Ovarian cancer is the fifth most common cause of cancer death in women worldwide and is the fourth leading cause of cancer related deaths in Malaysia. Ovarian cancer are classified based on histological types, because it is an important surrogate for genetic, prognostic, and predictive information about response to treatment. The malignant epithelial tumor, that is ovarian carcinoma (OC) is the most common group and the four most common subtypes of OC are serous, endometrioid, clear cell and mucinous. Among the OC, ovarian serous carcinoma (OSC) is the most common subtypes. Most of the women with OC presented with locally advanced disease or distant metastasized at the time of diagnosis. The two-tier grading system (also known as MDACC [M.D Anderson Cancer Center] grading system) has been accepted worldwide. Human epididymis 4 (HE4) is a new protein which is frequently overexpressed in ovarian neoplastic tissue. Previous studies have shown that there is significant difference of HE4 expression in low grade serous carcinoma (LGSC) and high grade serous carcinoma (HGSC). The aim of this study is to further evaluate the HE4 tissue expression in 2 different grades of OSC in Hospital Serdang.

This is a retrospective, cross-sectional study, of 71 cases histopathologically diagnosed as OSC comprising of 48 cases of HGSC and 23 cases of LGSC. Cases were collected from Hospital Serdang, from 1st January 2006 to 31st December 2013. All cases were examined for HE4 expression by immunohistochemistry using anti-HE4 (Polyclonal rabbit antibody dilution 1:40, Abcam).

**Introduction**

**Ovarian cancer**

Incidence and demography of ovