redness by 8.8% compared to Mupirocin ointment. Eleven patients are needed to be treated with Aloe vera with vitamin E cream to prevent one case of redness of the surgical wound.

As to edema, Mupirocin ointment reduced the risk of edema by 5.9% compared to placebo. Seventeen patients are needed to be treated to prevent once case of edema of the surgical wound. Aloe vera with vitamin E cream reduce the risk of edema by 5.9% compared to placebo. Seventeen patients are needed to be treated with Aloe vera with vitamin E cream to prevent one case of edema of the surgical wound.

As to wound discharge, placebo reduced the risk of occurrence of discharge by 2.9% better than Mupirocin ointment. Thirty four patients are needed to be treated with placebo to prevent one case of wound discharge. Aloe vera with vitamin E cream reduced the risk of occurrence of discharge by 2.9% better than Mupirocin ointment. Thirty four patients are needed to be treated with Aloe vera with Vitamin E cream to prevent one case of wound discharge.

As to granulation tissue, placebo reduced the non-formation of granulation tissue by 2.9% compared to Mupirocin ointment. Thirty four subjects are needed to be treated with placebo to prevent one case of non-formation of granulation tissue. Placebo reduced the non-formation of granulation tissue by 14.7% as compared to Aloe vera with vitamin E cream. 6.8 patients are needed to be treated with placebo to prevent one case of non-formation of granulation tissue. Mupirocin ointment reduced the risk of non-formation of granulation tissue by 7.6% compared to Aloe vera with vitamin E cream. 5.7 patients are needed to be treated with Mupirocin ointment to prevent one case of non-formation of granulation tissue.

<table>
<thead>
<tr>
<th>Wound healing characteristic</th>
<th>Absolute Risk Reduction (ARR)</th>
<th>Number Needed to Treat (NNT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1 - R2</td>
<td>1 / ARR</td>
</tr>
<tr>
<td>Redness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mupirocin ointment vs Placebo</td>
<td>3/34 - 1/34 = 5.9%</td>
<td>1/0.059 = 17</td>
</tr>
<tr>
<td>Aloe vera with vitamin E cream vs Placebo</td>
<td>1/34 - 0/34 = 2.9%</td>
<td>1/0.029 = 34</td>
</tr>
<tr>
<td>Mupirocin vs Aloe vera with vitamin E cream</td>
<td>3/34 - 0/34 = 8.8%</td>
<td>1/0.088 = 11</td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mupirocin ointment vs Placebo</td>
<td>3/34 - 1/34 = 5.9%</td>
<td>1/0.059 = 17</td>
</tr>
<tr>
<td>Aloe vera with vitamin E cream vs Placebo</td>
<td>3/34 - 1/34 = 5.9%</td>
<td>1/0.059 = 17</td>
</tr>
<tr>
<td>Mupirocin vs Aloe vera with vitamin E cream</td>
<td>1/34 - 1/34 = 0</td>
<td>1/0 = 0</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mupirocin ointment vs Placebo</td>
<td>2/34 - 1/34 = 2.9%</td>
<td>1.0/0.029 = 34</td>
</tr>
<tr>
<td>Aloe vera with vitamin E cream vs Placebo</td>
<td>1/34 - 1/34 = 0</td>
<td>1/0 = 0</td>
</tr>
<tr>
<td>Mupirocin vs Aloe vera with vitamin E cream</td>
<td>2/34 - 1/34 = 2.9%</td>
<td>1.0/0.029 = 34</td>
</tr>
<tr>
<td>Formation of granulation tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mupirocin ointment vs Placebo</td>
<td>19/34 - 18/34 = 2.9%</td>
<td>1.0/0.029 = 34</td>
</tr>
<tr>
<td>Aloe vera with vitamin E cream vs Placebo</td>
<td>24/34 - 19/34 = 14.7%</td>
<td>1.0/0.147 = 6.8</td>
</tr>
<tr>
<td>Mupirocin vs Aloe vera with vitamin E cream</td>
<td>24/34 - 18/34 = 17.6%</td>
<td>1.0/0.176 = 5.7</td>
</tr>
</tbody>
</table>
DISCUSSION

Wound healing is a complex process. The proliferative phase of wound healing, sometimes called the fibroblastic or collagen phase, is dominated by the formation of granulation tissue and epithelialisation. Its duration is as early as 48 hours following the injury and may be completed after 21 days. Formation of granulation tissue, consisting of inflammatory cells, fibroblasts and vascular tissue, in a matrix of collagen, fibronectin, glycosaminoglycans and proteoglycans, is a central event during this proliferative phase. This new tissue is fragile and easily damaged by infection, abrasion or drying. The granulation tissue that is seen in wounds results from capillary and fibroblast proliferation. As collagen content increases, the wound site is strengthened, a process that continues over several weeks. Thus, wound repair must occur in a physiologic environment conducive to tissue repair and regeneration.

Topical ointments play an important role in wound healing. An optimal wound environment lessens the duration of the inflammatory and proliferative phases. Proliferation of epidermal cells, fibroblasts and macrophage activity occurs most effectively in a slightly moist environment with minimal bacterial colonization. Applying a topical agent to a dressing can provide a moist environment to promote this healing process.

Topical antibiotics offer a useful alternative to oral and parenteral agents in certain conditions and have some advantages such as easy to use, lower side effects, higher drug concentrations in the infected area, lower risk of developing bacterial resistance and being economical. The goals of topical antimicrobial therapy are to control microbial colonization, thus preventing development of invasive infections, prophylaxis and treatment of wound infections, pyodermas, burn infections, and eradication of *S. aureus*.

Guidelines for antibiotic prophylaxis of surgical wounds uniformly recommend prophylaxis for all clean-contaminated, contaminated, and dirty procedures. Prophylaxis is considered optional for most clean procedures, although it may be indicated for certain at-risk patients and for clean procedures that fulfill specific risk criteria. The Infectious Diseases Society of America recommends mupirocin as the best topical agent for the treatment and prevention of *S. aureus* and *S. pyogenes* infections, followed by bacitracin zinc and neomycin, although resistance is emerging. On the other hand, botanical remedies have also long been used as topical preparations to enhance wound healing. These topical preparations may be administered in vehicles with high water content, such as moist compresses, hydrogels, and tinctures, or may be applied in formulations with high fat content such as lipophilic creams and ointments.

At present, there are clinicians and patients alike who believe that the answer to effective wound care is antibiotic ointment prior to occlusive dressing. Several postoperative wound care ointments and creams are being advertised to prevent bacterial infections and likewise promote wound healing. In this study, we assessed the effect of topical Mupirocin ointment and Aloe vera with Vitamin E cream on the incidence of wound infection, allergic contact dermatitis and wound healing characteristics. Moreover, both topical agents were also compared to a placebo to evaluate if needed application of topical ointment or cream makes a significant difference in the aforementioned wound healing parameters. The period of observation specifically covers only the proliferative phase of wound healing, about 48 hours to 21 days following the surgical procedure, which is the specific phase in which the topical agents play their most important role.

Mupirocin, an antibiotic produced by *Pseudomonas fluorescens*, is effective in the topical treatment of skin infections caused by a wide range of gram-positive and some gram-negative bacteria. Mupirocin is thought to be bactericidal at the 2% concentration used in the clinical ointment. On the other hand, aloe vera gel is one of the herbal medicines that are commonly incorporated in many cosmetic products for their wound-healing properties. It has been shown to significantly enhance keratinocyte proliferation and migration in wounds with the cell proliferation-stimulating activity shortening healing time. Its anti-inflammatory activity has been demonstrated in several studies making it particularly useful in wound care. The addition of Vitamin E functions as the major lipophilic antioxidant, preventing peroxidation of lipids and resulting in more stable cell membranes.

Based on randomized controlled trials, the use of topical triple-antibiotic ointments significantly decreases infection rates in minor contaminated wounds compared with a petrolatum control while plain petrolatum ointment is equivalent to triple-antibiotic ointment for sterile wounds as a post-procedure wound dressing. Numerous studies support the prophylactic application of topical antibiotics to wounds that are clean. Dire, et al.
compared topical bacitracin zinc (Bacitracin) and silver sulfadiazine (Silvadene) with petrolatum as a control in a well-conducted randomized controlled trial of 426 patients with uncomplicated wounds. Although wound infection rates described only as “stitch abscesses” were 17.6% for petrolatum, 5.5% for bacitracin (number needed to treat, NNT=8), 4.5% for Neosporin (NNT=8), and for Silvadene (NNT=18), there was no difference in rates of more serious infections between groups.7

In another randomized controlled trial by Smack, et al. of 1249 wounds from sterile dermatologic surgeries which compared white petrolatum with bacitracin zinc ointment prophylaxis, the study found no statistically significant differences in post-procedure infection rates (2% in petrolatum group vs 0.9% in bacitracin zinc group; 95% CI – 0.4 to 2.7).8 Hood, et al. compared Neosporin with Mupirocin (Bactroban) in preventing infections in uncomplicated soft tissue wounds in a small randomized prospective trial of 99 patients. The study found no statistical difference in infection rates.10

This study was conducted among patients with similar clean wounds after uncomplicated elective surgeries. The topical agents compared were Mupirocin ointment and aloe vera with vitamin E cream versus a placebo as control. As compared to the review of literature, there was no recorded case of wound infection among the patients enrolled in the different study groups at any time during the postoperative evaluation. There were no cases of presence of purulent suture site, suture abscess, cellulitis, infective necrosis, subcuticular abscess, regional lymphadenitis and sepsis which may signify wound infection. There were no patients diagnosed with surgical site infection.

Use of these frequently applied adjunctive medications to wound care is not without risks. One of the most common and easily observed adverse reactions is the development of an allergic contact dermatitis. Allergic contact dermatitis associated with topical antimicrobial agents is an increasing problem in postoperative wound care. Sheth, et al. reviewed the topical antimicrobial agents most commonly used postoperatively in North America and Europe. The incidence of allergic contact dermatitis from each agent was examined and guidelines were provided for the use of topical antimicrobials on closed and open wounds in the postoperative period. Neomycin was the most common cause of allergic contact dermatitis (11%). Bacitracin was also a common culprit (8%). Polymyxin B and mupirocin were not significant allergens. It was concluded that for closed wounds, the use of topical neomycin postoperatively should be avoided. White petrolatum is an efficacious and cost-effective alternative for closed wounds.33

In a randomized double blind study conducted by Baumann, et al. to determine whether topical applied vitamin E had effect on the cosmetic appearance of scars, results showed that topically applied vitamin E does not help in improving the cosmetic appearance of scars and leads to a high incidence of contact dermatitis. In 90% of cases in this study, topical vitamin E either had no effect on, or actually worsened the cosmetic appearance of scars. Of these studied, 33% developed contact dermatitis to vitamin E. It was concluded that topical vitamin E on surgical wounds should be discouraged.31

In this study, mupirocin and aloe vera were compared with vitamin E, there were no recorded cases of allergic contact dermatitis among the patients enrolled in the different study groups at any time during the postoperative evaluation. There were no cases of any itchy eczematous lesion confined to the site of contact with the topical agents being studied. There were likewise no itchy eczematous lesions that were outside the contact area and no reports of any generalized allergic reaction. Dixon, et al. further evaluated the effect of topical medications on wound healing characteristics. In a blinded randomized clinical trial of 1801 surgical wounds to evaluate the effect of applying ointment (Mupirocin) to a wound before occlusive dressing, in comparison with no ointment or sterile paraffin, wound infection, scar, hemorrhage, dehiscence and other complications were assessed at suture removal. Results showed that there were no significant differences in outcome for all endpoints evaluated. The infection rate was 1.4% with no ointment, 1.6% for paraffin and 2.3% for mupirocin (P=0.490). Total complication rates were 3.5%, 4.7% and 4.8% for no ointment, paraffin and mupirocin, respectively (P=0.590). There was no difference in postoperative pain, degree of inconvenience or overall level of satisfaction with treatment. They concluded that putting ointment on surgical wound before occlusive dressing, does not benefit the patient. They recommended that, in view of antibiotic resistance, mupirocin ointment is not indicated for clean surgical wounds.11

Similar to the study of Dixon, et al. there were no significant difference in the occurrence of redness or erythema, edema or swelling, presence of serous discharge and occurrence of dehiscence in the topical
use of any of the agents mupirocin ointment, aloe vera with vitamin E cream and the placebo. Moreover, comparing the occurrence of formation of granulation tissue among the three different topical agents, there was also no significant difference during its onset on the second week postoperative evaluation to the third week postoperative evaluation. As to the risk difference (ARR) and clinical advantage of the intervention (NNT), results for the different wound characteristics evaluated for wound healing were not consistent and did not show significant benefit for the patients.

During the course of the study, there were no complaints of postoperative pain needing further analgesia and no reports of any inconvenience causing discontinuation of use and eventual dropping out of the study.

CONCLUSION

This prospective double blind randomized placebo controlled trial showed that topical application of mupirocin ointment or aloe vera with vitamin E cream on primary clean incision wounds of uncomplicated elective surgeries made no difference in the incidence of wound infection, occurrence of allergic contact dermatitis and on the different wound healing characteristics when compared with placebo. Topical application of these agents before occlusive dressing immediately post operation and its use in daily wound care up to three weeks post operation did not show significant benefit for the patient.

RECOMMENDATIONS

Considering the issues of antibiotic resistance, risks of allergic contact dermatitis and the added expense, topical use of mupirocin ointment or aloe vera with vitamin E cream should no longer be applied to clean incision wounds of uncomplicated elective surgeries before occlusive dressing immediately post operation or in daily wound care.

For further studies, the investigators recommend that other commercially available topical products of different active ingredients be tested for their wound healing effects as they are advertised and marketed for such use. It is suggested that the long term effect on scar formation be likewise evaluated to include assessment of effects on pain experienced, convenience of application or use and cost effectiveness. Patient satisfaction must likewise be weighed against the medical risks.

REFERENCES


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Intraperitoneal Chemotherapy in the Philippines: Toxicity Profile Reviewed

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Department of Obstetrics and Gynecology, Dr. Jose R. Reyes Memorial Medical Center

Objective: To review the toxicity profile of intraperitoneal (IP) chemotherapy in Filipino patients with epithelial ovarian and fallopian tube carcinoma.

Patients and Methods: A retrospective case series of sixteen patients who received IP chemotherapy for epithelial ovarian and fallopian tube cancer between January 1, 2007 and July 31, 2010 was performed. IP chemotherapy regimen consisted of GOG 172 protocol (n=11); SWOG 8501/GOG 104 protocol (n=2); IV Paclitaxel 135 mg/m² day 1/IP Cisplatin 100 mg/m² day 2 (n=1); Fujiwara protocol (n=1); and IP Oxaliplatin 100mg/m² (n=1). Demographic profile was collected. Patients were assessed for toxicity.

Results: Sixteen patients underwent IP chemotherapy. The median age was 51 (range: 37-62). Six patients had stage III disease, 2 had stage I, 4 had persistence, 2 had recurrent ovarian cancer and one had stage II fallopian tube cancer. Median number of cycles given was 3.5. Seven out of 16 patients (43.75%) completed 6 cycles of chemotherapy, 5 out of 16 (31.25%) were still undergoing treatment. Fifty seven cycles were analyzed for toxicity. Maximal grade of adverse effects were: Grade(G) 2 anemia, 8.77%; G3 leukopenia, 1.75%; G3 anorexia/nausea/vomiting/dehydration, 5.26%; G3/4 diarrhea, 1.75%; G3 ileus/skin toxicity, 1.75%; G2/3 abdominal pain, 1.75%; G3/4 hypokalemia, 1.75%; and G3 fatigue, 3.5%. IP catheter-related infection occurred in one patient (1.75%), two (3.5%) had IP port re-insertion and one patient (1.75%) had leakage problem. No deaths were reported in this series.

Conclusion: Intraperitoneal chemotherapy is well-tolerated with acceptable toxicities in the treatment of ovarian and fallopian tube carcinoma in the Philippines.

Key words: intraperitoneal chemotherapy, ovarian cancer, toxicity

Ovarian carcinoma is the leading cause of gynecologic cancer deaths globally.¹ In the United States alone, approximately 21,880 new cases of ovarian cancer resulting in 13,850 deaths was estimated to occur this 2010.² In the Philippines, on the other hand, about 3,283 new cases were diagnosed and 1,918 died from it in 2005.³ Majority of these cases are diagnosed in its advanced stage (FIGO Stage III or IV) due to the silent nature of the disease. The current standard of treatment after primary maximal cytoreductive surgery is still systemic administration of combination platinum-taxane (Paclitaxel) regimen.⁴ Most of the patients (up to 80%) will have a complete response after this treatment but some will eventually have a persistent disease, or have a relapse and later die of the disease due to platinum-based chemotherapy resistance. Over-all five year survival rate is 30-46% even for those with minimal residual intraperitoneal disease.²,⁵ Cure is hardly attained despite great attempts to improve the outcome of these patients with the use of various combination systemic chemotherapeutic agents.

Results of multicenter randomized phase III trials showed that the intraperitoneal (IP) route of administering cytotoxic drugs is a rational approach in the primary chemotherapeutic management of optimally debulked advanced stage epithelial ovarian cancer. Taking into account the intraperitoneal spread of the disease primarily through exfoliation and implantation of malignant cells to the peritoneal cavity, and that the peritoneal cavity can be exposed to sustained high drug concentrations than can be safely attained with systemic delivery.⁶,⁷ Furthermore,
IP cisplatin-based chemotherapy regimen has significantly improved the overall survival outcome of diagnosed ovarian cancer cases with reasonable certainty compared to standard intravenous (IV) chemotherapy (65.6 vs. 49.7 months). Nonetheless, this approach has not gained wide acceptance mainly due to treatment-related toxicities which may influence the completion of treatment, affect the quality of life, and significantly increase the overall cost of treating the disease.

**Objective of the Study**

**General Objective**

To review the toxicity profile of intraperitoneal chemotherapy in Filipino patients with epithelial ovarian and fallopian tube carcinoma.

**Specific Objectives**

1. To determine the hematologic and non-hematologic adverse effects of intraperitoneal chemotherapy.

2. To determine the IP catheter-related adverse effects of intraperitoneal chemotherapy.

**MATERIALS AND METHODS**

A retrospective review of the records of 16 patients who received intraperitoneal chemotherapy between January 1, 2007 and July 31, 2010 was done. Patients received IP chemotherapy as adjuvant treatment after primary maximal cytoreductive surgery (n=10) or for treatment of bulky persistent (n=4) or recurrent (n=2) disease. Ten of the 16 (62.5%) patients were initially diagnosed with stage III ovarian cancer, 2 of the 16 (18.75%) with stage II disease, 3 of the 16 (18.75%) with stage I disease and 1 of the 16 (6.25%) with stage 1 fallopian tube cancer. All underwent a platinum-based chemotherapy after primary cytoreduction except for 1, who was given doxorubicin for 5 cycles. IP treatment consisted of a platinum-based combination therapy using one of the following: 1. Gynecologic Oncology Group (GOG) 172 protocol: Cisplatin 100 mg/m² on Day 2 + IP Paclitaxel 60 mg/m² on Day 8 + IV Paclitaxel 135 mg/m² (n=11); 2. Southwest Oncology Group (SWOG) 8501/GOG 104 protocol: Cisplatin 100 mg/m² on Day 2 + IV Cyclophosphamide 600 mg/m² (n=2); 3. Cisplatin 100 mg/m² on Day 2 + IV Paclitaxel 135 mg/m² (n=1); and 4. Fujiwara protocol: Carboplatin AUC 5 on Day 2 + IV Paclitaxel 175 mg/m² (n=1); 5. Oxaliplatin 100mg/m² (n=1). All patients had normal bone marrow reserve and normal liver, renal, respiratory and cardiac functions prior to IP chemotherapy. One patient had previous bowel resection prior to IP therapy. Hematologic and non-hematologic including IP catheter-related toxicity data were assessed during and after each cycle based on the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) for Adverse Events version 3.0 grading system.

Descriptive statistics were generated for all variables. Frequencies and percentages were computed for nominal data and mean, median and range were computed for numerical data.

**RESULTS**

**A. Patient Demographics**

The clinical characteristics of the patients are listed in Table 1. The mean age was 51 years (range: 37-62 years). Six patients had stage III ovarian cancer, 1 had stage 2 disease, 2 had stage I disease, 4 had persistence, 2 had a recurrent disease and 1 had stage 2 fallopian tube cancer. Twenty five percent of the patients were of the endometrioid type, followed by papillary serous (18.75%), and serous and clear cell histology (12.5%). The other histologic types namely, mixed epithelial, mucinous cystic tumor of low malignant potential with diffuse peritoneal adenomucinosis (DPAM), mucinous tumor of low malignant potential synchronous with adenocarcinoma, cervix, and adenocarcinoma, fallopian tube had 7% respectively. More than half (56.25%) of the population had tumor size less than or equal to 1 cm prior to initiation of IP treatment and 31.25% had greater than 1 cm. The Eastern Cooperative Oncology Group (ECOG) performance status ranged from 0 to 1. The median number of treatment cycles was 3.5 (range: 1-6). Seven of the 16 patients (43.75%) completed the scheduled 6 cycles of treatment and 5 of the 16 (31.25%) are still undergoing treatment.

The toxicity data indicated in Tables 2 and 3 are the worst grades recorded during and after the treatment cycle. Toxicity data were available in a total of 57 cycles.
Grade (G) 2 anemia occurred in 8.77% of patients, 3.5% had G1 leukopenia, 7.01% had G2 and 1.75% had G3 leukopenia. Grade 1 thrombocytopenia was observed in 1.75% of patients. Febrile neutropenia was noted in one patient. These patients responded well to granulocyte colony-stimulating factor, antibiotics, blood transfusion and use of erythropoietin.

The incidences of maximal grade of adverse effects seen in all cycles were as follows: G3 anorexia/nausea/vomiting/dehydration, 5.26%; G3/4 diarrhea, 1.75%; G3 ileus, 1.75%; G3 abdominal pain, 1.75%; G3/4 hypokalemia, 1.75%; G3 hypoalbuminemia, 1.75%; G3 skin toxicity, 1.75% and G3 fatigue, 3.5%. These were easily managed medically by IV hydration, correction of electrolytes and giving of analgesics. IP catheter-related complication occurred in 4 patients (7%). One patient had severe peritonitis and was treated with systemic antibiotics, and removal of the IP catheter. Two patients had IP port re-insertion due to displacement and one patient had leakage in the mini-lap site. There were no inflow IP catheter obstruction, and kinking observed in this series. The toxicities observed caused the delays and discontinuation of the IP therapy of some of the patients. However, no treatment related deaths were noted in the study population.

**DISCUSSION**

Antineoplastic agents, regardless of route of administration, act at different phases of the cell cycle. They are most effective at killing tumor cells that are rapidly dividing. Yet, they can not perfectly distinguish normal cells (especially those that naturally divide rapidly) from tumor cells. Damage caused by these agents to these normal tissues results to a wide range of side effects. Likewise, escalating the dose may directly increase these adverse effects. Toxicities encountered that are severe enough will limit the dose a patient may receive and further influence the completion of a scheduled treatment.

Intraperitoneal chemotherapy provides a venue for giving prolonged higher drug concentrations that cannot be safely achieved through systemic administration while normal tissues like the bone marrow are relatively spared. The proposed mechanism is the destruction of cancer cells layer by layer with each subsequent infusion. IP drugs penetrate to a depth of few millimeters beneath the tumor surface, go into the capillaries adjacent to the peritoneum and systemic circulation, and then return to the inner core of tumor tissue through tumor microcirculation.

Platinum-based agents, like cisplatin and carboplatin, used for intraperitoneal chemotherapy for ovarian carcinoma are relatively small molecules and are water-soluble. These agents easily enter the systemic circulation resulting in systemic exposure that is prolonged but lower than the intravenous route. This may be considered as one route for systemic chemotherapy with the added benefit of peritoneal and tumor exposure to high drug concentration while in the abdominal cavity. Howell, et al. in 1982 noted that the peak peritoneal concentration of free reactive cisplatin was approximately 21-fold higher than the plasma level.

In an animal study by Pretorius, et al., mode of administration did not affect systemic toxicity since the changes in renal and bone marrow functions were identical in the two groups. Drug levels within the tissues were similar except for the tissues lining the peritoneal cavity which has a 2.5-8 times higher levels than IV therapy.

Phase II trials on intraperitoneal therapy were mainly cisplatin-based. Results showed that a proportion of patients with small-volume residual ovarian cancer could achieve surgically documented complete response to second line IP chemotherapy when this clinical state had not been achieved after primary platinum-based systemic chemotherapy. A subset of these patients was observed to have prolonged survival. These trials marked the birth of 8 consecutive randomized phase III trials that have documented superior overall survival and progression-free survival in patients with stage III optimally debulked ovarian cancer given intraperitoneal chemotherapy.

A recent meta-analysis of these trials conducted by the National Cancer Institute (NCI) US/GOG and released as a clinical announcement from NCI last January 2006 showed that across all studies, IP therapy was associated with a 21.6% decrease in the risk of death (hazard ratio = 0.79; 95% confidence interval [CI] 0.70–0.89). The expected median duration of survival for women with optimally debulked ovarian cancer given standard treatment is approximately 4 years. Consequently, this decrease in the overall death rate is expected to translate to about a 12-month increase in overall median survival. The significant improvement in overall survival with IP therapy, however, was associated with higher fever and gastrointestinal (GIT) toxicity only.

Three of the most important trials in this meta-analysis includes the SWOG 8501/GOG 104
### Table 1: Demographic profile of patients

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<td><strong>IP Regimen Used</strong></td>
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<tr>
<td>a. IP cisplatin 100 mg/m² + IP paclitaxel 60 mg/m² + IV paclitaxel 135 mg/m² (GOG 172)</td>
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<tr>
<td>b. IP cisplatin 100 mg/m² + IV cyclophosphamide 600 mg/m² (GOG 104)</td>
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<td>c. IP cisplatin 100 mg/m² + IV paclitaxel 135 mg/m²</td>
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<td>d. IP carboplatin AUC 5 + IV paclitaxel 175 mg/m² (Fujiwara Protocol)</td>
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<td>e. IP oxaliplatin 100mg/m²</td>
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<tr>
<td>Goiter</td>
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intergroup trial. Alberts, et al.\textsuperscript{18}, in 1996, reported that patients with small-volume disease (largest residual tumor nodule <2cm in maximum diameter) after surgical cytoreduction given intraperitoneal cisplatin showed statistically significant 8-month improvement in overall survival (median of 49 vs. 41 months, P=.02) compared to the IV regimen. There was also a lower incidence of neutropenia and tinnitus but a higher incidence of abdominal discomfort in the IP arm. This was followed by the GOG-led intergroup trial (GOG-114/SWOG 9227) published by Markman, et al. in 2001.\textsuperscript{19} Comparison of IV paclitaxel plus cisplatin to two courses of IV carboplatin with six courses of IV paclitaxel plus IP cisplatin showed that the IP arm was associated with a 28-month progression-free survival and a 63-month overall survival, both of which was superior to the 22-month progression–free survival and 52-month overall survival with IV-administered drugs (\(P=0.02\) and \(P=0.05\), respectively). Significant myelotoxicity and a great proportion of patients failed to complete the prescribed treatment in the IP arm but these were attributed to the two initial courses of carboplatin.\textsuperscript{19}

In 2006, Armstrong, et al.\textsuperscript{13} published the third phase III randomized trial of IP cisplatin for the treatment of newly diagnosed stage III ovarian cancer (GOG-172) which sparked renewed interest in IP as a route for chemotherapy. The trial compared the IP regimen of IV paclitaxel at 135 mg/m\textsuperscript{2} over 24 hours on day 1 + IP cisplatin at 100 mg/m\textsuperscript{2} on day 2 + IP paclitaxel at 60 mg/m\textsuperscript{2} on day 8 to the IV regimen of paclitaxel at 135 mg/m\textsuperscript{2} over 24 hours IV on day 1 + cisplatin at 75 mg/m\textsuperscript{2} IV on day 2. It demonstrated the longest median survival in any randomized study of primary chemotherapy for

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### Table 2. Hematologic toxicity of patients who underwent IP chemotherapy.

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Frequency (N=57)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood and Bone Marrow</strong></td>
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<tr>
<td>Hemoglobin</td>
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</tr>
<tr>
<td>G1</td>
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<tr>
<td>G2</td>
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<td>G3</td>
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<tr>
<td><strong>Leukocytes</strong></td>
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</tr>
<tr>
<td>G1</td>
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<td>3.5</td>
</tr>
<tr>
<td>G2</td>
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<tr>
<td><strong>Platelets</strong></td>
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<tr>
<td>G1</td>
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### Table 3. Non-hematologic toxicity of patients who underwent IP chemotherapy.

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<th>Adverse Event</th>
<th>Frequency (N=57)</th>
<th>Percentage (%)</th>
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</thead>
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<tr>
<td>G2</td>
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<td>1.75</td>
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<td>G3</td>
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<tr>
<td>Nausea</td>
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<tr>
<td>G1</td>
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<td>G2</td>
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<tr>
<td>Vomiting</td>
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<tr>
<td>G2</td>
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<td>1.75</td>
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advanced ovarian cancer to date, i.e. 65.6 months (IP arm) vs. 49.7 months (IV arm) (P=0.3). Relative risk of death was 0.75 (95% CI = 0.58-0.97, P=0.3) which translates to a 25% reduction in the risk of death and a 5.5-month progression-free survival associated with the IP arm (23.8 vs. 18.3 months, P=0.5). In spite of this, higher toxicities and worse quality of life before cycle four and three to six weeks after the treatment were observed in the IP arm. In particular, patients experienced greater G3/4 pain (11% vs. 1%), fatigue (18% vs. 4%), leukopenia (76% vs. 64%), infection (16% vs. 6%), platelet (12% vs. 4%), gastrointestinal (46% vs. 24%), metabolic (27% vs. 7%) and neurologic toxicities (19% vs. 9%). As a result of these toxicities, 48% had three or fewer IP treatment and only 42% completed the planned six cycles. The quality of life a year after the treatment, though, was similar to those women treated with the standard IV chemotherapy.8,13

Based on these trials, these toxicities may be summed up as cisplatin-related toxicities, paclitaxel-related toxicities, and catheter-related complications. IP cisplatin-related toxicities include nephrotoxicity and neurotoxicity. IP paclitaxel-related toxicities were severe abdominal pain and severe neurotoxicity. All were short-lived and controllable. IP catheter-related complications include infection, inflow obstruction, leakage, extrusion, and severe pain. Fortunately, these complications were not serious.11

Four years after the excitement over GOG 172, IP chemotherapy still has not been adopted into standard practice across the United States. The best data sources suggest that less than one percent of women with ovarian cancer receive IP chemotherapy. Among the reasons cited behind this failure to adopt IP are: the lack of expertise in IP catheter placement, the more complex and time-consuming administration of IP chemotherapy and the greater toxicity associated with the IP chemotherapy.

Subsequently, IP chemotherapy was adopted by the Mayo Clinic using the GOG 172 regimen as its new standard of care for optimally-debulked ovarian cancer patients. This led Aletti, et al., to retrospectively evaluate the completion rates and toxic effects of IP chemotherapy in a cross section of patients with ovarian cancer. They noted that IP chemotherapy was recommended in 59 of 89 (66%) patients. The reasons for not recommending IP chemotherapy included postoperative complications with slow recovery in 7 of 89 (8%) patients, poor nutritional/functional status in 5 (6%) patients, and extensive surgery including large bowel resection in 9 (10%) patients. Twenty eight of whom (47%) were started on IP chemotherapy, 14 of 28 (50%) patients received 3 or more cycles and only 8 of 28 (33%) completed the six-cycle course. IP chemotherapy was discontinued because of grade 3/4 nephrotoxicity in 5 of 20 (25%), severe abdominal pain in 5 of 20 (25%), port infection or malfunction in 5 of 20 (25%), including one patient with bowel perforation by intraperitoneal catheter, a combination of the above in 1 of 20 (5%), and other in 4 of 20 (20%). They surmised that the IP chemotherapy regimen used has high morbidity with only a small percentage of patients completing the treatment. Therefore, should be recommended in a selective population of patients with ovarian cancer.20

Gray, et al., on the other hand, studied the tolerability and feasibility of alternative IP treatment strategies considering the toxicity and poor patient tolerability of GOG 172. They found out that 9 patients (36%) given IP Cisplatin either had a change in or discontinuation of the regimen secondary to abdominal pain (2), port malfunction (2), neuropathy (2), progression (1), nausea (1) and port infection (1). But, 4 (44%) of these patients who were shifted to IP Carboplatin were able to continue IP therapy. Conversely, the IP Carboplatin group had only one patient (7%) who received one less planned cycle due to port malfunction. The difference in the completion rates was significant (P=0.048). Grade 3 non-hematologic toxicity was also more common in the IP Cisplatin group (20% vs. 0%, P=NS). Complete response was 92% for both groups, with three patients having partial responses (8%). Recurrence rates in the IP Carboplatin and Cisplatin groups were 27 and 38%, respectively (P=NS), at a median follow-up of 18.3 months. Although, the median progression-free interval and overall survival have not yet been reached for both groups they concluded that using IP Carboplatin or dropping Day 8 IP paclitaxel may have less toxicity and less discontinuation of therapy without compromise of progression-free and overall survival.21

In our study, several toxicities were observed. Bone marrow suppression was demonstrated by maximal G2 anemia and G3 leukopenia. Non-hematologic adverse events noted were G3/4 GIT toxicity, G2/3 abdominal pain, G3/4 hypokalemia, G3 hypoalbuminemia, G3 skin toxicity and G3 fatigue. IP catheter-related severe peritonitis occurred in only 1/16 (1.75%) patients. Likewise, one patient had leakage in the mini-lap site and two (3.5%) had IP port re-insertion due to displacement. Nearly all of the toxicities were seen in 2/16 patients in the series. One of whom had a persistent disease,
previously treated with platinum based agents, and had a poorer ECOG performance status of 1 compared to the rest of the patients. IP therapy was discontinued for this patient after one cycle of treatment due to the observed toxicities specifically severe peritonitis. She was shifted to standard IV therapy and the IP catheter was removed. The other patient was able to complete 4 cycles before the said toxicities occurred. She was shifted to the standard IV therapy and was followed by 2 IP Paclitaxel as consolidation treatment.

There was also a patient who had a skin dehiscence of about 1.0 cm in greatest diameter overlying the IP port with passage of clear fluid. This fluid collection was presumed to be a seroma which drained after a skin break secondary to multiple punctures on the skin over the IP port site. It was also speculated that the technique of imbedding the port may have something to do with the dehiscence. It was observed that the port was immediately overlying the skin. Imbedding the port under the subcutaneous tissue might have provided additional protection from multiple punctures. Another patient, however, had fascial dehiscence requiring repair with a prolene mesh.

Two other patients had port access problem due to displacement. On IP port re-insertion, it was noted that the port was not properly anchored to the fascia. Observance of proper port insertion technique is essential to avoid such occurrences. One had leakage problem in the mini-lap site which was most likely due to poor wound healing and severe hypoalbuminemia. This patient refused further treatment due to financial instability.

There were no inflow IP catheter obstruction, and kinking problem noted in this series. No treatment related deaths were also observed. The G3/4 toxicities were seen in only a minority of the patients. In general, the toxicities observed were transient, tolerable and manageable by IV hydration, granulocyte-colony stimulating factors (g-csf), blood transfusion, analgesics, erythropoietin and antibiotics.

Significantly, more toxic events are expected to be seen in patients having IP therapy than in IV therapy. The IP regimen was designed to provide an intensive therapy that cannot be safely given intravenously. This ability to give higher and more frequent dosing when using this approach is one of the benefits of IP therapy i.e., the dose-intensity/dose-density cannot be provided in the standard IV route. A higher dose of cisplatin is given because the capillary uptake of cisplatin from peritoneal surfaces is slow and incomplete, resulting in systemic exposure that is prolonged. GIT related adverse effects mainly abdominal pain appears to be inevitable because of nature of the IP therapy itself. It is related to the infusion of cytotoxic drugs resulting to abdominal distension especially when pain develops immediately after delivery of the treatment volume i.e. two liters in most of the trials. Fifty six per cent (56%) of the patients in this series experienced abdominal pain after infusion. Most were temporary but, one patient had G3 abdominal pain and refused further IP treatment after one cycle due to this problem. Her ECOG performance status was 1 and tumor size was more than 10 cm prior to IP therapy. It is probable that the tumor size prior to IP therapy was a factor to the intolerability of this patient to the volume of infusate. Another patient also had G2 abdominal pain during and after infusion of Oxaliplatin which improved by giving pain relievers. Although the optimal volume of infusate is not known, the observation of Fujiwara, et al. that decreasing the infusate volume to 500ml to 1 liter can improve the tolerability of the treatment in Asians who generally have smaller stature than the Caucasians.

Still, majority of the toxicities reported in this study were observed in the group given the GOG 172 regimen. The least adverse event was noted in one patient who received IP Carboplatin following the Fujiwara protocol. This further supports earlier studies of Fujiwara and recently Gray on the more acceptable toxicity profile of IP Carboplatin.

### CONCLUSION

In the Philippines, intraperitoneal chemotherapy is a novel treatment for ovarian carcinoma. Only a handful of cases have been attempted despite evidence confirming the significant improvement in overall survival of patients with optimally debulked advanced ovarian cancer given IP chemotherapy. It may not be the ideal treatment for all patients, but should be offered as a practical treatment choice. Patients are entitled to unprejudiced information on the survival and toxicity differences of IP and standard IV therapy. The IP therapy in this study was generally well-tolerated with acceptable toxicities that were short-term and manageable. Although this was a limited study, we may, in a limited way, conclude that IP therapy may be a reasonable treatment option, given the acceptable toxicity profile.
seen versus a significant improvement in overall survival.

REFERENCES

Vaginal Myoma Masked by Condyloma Acuminata: A Case Report

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Leiomyoma of the vagina is rare tumor. There are only approximately 330 cases reported worldwide. Diagnosis is simple but unsuspected. These lesions are benign smooth muscle neoplasms, usually solitary, slow growing, and in many cases, asymptomatic. Histologically, they resemble uterine leiomyoma.

We report a case of a 38-year old nulligravid who presented with a two-year history of a painless anterior vaginal mass and abdominal enlargement. Incisional biopsy of the mass done and revealed condyloma acuminate. Ultrasonography revealed an impression of vaginal myoma. She underwent exploratory laparotomy, total hysterectomy for myoma uteri and subsequent transvaginal excision and enucleation of the vaginal mass. Histopathologic results revealed a vaginal Leiomyoma co-existing with multiple uterine Leiomyoma co-existing with multiple uterine Leiomyoma. Condyloma acuminata was also found on the vaginal epithelium overlying the tumor. Diagnosis was confirmed with histologic evaluation.

Key words: Vaginal leiomyoma, leiomyoma, condyloma acuminata, enucleation

Leiomyoma is a benign solid tumor of the smooth muscle and represents the most frequent gynecologic tumor.1 Although the uterus is the most common site of origin, leiomyomas can develop at any site where there is smooth muscle cell.2 Vaginal leiomyoma is a rare entity. It usually arises from the anterior vaginal wall, but is not always associated with leiomyomas arising from any other sites, such as the uterus, cervix, round ligament, ovary, inguinal canal, and vulva. A histopathologic study can confirm the correct diagnosis. These tumors are frequently misdiagnosed and sarcomatous changes can occur. Because of its rarity, the diagnosis and management of an extrauterine leiomyoma is even more challenging. An uncommon case of a vaginal leiomyoma with concomitant Condyloma acuminata as discussed here.

THE CASE

This is a case of a 38-year old nulligravid Filipino woman who presented with a slowly-growing vaginal mass.

The patient's condition started two years prior to admission when she noted a firm, painless mass, about three centimeters in diameter, protruding from her introitus. This was accompanied by a gradually enlarging abdomen. There were no other symptoms such as bleeding, or those urinary or compressive in nature. The patient did not consult until six months prior to admission when the vaginal mass gradually enlarged to about six centimeters in diameter, and still with no accompanying symptoms. There were no bladder and bowel changes, no weight loss, and no changes in menstrual pattern. She consulted a local health center in Cavite wherein physical examination and ultrasound revealed myoma uteri and uterine prolapse. The patient was advised observation but was lost to follow-up. The progressive enlargement of the abdomen and vaginal mass eventually prompted the consult at our institution.

Her past medical history was unremarkable aside from the multiple warts on her face and neck, which she had for the past several years. Her mother and sibling also had multiple warts all over their bodies. There were no other heredofamilial diseases. She was
a non-smoker and non-alcoholic beverage drinker. She denied illicit drug use. She also denied sexual contact.

On physical examination, the abdomen was globular and nontender. There was a 20cm x 15cm, firm, movable, intra-abdominal mass extending from the symphysis pubis up to three fingerbreadths below the xiphoid process (Figure 1). On vaginal inspection, there was an 8cm x 6cm x 5cm, pinkish, oval-shaped mass on the introitus (Figures 2 & 3). The mass was generally smooth, but on further inspection, there was note of multiple, pinkish, soft papules (Figure 4) measuring 0.1cm - 0.3cm in diameter covering the upper middle surface of the mass, inferior to the urethral area. The hymen had been stretched out by the huge prolapsing mass. Upon internal examination, it was noted that the mass was slightly movable and its antero-superior portion was attached directly to the lower third of the anterior vaginal wall (Figure 5). By lifting the mass anteriorly, two fingers could be inserted into the vaginal canal below the posterior border of the mass, but there was difficulty in assessing the lateral vaginal walls. One-fourth of the mass occupied the lower third of the vagina while the remainder of the mass was seen protruding at the introitus (Figure 6). The remainder of the vaginal wall was smooth. The cervix was smooth, firm, and closed. The uterus was enlarged to 24 weeks. There was no adnexal mass or tenderness. Vaginal speculum could not be inserted due to the obstructing mass. There was no lymphadenopathy.

The admitting impression was Nulligravid, Vaginal Mass rule out Malignancy, Myoma uteri.

Two separate punch biopsies on the middle and lower portions of the vaginal mass were done on separate occasions. Both revealed insignificant findings i.e., strips of stratified squamous epithelium and acute and chronic inflammation, respectively. Hence, a repeat incisional biopsy involving the deeper tissues of the mass was made which revealed
Figure 3. Closer view of the mass showing the (A, arrow) urethra, (B) lateral and (C) be occupying the middle anterior vaginal wall.

Figure 4. There were multiple, pinkish, soft, papules measuring 0.1cm-0.3cm diameter covering the upper middle surface of the mass, inferior to the urethral area (arrow).

Figure 5. On internal examination, the viginal vault was accessed below the inferior border of the firm, non-tender mass. A foley catheter was inserted on the urethra (arrow).
condyloma acuminate. A transvaginal/transabdominal ultrasound done in our institution showed intramural myoma uteri and a vaginal myoma (Figure 7). Work-up for sexually transmitted diseases including HbsAg, VDRL, and HIV screening was negative. Other laboratory results were normal.

Pre-operative working impression was Nulligravid, Myoma Uteri, Vaginal Mass probably Vaginal Leiomyoma vs Condyloma Acuminata, status post incisional biopsy-Condyloma Acuminata.

The patient underwent exploratory laparotomy, total abdominal hysterectomy (Figure 8) followed by excision and enucleation of the vaginal mass via transvaginal approach (Figure 9). Intra-operative findings showed multiple myoma uteri, intramural and submucous component (Figures 10 & 11), the largest of which measured 14cm x 9.5cm x 7.5cm. On cut-section, the specimen revealed whorled, white-tan masses with well-defined capsules (Figure 11).

Figure 6. One-fourth of the mass occupies the lower third of the vagina while the remaining three-fourth is seen protruding on the introitus.

Figure 7. Ultrasonography showed (A) anterior intramural mass and (B) well-circumscribed hypoechoic solid mass arising from the anterior vaginal wall.

Figure 8. Total abdominal hysterectomy for myoma uteri showing the enlarged uterus with grossly normal ovaries and fallopian tubes (arrows).
Figure 9. (A),(B) Excision and enucleation of the vaginal mass. A midline vertical incision is made on the anterior vaginal wall. The vaginal epithelium is dissected sharply off the fibromuscular layer, looking for an apparent cleavage plane. The mass is grasped with tenaculum, to achieve tissue traction and create tissue tension between the myoma and the vaginal wall.

Figure 9. (C) The meticulous sharp and blunt dissection of the pseudocapsule surrounding the myoma using cautery Matzenbaum scissors.

Figure 9. (E) Following removal of the tumor, excess tissue was trimmed. (F) Ligation of bleeders minimized blood loss.
Figure 9. (G) (H) The ability to precisely enucleate the mass and repair the defect with multilayer suturing is crucial. The vaginal mucosa reapproximated using 2-0 delayed absorbable suture.

Figure 10. The serosal surface of the uterus is smooth and regularly enlarged to 16.5cm x 15cm x 14cm.

Figure 11. Cut section of the uterus showing whorled white-tan cut surface (arrow) that bulge above the surrounding myometrium.

During the transvaginal approach, the urethra was superior to the mass, and the foley catheter was inserted without difficulty. The excised vaginal mass measured 8cm x 7cm x 5.5cm (Figure 12). On its upper middle surface were multiple, pinkish papules. On cut-section, the specimen was solid, with well-defined capsule, cream-white to tan color, and with whorled-like pattern (Figure 13).

Histopathologic examination of both uterine and vaginal specimens showed leiomyoma (Figures 14 & 15) and Condyloma acuminata on the overlying vaginal epithelium (Figure 16).
The patient was discharged well on the fourth postoperative day (Figure 17) with good bowel and bladder function. Upon follow-up on and third postoperative weeks (Figures 18 & 19), the vaginal wound was intact with normal-looking external genitalia and the patient was voiding normally. She was referred to the Dermatology department for evaluation and work-up of the warts on the other parts of her body. The plan was to perform biopsy.

Vaginal leiomyoma is a rare tumor with variable clinical presentation and broad differentials that can lead to pre-operative misdiagnosis. Diagnosis is simple, but usually unsuspected. The tumor can sometimes have unusual presentation that is largely responsible for the relative difficulty in pre-operative diagnosis. It may be confused with a large variety of benign conditions. When presented with a midline anterior vaginal mass, the differential diagnoses include uterine prolapse, cystocele, urethrocystocele, urethral diverticulum, fibroepithelial polyp, Gartner duct cyst, Batholin's gland cyst, Skene duct abscess, cervical myoma, or vaginal malignancy. Gupta, et al. reported a case of a vaginal myoma thought to be an ovarian tumor. Shirvani and Winters reported a case of a vaginal myoma mimicking a urethral diverticulum. Njeh, et al. even called the vaginal myoma as a "female prostate" as it presented as an intravesical defect revealed in cystogram resembling a hypertrophied prostate. Jeng, et al. and Leron and Staton each reported a case of vagina myoma which was initially misdiagnosed as uterine prolapse.

In this case, there was likewise dilemma on the pre-operative diagnosis. Initially, the tumor was not examined well and was mistaken for a uterine prolapse. In uterine prolapse, the cervix and the body of the uterus are usually visible in the introitus, which was not seen in our patient. The mass could not be repositioned inside the vault and could not be affected by valsalva maneuver and coughing in contrast to a uterine prolapse. In addition, vaginal birth is the most frequently-cited risk factor to uterine prolapse and our patient is a nulligravid. Hence, uterine prolapse was ruled out.

Another factor which misled us with our diagnosis was the result of the incisional biopsy of Condyloma acuminata. The classic presentation of Condyloma varies from pinhead size papules to large cauliflower-like pedunculated masses very unlike our patient's generally smooth oval-shaped mass. Furthermore, risk factors for acquiring condyloma acuminata such as smoking, oral contraceptives, multiple sexual partners, and early coital age were not present in our patient. However, an unusual
Figure 16. (A)(B) Histopathology of condyloma acuminata- showing parabasalar hyperplasia with prominent intracellular bridges. Above the basilar layer, koilocytotic cells (arrow) with prominent perinuclear halos are found in the more superficial epithelium. (C) Magnified view.

Figure 17. Post-operatively.
presentation of condyloma acuminata was still considered.

The first case of myoma of the vagina was reported in 1733 by Denys de leyden.$^{4,5,7,12,13}$ Only about 330 cases of vaginal leiomyoma were reported worldwide.$^{4}$ Bennett and Ehrlich found only 9 cases in 50,000 surgical specimens and only 1 case in 15,000 autopsies reviewed at John Hopkins Hospital.$^{5,7,13,14}$ In a literature search using PubMed and Medline, there were only 72 cases of published articles about vaginal leiomyoma. No local study has been published in the Philippine Journal of Obstetrics and Gynecology from 1976 to 2009. This is the first reported case in our institution.

The etiology of vaginal leiomyoma is unknown, but it is said to have come from the smooth muscle components of the vulva and vagina, smooth muscle tissues, smooth muscle of the bladder or urethra, round ligament remnants, erectile tissue, and blood vessels.$^{15}$ Vaginal leiomyoma is most common in Caucasians while uterine leiomyoma is most common in black women.$^{2}$ Hispanic and Asian women have similar rates as White women.$^{1}$ Like uterine leiomyoma, they occur most frequently

Figure 18. Two weeks post-operatively, normal looking external genitalia, wound well coaptated with good bladder function.

Figure 19. Three weeks post operatively, wound completely healed and patient voided normally.
between 35-50 years of age.5 Risk factors include increasing age, early menarche, low parity, Tamoxifen use, obesity, and, in some studies, a high-fat diet.1 In our case, the patient is a 38-year old nulligravid obese, who is fond of eating high-fat diet.

Vaginal leiomyoma may occur anywhere within the vagina, usually situated in the submucosal region. These tumors are most commonly located in the midline anterior vaginal wall.3,5,16 A single case of a vaginal myoma arising from the lateral wall was reported by Kaufman, et al.2 Posterior location is more associated with malignancy,17 although there was one report of benign vaginal myoma on the posterior wall.7 They are not always associated with leiomyomas arising from other sites, such as the uterus, cervix, round ligament, ovary, inguinal canal, and vulva.5,18 They are usually solitary.5 Their sizes vary from 0.5cm to 15cm in diameter, averaging about 3cm to 4cm.5,19,20 They can grow to as large as 20cm in diameter.19 The patient in our case presented with a myoma located in the anterior vaginal wall and a concomitant multiple myoma uteri.

Vaginal myoma usually presents as a mass per vagina. Since most are relatively small, they are often asymptomatic in many cases. When large symptoms arise due to compression, they include pain, vaginal discharge or bleeding, dyspareunia, sensation of pressure, obstruction of the vagina, constipation, or urinary tract symptoms such as frequency, urgency, dysuria, bladder neck obstruction, urinary retention, and incontinence.5 Our patient, even with a large 8cm x 6cm myoma, was asymptomatic. The lack of symptoms may be attributed to the elasticity of the vagina.20

An unusual presentation reported was gluteal swelling with purulent vaginal discharge.16 The most useful modalities for detecting extraterine leiomyomas are ultrasonography, computed tomography and magnetic resonance imaging.21 Pre-operative imaging and careful examination may help rule out malignancy. Ultrasonography is extremely useful to depict the morphology and anatomic location and to reveal the heterogenous echo texture consistent with myoma.9 Magnetic resonance imaging will show a homogenous lesion with similar signal intensity than that of the myometrium.7 In our case, we used ultrasound to confirm our clinical diagnosis of vaginal leiomyoma and showed a well-circumscribed hypoechoic mass protruding from the introitus measuring 6.6cm x 6.3cm. The echogenic mass in the vaginal wall was clearly separated from the uterus, cervix, and bladder (Figure 7).

In cases where there are urinary symptoms such as difficulty in micturition, a urethroscopy or voiding cystourethrogram can be done to exclude a suburethral diverticulum.5 The urinary tract and the course of the ureter can also be evaluated using an intravenous pyelogram.5,22 Other modalities such as punch biopsy, incisional biopsy or needle biopsy5 may also help rule out malignancy, or if the diagnosis cannot be made confidently by visual inspection and non-invasive methods. However, it may sometimes mislead the diagnosis.

In this case, punch biopsy was performed twice, revealing insignificant findings. On the third attempt, an incisional biopsy was done, and revealed Condyloma acuminata. Although it ruled out malignancy, it made our working impression confusing because we also considered the mass as Condyloma acuminata. It is important that we managed our patient properly. Whether the tumor is myoma or Condyloma, local excision and enucleation is an adequate treatment. However, for malignant tumors, a more radical procedure with wider margins is appropriate.

For vaginal myoma, surgical excision and enucleation using transvaginal approach is the treatment of choice.5,6,23,24 If the tumor is located high in the anterior vaginal wall or if the base of the tumor is not accessible, an abdominal route is preferable.22 Prior to surgery, urethral cathetherization is done as a guide in order to avoid urethral injury (Figure 5). A midline vertical incision is made on the anterior vaginal wall, with length enough to accommodate the appropriate diameter of the tumor. The vaginal epithelium is dissected sharply off the fibromuscular layer. Tumor excision and enucleation follows, looking for an apparent cleavage plane. The mass is grasped with tenaculum, to achieve tissue traction and create tissue tension between the myoma and the vaginal wall. Then, meticulous sharp and blunt dissection of the pseudocapsule surrounding the myoma is carried out using cautery and Metzenbaum scissors. Following removal of the tumor, excess tissue is trimmed. Ligation of bleeders minimizes blood loss. The ability to precisely enucleate the mass and repair the defect with multilayer suturing is crucial. The vaginal mucosa is re-approximated using 2-0 delayed absorbable suture. The operation is not easy. Excision and enucleation of the mass while sparing the urethra and the bladder wall can be a challenge. These structures lie very close to the vaginal mass; damaging any of these can leave the patient with incontinence and urologic problems. On
the other hand, failure to re-approximate the tissues precisely can lead to cystocoele and urinary retention. In addition, morcella of the mass must be avoided. The mass should be completely excised and malignant transformation.16

The gross and microscopic appearances of vaginal leiomyomas resemble those of their uterine counterparts. On cut-section, the surface has a glistening, pearl-white appearance, with the smooth muscle arranged in trabeculated or whorled configuration. They are well-circumscribed, firm masses that may occasionally contain foci of necrosis, edema, or hyalinization. Microscopically, they are composed of interlacing fascicles of spindle-shaped cells, with elongated, oval nuclei and little or no mitotic activity or nuclear pleomorphism.25 There is no prominent increase in cellularity; but unlike in uterine leiomyoma, there is abundance of capillary-sized vessels. It is recommended that the diagnosis of vaginal leiomyoma be reserved for those tumors fewer than 5 mitoses per 10 hpf.26 The tumors are usually slow-growing and malignant conversion is extremely rare. However, it should also be noted that an increased mitotic activity in the absence of aggressive behavior may be present in vaginal myomas during pregnancy. This is true of uterine leiomyoma, the vaginal lesions are estrogen-dependent. Hence, they can grow rapidly during pregnancy and regress after menopause.

It is possible that a leiomyoma may actually be a smooth muscle tumor of unknown malignant potential or a leiomyoma affects the management of the tumor.27 Therefore, it is important to rule out malignancy. Sarcomatous changes, tumor recurrence and rapid enlargement usually indicate malignancy. Recurrence is uncommon but reported.5 In general, sarcomas represent 2-3% of all gynecologic malignancies and only 10% occur outside the uterus. However, Liu reported an incidence of 9.1% malignant transformation of vaginal leiomyoma.28 Thus, a thorough histologic evaluation and subsequent definitive management are warranted.

Another interesting point of this case was the concurrent presence of Condyloma acuminata on the vaginal myoma. No such reported case is published to date. Condyloma acuminata is the most common viral sexually transmitted disease.29 It is caused by Human Papilloma Virus (HPV). In more than 90% of cases, they are caused by HPV types 6 and 11, which are considered low-risk types because they are not associated with increased risk of cancer. However, a person may be infected with more than one type of HPV at the same time. Transmission of HPV is primarily through sexual contact by direct skin to skin contact from an infected individual. The virus can also be transmitted from mother to infant during childbirth. Our patient is nulligravid and denied history of sexual contact to explain the acquisition of the lesion. However, infection by direct manual contact or indirectly by fomites rarely may occur.29 Some researches suggest that genital HPV can be transmitted through digital penetration, transfer of body fluids and other non-sexual routes such as via fomites, i.e., inanimate objects like towels or underwear. More research must be conducted to examine these modes of transmission. Conditions that are known to predispose women to infection with HPV include immunosuppression, diabetes, pregnancy, and local trauma.15 The presence of the large myoma exposed the vaginal mucosa to the outside environment, which may take it prone to infection and trauma. It is also possible that the quality of the area may be a factor, i.e., skin is much repellant rather than the mucosa. It is also interesting to note that the patient had multiple warts on other parts of her body. The relation of these warts to the lesion on the vagina could not be ignored, but there is no supporting evidence to prove this yet. It is possible that the cutaneous manifestations of HPV are not only dependent on subtype but also site-dependent. Nonetheless, the management of condyloma is surgical excision which has the highest success rate and lowest recurrence rate.

**SUMMARY AND CONCLUSION**

Vaginal leiomyomas are infrequent; nevertheless, they should not be taken for granted. These tumors have variable presentation and can be misdiagnosed with a variety of other conditions. A complete history and physical examination supported by laboratories are important in the diagnosis. Therefore, when presented with a vaginal mass, we should consider vaginal leiomyoma as one of the differentials. Whenever detected, it has to be removed immediately to prevent further growth and transformation to malignancy. Complete surgical excision via the transvaginal approach is the treatment of choice followed by careful histologic evaluation to exclude malignancy. Early detection prevents larger lesions that may produce symptoms; this will improve the quality of life of the patient. Proper advice is also recommended. On the other hand, the co-existence of the Condyloma acuminata and vaginal leiomyoma should be further investigated.
REFERENCES

Collision tumors represent the coexistence of two or three adjacent but histologically distinct tumors, without histologic admixture in an organ. Collision tumors have been reported in various organs, but are rarely seen in the ovaries. Reported cases consisted of a benign mature ovarian teratoma and an ovarian cystadenoma or cystadenocarcinoma. Primary carcinoid tumors of the ovary, on the other hand, are uncommon, with a reported incidence of 0.3%. They are usually unilateral and are found in association with mature cystic teratoma, forming a solid nodule within it. Primary ovarian carcinoids rarely metastasize and are treated as ovarian tumors with low malignant potential. This is a rare case of a triple collision tumor of the ovary in a 63 year old multigravid who complained of a gradually enlarging abdominal mass. Total abdominal hysterectomy with bilateral salpingo-oophorectomy and complete surgical staging was performed. Histopathology revealed coexistence of a mucinous tumor of low malignant potential, trabecular-type carcinoid tumor, and mature cystic teratoma in the right ovary. The possibility of the occurrence of such collision tumors must always be kept in mind intraoperatively and during pathologic examination to avoid misdiagnosis of these cases.

Key words: Collision tumor, mature cystic teratoma, carcinoid tumor, mucinous tumor

Collision tumors represent the coexistence of two or three adjacent but histologically distinct tumors. The components are considered as different primary neoplasms that are separated from one another by their basal lamina or an intervening stroma. Collision tumors of the ovary are rare entities and most commonly consist of a benign mature ovarian teratoma and an ovarian cystadenoma or cystadenocarcinoma. Other histologic combinations have also been reported, with the mechanism of origin still uncertain.

This is a rare case of a triple collision tumor in a postmenopausal multigravid who underwent surgery for an enlarging multiloculated mass in the right ovary. The histopathology examination revealed the coexistence of a mucinous cystadenoma with focus of borderline lesion, carcinoid tumor and mature cystic teratoma.

THE CASE

A 63 year old multigravid was admitted due to abdominal enlargement. The condition started a year prior to admission, when she palpated a non-tender mass approximately 5cm at the hypogastric area. There was no weight loss, anorexia, skin flushing, nausea, vomiting, or abdominal pain. There were no changes in bowel or bladder habits. No consult was done. The mass was noted to enlarge gradually, prompting consultation at our institution two weeks prior to admission. She had no co-morbid medical illness and had no previous surgeries. Family history was unremarkable. She had her menarche at 14 years old. Subsequent menstrual cycles came at regular monthly intervals with moderate flow. She experienced menopause at the age of 59. She is a gravida 10 para 10. All pregnancies were carried to term, delivered vaginally, with no complications noted.

On physical examination, the patient was conscious, coherent, ambulatory, not in cardiorespiratory distress, with an Eastern Cooperative Oncology Group (ECOG) performance score of zero. Vital signs were stable. She had pink palpebral conjunctivae and anicteric sclerae. There were no palpable cervical or supraclavicular lymph
nodes. She had symmetrical chest expansion with clear breath sounds, no rales or wheezes. She had an adynamic precordium with distinct heart sounds and no murmurs. The abdomen was flabby and soft, with normoactive bowel sounds. Abdominal palpation revealed a mass at the hypogastrium, extending up to 4 cm below the umbilicus. The mass was predominantly cystic with solid areas, irregular in shape, and movable. There were no palpable inguinal lymph nodes. She had normal external genitalia. On speculum examination, the cervix was pink with no lesion or discharge noted. On internal examination, she had a smooth parous vagina. The cervix was firm, smooth, short and closed. There was a pelvoabdominal mass approximately 14 cm, firm, movable and non-tender. The corpus and adnexae were difficult to assess. Transvaginal ultrasound was requested, which showed bilateral ovarian masses: 5.0 cm x 4.22 cm x 4.14 cm in the right ovary, with a Sassone score of 10, and 11.01 cm x 9.95 cm x 9.90 cm in the left ovary, with a Sassone score of 12. There was also note of a posterior pelvic wall mass which could be rectal, parasitic myoma, or a part of the ovarian pathology. Serum CA 125 was normal at 26.4 U/mL. The patient was subsequently admitted for laparotomy, with an admitting impression of ovarian new growth, probably malignant.

The patient underwent exploratory laparotomy on her 2nd hospital day. On exploration, there was 50 cc ascitic fluid. Peritoneal fluid was collected for cytology. The liver, subdiaphragmatic surfaces, spleen, kidneys, stomach, omentum, intestines and appendix were grossly normal on inspection and palpation. There were no adhesions noted. The right ovary was converted to a 10 cm x 11 cm x 10 cm predominantly cystic multiloculated mass with intact capsule (Figure 1). Cut section revealed the locules to contain sebum and hair, others contained mucin. There was note of a 5 cm yellowish solid area within (Figure 2). The left ovary measured 4 cm x 2 cm x 1 cm and was grossly normal. Both fallopian tubes were unremarkable. The uterus was small. Right salpingooophorectomy was done. The right ovary and fallopian tube were submitted for frozen section, which revealed malignant carcinoid tumor, rule out adenocarcinoma, mucinous tumor, and mature cystic teratoma. The carcinoid tumor was noted to be within the ovarian stroma. The patient was referred intra-operatively to a gynecologic oncologist. Total abdominal hysterectomy with left salpingooophorectomy, bilateral pelvic lymph node dissection, paraaortic lymph node sampling, infracolic omentectomy, and random peritoneal biopsies were done. The harvested lymph nodes were not suspicious for malignancy. A 2.5 cm x 2.5 cm, ovoid cystic mass was noted at the left pararectosigmoid area, containing gelatinous fluid within. Intraoperative referral to surgery was done. The specimen was likewise submitted for frozen section, revealing simple cyst with chronic inflammation. Estimated blood loss was 700 cc.

Postoperatively, two units of packed red blood cell were transfused. The patient developed fever (38.2°C) on her second postoperative day. Bibasal
crackles were noted on physical examination. Chest x-ray revealed haziness in the left paracardiac area for which pneumonia is not ruled out. She was then given Azithromycin. The rest of the patient's hospital stay was unremarkable. She was discharged on the 12th postoperative day in good general condition.

Pathology

Microscopic examination of the locules of the right ovary showed areas with a single layer of tall columnar mucin-containing cells with basally located small nuclei (Figure 3). Other areas showed multilayering of the epithelium with atypical hyperchromatic nuclei but with intact basement membrane (Figures 4 & 5), consistent with borderline mucinous tumor. Found in the same ovary are mature elements of sebaceous gland, smooth muscle and adipose tissues, which are consistent with mature teratoma (Figure 6). Adjacent to the teratoma is a cellular mass which is the carcinoid tumor composed of a gland-like structure (Figure 6). The carcinoid tumor was noted to be of the trabecular pattern, with long ramifying and anastomosing cords surrounded by connective tissue stroma (Figure 7).

Figure 3. An area of mucinous cystadenoma in the right ovary showing a single layer of tall columnar mucin-containing cells with basally located small nuclei (100x).

Figure 4. Other areas of the right ovary showed multilayering of the epithelium (100x).

Figure 5. Higher magnification of the area shown in Figure 4 showed multilayering of the epithelium with atypical hyperchromatic nuclei but with intact basement membrane (450x).

Figure 6. Other areas of the ovary showed mature elements of (A) sebaceous gland, (B) smooth muscle and (C) adipose tissue. Adjacent to the teratoma is a cellular mass which is the (D) carcinoid tumor, composed of uniform gland-like structures (100x).

Figure 7. Higher magnification of the carcinoid tumor, of the trabecular pattern, with long ramifying and anastomosing cords surrounded by connective tissue stroma (100x).
Final histopathologic diagnosis showed Mucinous cystadenoma with small focus of borderline mucinous lesion, carcinoid tumor, and mature cystic teratoma in the right ovary; unremarkable right and left fallopian tubes; Paracortical cyst and corpora albicantia, left ovary; Senile cystic atrophy, endometrium, atrophic myometrium, chronic cervicitis with focal squamous metaplasia and nabothian cyst. All lymph nodes recovered were negative for malignant involvement. The pararectosigmoid mass was a simple cyst with chronic inflammation and foreign body reaction. No malignant cells were seen on cytology.

Final diagnosis for this case is a triple collision tumor of the right ovary. Mucinous tumor of low malignant potential, stage IA, Primary Carcinoid Tumor of the ovary, stage IA, Mature cystic teratoma.

**DISCUSSION**

Collision tumors represent a coexistence of two adjacent but histologically distinct tumors, without histologic admixture in an organ.¹ The different components are considered separate primary neoplasms which are histologically distinct and separated from each other.² These tumors have been described as case reports occurring in various organs such as the esophagus, stomach, liver, bone, kidneys, brain, lungs and thyroid gland.³ Collision tumors involving the ovaries are rare, with a teratoma together with cystadenoma or cystadenocarcinoma as the most common histologic combination.³ The first case of triple collision tumor of the ovary had been reported in Greece in 2008. Their case consisted of mature cystic teratoma and serous cystadenoma of the right ovary and hemorrhagic follicular cyst in the left ovary.⁵

This is probably the first ever reported case of a triple collision tumor occurring in a single ovary.

Mature cystic teratoma is the most common germ cell tumor of the ovary, comprising more than 20% of all ovarian neoplasms. It may occur at any age, with a peak incidence in the first two decades of life.⁶ Microscopically, it is composed of tissues that originate from the ectoderm, mesoderm, or endoderm, found in variable proportions within the tumor. Malignancy complicates 1-2% of these tumors and may occur either by malignant transformation of one of the component benign element, or a malignant lesion may co-exist with the benign teratoma.⁷ Malignant transformation of mature cystic teratomas is an uncommon complication found in approximately 1-3% of all mature cystic teratomas.

Majority of these cases occur in women more than 40 years of age, with a peak incidence in the fifth and sixth decades of life.⁵ Tumor diameter of more than 10 cm, bilateral ovarian involvement, presence of multiple dermoid cysts, and rapid growth, are associated with a relatively higher risk of developing ovarian malignancies.⁸⁻¹° Cysts that are unusually adherent, containing solid areas or firm, friable, myxomatous or variegated portions, typically harbor malignancy. Furthermore, the presence of nodular, papillary or cauliflower-like growths protruding into the cyst cavity, or nodules or plaques along the cyst wall, penetrating through the outer surface of the ovarian capsule, or invading adjacent organs, are reliable markers of malignancy.⁷

Although any of the component tissues of the teratoma has the potential to undergo malignant transformation, squamous cell carcinoma is the most commonly associated cancer, arising from the squamous lining of the cyst wall. It represents 75% of cases.¹⁰ Other tumors that may arise from a mature cystic teratoma include adenocarcinoma (6.8%), basal cell carcinoma, adenosquamous carcinoma, thyroid carcinoma, sebaceous carcinoma, malignant melanoma, sarcoma, carcinoid tumor, neuroectodermal tumor and mucinous tumors.¹⁰,¹¹ Imaging by CT and MRI will show a fat-containing tumor with enhancing irregularly margined solid component that tends to be relatively large and to show extensive transmural extension and direct invasion of adjacent organs. In particular, contrast enhancement of the Rokitansky protuberance should raise the suspicion of a possible malignant transformation. This protuberance is a common site of malignant transformation and should be adequately evaluated during pathologic analysis.¹²

Mucinous ovarian tumors have also been found in association with mature cystic teratoma. The mucinous epithelium in such cases may be histologically benign, borderline or malignant.¹¹ Only two cases of mucinous tumor of low malignant potential associated with a mature cystic teratoma, as seen in our case, have been reported in literature.¹³,¹⁴
Primary carcinoid tumors of the ovary are rare, comprising less than one percent of primary ovarian neoplasms, and 0.3% of all carcinoid tumors.\textsuperscript{15} Majority of these tumors occur in association with mature cystic teratoma, although carcinoid tumors occurring in the pure form, unassociated with teratoma, have also been described. The case presented showed carcinoid tumor in association with a mature cystic teratoma in the same ovary.

These tumors may be classified into four types according to their morphologic and clinicopathologic features: insular or islet carcinoid (midgut derivation), trabecular (foregut and hindgut derivation), strumal (carcinoid tumor combined with thyroid tissue), and mucinous (goblet, adenocarcinoid).\textsuperscript{16} The insular type is most common, followed by the strumal type. Our patient demonstrated the trabecular type carcinoid tumor. As expected, our index patient did not develop any symptom attributable to the carcinoid syndrome, as this has been found to be associated only with the insular type.

Majority of patients are in the postmenopausal age group, as in this case. Imaging appearance of carcinoid tumors have not been well-described due to the rarity of cases. In general, they are solid lesions and are difficult to distinguish from solid malignancies sonologically and radiographically, although the presence of necrosis is more common among the latter. Nevertheless, carcinoid tumors of the mucinous type will have higher signal intensity on T2 weighted MR image compared with other solid tumors because of its high-signal intensity mucin content.\textsuperscript{17}

Primary ovarian carcinoids are usually unilateral. Grossly, they form a solid nodule within a cystic teratoma (Figure 2), or may be found as a solid yellow-gray mass when in the pure form.\textsuperscript{16} Microscopically, these tumors are similar to carcinoid tumors of the other organs. Secretory granules are seen within the tumor cells. Serotonin and hormonal peptides may be demonstrated by immunocytochemical analysis.\textsuperscript{17}

In its pure form, where the carcinomatoid component is the exclusive element of the tumor, careful evaluation must be done in order to distinguish them from metastasis from other primary sites.\textsuperscript{18} Metastatic carcinoids are usually bilateral, with scattered tumor deposits present in both ovaries.\textsuperscript{16} The synchronous presence of other teratomatous components favors a primary origin of the tumor.\textsuperscript{18} Carcinoid tumors are slow-growing, rarely metastasize, and are treated as an ovarian neoplasm with low malignant potential.\textsuperscript{16}

We are presented with a postmenopausal patient who underwent completion surgery and complete surgical staging for a malignant ovarian tumor. Surgery is the standard primary treatment for ovarian malignancies. The type and extent of surgery depend on whether a woman is still desirous of future pregnancy. For women who do not plan to have children or those who already completed their family size, total abdominal hysterectomy with bilateral salpingooophorectomy is performed. Complete surgical staging should be done if the diagnosis of malignancy is suspected, or confirmed by intraoperative frozen section examination.\textsuperscript{19}

In the case presented, histopathologic examination revealed the presence of a mature cystic teratoma, trabecular-type carcinoid tumor, and mucinous tumor of low malignant potential in the same ovary. The presence of all these three histologically distinct tumors in a single organ is a rare finding. The unilaterality of the tumor, absence of scattered tumor deposits, the association with a mature cystic teratoma, the absence of an extra-ovarian pathology, and the absence of lymphovascular space invasion, support the diagnosis that this tumor is primarily ovarian in origin, and is not metastatic from other sites.

The embryology and histogenesis of the carcinoid and adenocarcinoma components is of academic interest. Theoretically, both of these tumors may have arisen as a consequence of transformation of the teratoma. Through the years, different theories have been postulated to explain the origin of teratomas. Willis (1967), following Askenazy (1907), concluded that they arise from totipotential cells which become scattered throughout the various parts of the body during embryonic life. These cells normally remain dormant, but are later capable of further growth and differentiation if suitably stimulated. Subsequently, Pierce and Abell (1970) and Teilum (1971) theorized that teratomas arise from germ cells, being the only truly totipotential cells in the body. These germ cells first appear in the embryo along the wall of the yolk sac. They then migrate around the hinder end of the primitive gut to the genital ridge on the posterior abdominal wall. Here they congregate and are absorbed into the developing gonad which later descends to the pelvis or scrotum. It has been suggested that during this migration, some germ cells may get left behind on the journey or may stray too far and come to rest at various sites along the dorsal wall of the embryo.
near the midline. If these cells do not degenerate but remain viable, they may give rise to tumors in precisely these locations, that is, the retroperitoneum, sacral region, mediastinum, and pineal region. These cells may likewise differentiate along various germ lines, essentially recapitulating any tissue of the body derived from any of the germ cell layer (ectoderm, mesoderm, endoderm). Examples include hair, teeth, fat, skin, muscle, and endocrine tissue.

More recent evidence on the teratomatous origin of the carcinoid and adenocarcinoma components had been reported. Vang, et al. noted that a certain subset of mucinous tumors associated with teratomas exhibiting morphologic and immunohistochemical features of lower intestinal tract-type mucinous tumors may be teratomatous in origin. On the other hand, they may be independent epithelial and endodermal tumors that merely co-existed with the teratoma. Tumors that express CK7 with or without CK20 expression may be derived from upper gastrointestinal tract-type or sinonasal-type teratomatous elements, but could be independent tumors of surface epithelial-stromal origin. Tang, et al. reported a case of a mature cystic teratoma associated with complete colonic wall and mucinous cystadenoma. The authors demonstrated a transition zone between these two lesions, which was composed of mucin-producing cells with typical architecture of mucinous cystadenoma with scattered goblet cells and Paneth cells. Immunohistochemical analysis showed that this zone stained positive for both CK7 and CK20, and was focally positive for chromogranin. The authors concluded that the mucinous cystadenoma component most likely originated from the colonic epithelium of the teratoma. However, in our case, no transition zone as such can be demonstrated. Furthermore, each of the component tumors is histologically distinct and separated from one another as they are confined in separate locules, and are separated by narrow stroma. Although the tumors have a common point of meeting or contact, for the most part, they are noted to be separate from one another. Therefore, the case presented is that of a rare case of a triple collision tumor of the ovary.

No adjuvant treatment is warranted in this case since both the mucinous tumor and the carcinoid tumor are stage IA. Almost all borderline mucinous tumors are stage I and have an excellent prognosis following surgical treatment with reported metastatic rates of 0-3% for those without non-invasive carcinoma and 0-7% for those with non-invasive carcinoma. In one of the largest reported series of stage I pure borderline tumors devoid of microinvasion or non-invasive carcinoma, corrected actuarial survival rates were 98% at 5 years and 96% at 10 years. Likewise, carcinoid tumors are indolent tumors that rarely metastasize and are considered as tumors with low malignant potential.

Following the local guidelines for surveillance of gynecologic malignancies, monitoring for recurrence of disease is composed of regular follow-up with pelvic examination every 3 months for the first postoperative year, every 4 months during the second year, every 6 months during the third to the fifth year, then yearly thereafter. Tumor marker determination such as CA 125 and CA 19-9 will also be done every follow-up visit. CT scan or MRI is recommended for the first three years, or when indicated. Chest X-ray is also done, depending on patient signs or symptoms. Our patient was advised to undergo close surveillance as mentioned, unfortunately, she was lost to follow-up.

In summary, this is a rare case of a triple collision tumor of the ovary consisting of a mature cystic teratoma, mucinous tumor of low malignant potential, and trabecular-type carcinoid tumor in a postmenopausal patient who underwent complete surgical staging procedure. Knowledge of the existence of such tumors is important to increase the surveillance level of the surgeon and the pathologist, and prevent missing the diagnosis of other coexisting tumor types that might impact patient treatment and prognosis.

REFERENCES


Keynote Address*

AUGUSTO M. MANALO, MD**

Dr. Regta Pichay, President of the Philippine Obstetrical and Gynecological Society, Dr. Sylvia Carnero, Vice President and Overall Chairman of the Organizing Committee for this Annual Convention, other members of the illustrious Board of Trustees, Distinguished Guests, Colleagues and Friends.

I feel greatly honored, and humbled, that I should be asked to deliver the Keynote Address for this year’s Annual Convention.

I have been tasked to speak on Obstetric Gynecologic Practice in the Philippines - Past, Present and Future. In this presentation, I hope you will allow me to define the limits of the terms - Past, Present and Future. I will not talk about the distant Past, nor will I talk much about the still foreseeable future. I would like to assume a position where “I can easily look back, and just as easily look ahead as if I were standing at a doorway between two familiar rooms.”

The Philippines has shared, with other countries of the world, the very heavy burden of caring for its women, particularly during pregnancy and childbirth. This burden, and how we have coped with it, is most glaringly reflected in mortality and morbidity figures that are very now and then released by reputable health organizations.

According to a recent WHO and UNICEF publication, about 585,000 maternal deaths occur annually throughout the world. About 99% take place in developing countries. About 300,000 take place in Asia, especially South Asia. Most of the remaining take place in Africa.

According to Fathalla, these figures, staggering as they are, only represent “the tip of an iceberg of maternal morbidity and suffering.”

The WHO also estimates that every year, about 150,000,000 women suffer long term disabilities as a result of pregnancy and childbirth.

In the Philippines, maternal mortality and morbidity figures are no less disheartening... Especially when we consider that supposedly updated reports reveal gross underestimates of the previous ones. Every now and then, we read in the newspapers that great difficulty encountered by the employees of the National Statistics Office in penetrating certain areas in our remote provinces, and even in Metro Manila.

In the 1993 Philippine National Safe Motherhood Survey, 94% of women interviewed admitted having had prenatal care - 58% from midwives, 35% from doctors who were not necessarily obstetrician gynecologists, 28% from hilots and 5% from nurses. About 70% of deliveries took place at home - 52% were attended by hilots, 35% by midwives, 26% by doctors (again, not necessarily obstetrician gynecologists) and 15% by nurses. The rest were attended by relatives.

This indeed is a very disappointing report, considering that they were gathered only shortly before 1993. We would expect more participation from the professionals: doctors, nurses and midwives. The Philippine Obstetrical and Gynecological Society, has been certifying fellows and diplomates and accrediting residency programs long before that survey was made. Obstetrics and Gynecology has always been a major part of the subjects taught in

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* Delivered during 2010 Annual Convention and 64th Anniversary Celebration, Philippine Obstetrical and Gynecological Society (Foundation Inc.), November 10, 2010, Philippine International Convention Center.

** POGS President and Professor Emeritus, Department of Obstetrics and Gynecology, Philippine General Hospital and College of Medicine, University of the Philippines Manila.
medical schools. There seems to be no reason why basic needs of our women, like prenatal care cannot be provided by these professionals.

There seem to be a number of reasons for the figures in the survey.
1. sampling was not extensive enough
2. inaccessibility of professional care givers
3. lack of public health education

To me, the most important reason for the discrepancy between the expected and the actual participation of professionals is inadequate sampling of women interviewed.

We are all aware that the concerns of the obstetrician-gynecologists of today, of the recent past and of the foreseeable future should not be limited to the care of the pregnant women and assuring their safe delivery. They encompass much more.

1. The treatment of abortion, spontaneous or septic-induced
2. The recognition and treatment of pelvic infection, including sexually transmitted infections
3. Family planning
4. Cancer prevention and screening
5. Treatment of benign gynecologic conditions
6. Care of the aging women
7. Other functions of a family physician

Generally, the treatment of septic induced abortion, because of its social implication, is handled only by one obstetrician-gynecologist in secrecy and shared only with other physician during the last stages of the disease. The incidence of septic-induced abortion in the Philippines, particularly in Manila and other major cities is much more than what is reported.

Similarly, the incidence of sexually transmitted infection is much more than what is reported; and the obstetrician-gynecologist, very often cannot just share the burden of diagnosis and treatment with other physicians.

In Family Planning because of religious and moral convictions of the physicians, there are also limitations not only in the practice but also in advocacy.

In cancer prevention and screening, we still use very antiquated measures. For years, it has been accepted that our screening programs have targeted less than 10% of our susceptible population. These, in fact, is the reason why the incidence of cancer of the cervix, our most frequent gynecologic malignancy, is still the same as it was 20 or 30 years ago.

The treatment of benign gynecologic conditions appear to be the more clear-cut concern of the obstetrician gynecologist, unless we take into consideration alternative approaches to improve treatment results.

The care of the aging women appears to be a neglected concern. It is generally assumed that the close family ties and respect for elders will automatically take care of those women. What seems to be neglected are the particular needs of these women.

I am sure you will agree with me that is really a very tremendous task that falls on the shoulders of the Obstetrician-Gynecologists of the Past, the Present and maybe the Future. The task is made more difficult by the geographical location of the practice of many of our physicians. In many regions, they have to harness their own resources to be able to deliver good obstetric and gynecologic care. Because of the lack of a good referral system, they may be forced to handle cases that are beyond their competence.

Our country is made up of more than 7,000 islands, most of which are inhabited. Because of their inaccessibility, they are seldom reached by medical, including obstetrical and gynecological services. According to reliable sources, in many of these places, people live and die without seeing a doctor, or any health care provider.

In many of the bigger islands, communities are separated by mountains and forests, and roads that are not good enough for transport and establishment of health service facilities.

This has been a major feedback from the participants of the Regional Reproductive Health Workshops held in many regions of the country from 1996 to 1997, sponsored by the Asia Oceania Federation of Obstetrics and Gynecology (AOFOG) and the Philippine Obstetrical and Gynecological Society (POGS). I was Country Coordinator for the Workshops and had the privilege to hear the feedbacks/complaints from all the participants. The participants consisted of health care providers as well as health care recipients.

Among the items that they strongly wish would happen in their region were:
1. Improved transportation and communication
2. Access to national and regional statistics
3. Adequate public health education
4. Upgrading of competencies of TBAs and Barangay Health Workers
5. Continuing health professional education
6. Upgrading and making more accessible health care facilities
7. Improved systems of referrals
8. Increased sensitivity to Filipino culture and values

While these workshops were designed to sensitize the health care providers in the different regions, we have realized that sensitization was not enough. What was more important was the resources - the health resources poured into these regions. Without those resources, the improvement in Obstetric and Gynecologic practices will be very difficult, if not impossible to accomplish in those regions.

Moved, perhaps, by the very sad plight of developing countries, some member states of the United Nations (UN) gathered at the Millennium Summit to affirm commitments towards reducing Poverty and the worst forms of human deprivation. The Summit adopted the UN Millennium Declaration which embodies specific targets and milestones in eliminating poverty worldwide. Time-bound and measurable indicators were identified serving as bases for progress reports by member States.

Important concerns identified were:
1. Eradicate extreme poverty and sufferings
2. Achieve universal primary education
3. Promote gender equality and empower women
4. Reduce child mortality
5. Improve maternal health
6. Combat HIV/AIDS, malaria and other diseases
7. Ensure environmental sustainability
8. Develop a global partnership for development

Many countries took advantage of this, some overenthusiastically forgetting that resources are needed to generate other resources to fuel most of these projects. Some countries were hesitant, thinking that this was just a way of different countries to exert dominance over them.

The Philippines has literary slept during the first few years of its implantation. It has allowed too much water to pass under the bridge. It has tolerated corruption in both our central and local government. What are we going to do with the time that we have lost? The answer is very Filipino: “Huli man ay naihahabol din.”

I think I have overemphasized the difficulties of rural practice of obstetrics and gynecology in the Philippines. There is an urban practice too which is just as challenging.

We have 93 accredited training programs which contribute specialists which tend to crowd or over crowd Metro Manila and other big cities. As of this writing, we have 1,952 fellows, 293 life members and 257 diplomates, most of whom are in Metro Manila and other major cities. Some of them have deviated from patient care. Some of them teach in Medical Schools and some are consultants in residency training programs. Some go into research. Many times, the results of those researches become sources of pride in the International Brotherhood of Obstetrician-Gynecologists.

Indeed, we have as many types of obstetricians and gynecologists now as there are stars in the sky. However, this much I can say for them, no matter how much influence, the lure of globalization has for them, they still manage to retain the value that has been inculcated in them since their student days - nationalism.

No matter what present preoccupation they are in, they wake up in the night and seem to hear the cries of our women in the remote islands and in the isolated mountain villages - like the mournful sounds of distant dreams.

They have responded by organizing, regularly, medical and surgical missions, and engaging in community service activities that penetrate our most remote communities.

I am reminded of the writings of John Donne, writings that have provided inspiration for Ernest Hemingway’s immortal novel - “For Whom the Bell Tolls.” To paraphrase him, no man is an island, entire in itself. Every man is part of the continent. Every man is part of another man because they are both parts of creation. If a man dies, you cannot say, even to yourself, that you are not responsible for his death. So, why should you grieve for him? The truth is, when he dies, a part of you dies with him. You become a lesser man. Therefore, in your quiet evenings, when you hear the mournful sounds of the bell, do not tend to know for whom the bell tolls. It tolls for thee.
Erratum

In the January - March, 2009 issue of the Philippine Journal of Obstetrics and Gynecology, the author’s name (in bold font) in the following articles was inadvertently omitted. We apologize for the oversight.

1. Use of Anemia Scoring Index to Predict Severity of Anemia Among Emergency Ob-Gyne Patients
   Sigrid Tan Aguirre, MD; Ana Victoria V. Dy Echo, MD and Jericho Thaddeus P. Luna, MD, FPOGS

2. Endometriotic Cyst Arising from an Ectopic Ovary in the Liver
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SPECIAL ARTICLE

Keynote Address
Augusto M. Manalo, MD
Instructions for Authors

1. **Aims and Scope**

   The Philippine Journal of Obstetrics and Gynecology is the official publication of the Philippine Obstetrical and Gynecological Society, Inc (POGS). It is a peer-reviewed journal that covers all aspects in obstetrics and gynecology and features original research papers, interesting case reports, clinical reviews and guidelines, as well as correspondences. The journal is published quarterly and sent as third class mail to all POGS members.

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   All manuscripts, editorial business and correspondences should be sent to

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6. **Pre-Submission English-Language Editing**

   Only manuscripts in the English language with American English spelling will be accepted. Accepted manuscripts will be edited according to journal style. When major revisions are needed, the manuscript will be returned back to the author(s) for corrections and generally with accompanying comments from the reviewer(s).

7. **Authorship**

   All authors named in the manuscript should have significantly contributed to its preparation and writing. All authors should also agree on the contents of the manuscripts.

8. **Preparation of the Manuscript**

   Manuscript should be in the following format:

   - Typewritten in Arial font #12 on 8 1/2 x 11 inch white bond paper substance 20.
   - Double-spaced with 1 1/4 margin on its right, left, top and bottom sides.
   - The use of Word and its tools such as Spelling and Grammar, Word count, Language (Thesaurus) are encouraged.

9. **Parts of the Manuscript**

   a. **Title page**

   i. The title page should contain title of the paper, full name of all authors, their titles and
affiliations, and academic qualifications, contact numbers of the authors including e-mail, mailing address and telephone and fax numbers.

 ii. The title should be short and contains the key words.

 iii. If the paper has been presented in a scientific meeting, insert a footnote on the name, address and date of the presentation.

b. Abstract and key words

 i. The structured abstract of a research study should contain a maximum of 150 words. If should state the objectives, basic procedures, main findings and conclusion of the study, under the headings: Aim, Methods, Results, Conclusion. Case reports should have an unstructured abstract with a maximum of 75 words.

 ii. For indexing, a maximum of 5 key words should be written alphabetically below the abstract. It is recommended that the key words be taken from the US National Library of Medicine’s Medical Subject Headings (MeSH) browser list (http://www.nlm.nih.gov/mesh/meshhome.html).

c. Text

 i. Sections.

 The manuscript should be divided into the following subsections: Introduction, Objectives, Material and Methods, Results, Discussion, Acknowledgements, References.

 Generally, the introductory section should be concise and focused on the specific subject of the manuscript. The Result section should contain only findings borne by the study. The Discussion section attempts to explain the findings of the study based on current knowledge. It should not be a literature review.

 Whenever appropriate, the metric system, Systeme International (SI) units, and temperature in degree Celsius are used. Non-proprietary (generic) names should be used for medical substances, unless the use of a specific brand name is important. In the latter situation, the pharmaceutical interest should be declared.

 When acronyms or abbreviations are used, the acronym enclosed in parenthesis should initially follow the entire phrase or group of words. Abbreviations should be spelled out no matter how common they are.

 ii. Tables and Figures

 Tables and figures should be appropriately labeled and numbered consecutively. The contents of the table should complement the text but avoiding any redundancy. The tables should have concise but comprehensive legend. Column headings should be short with units in parenthesis, or statistical headings well-defined. Footnote symbols should be used (see Word, Insert, Reference, Footnote) except for the asterisk *(which should be reserved for P values (0.03**)). Choose the most important tables, with a maximum of 3 tables per manuscript. When presenting photograph of subjects or specimen, any identifying mark should be cropped, or an eye bar should be used to prevent the subject from being recognized. Use arrows to emphasize subtle pictures.

d. Acknowledgement

 i. The source of funding or grants should be acknowledged. As earlier started when brand names of drugs are used, the author should declare pharmaceutical interest, his industrial links or affiliations. Other contributing authors or institutions should also be acknowledged. Acknowledgement of writing inspirations is not allowed.

e. References

 i. Use the Vancouver style of referencing. In the text, the reference should be cited using superscript Arabic numerals placed after the sentence and in the order of their appearance. Write all names of authors if they number 6 or less. If > 6 names, cite the first 3 and use et al for the rest.

 ii. For abbreviating names of Journals, use the Index Medicus style.

 iii. Unpublished work should not be listed under references. The citation should appear in the text and enclosed in parenthesis (e.g. Cruz J dl, 2008, unpublished data).

 iv. The use of Endnote under Insert in Word is recommended.

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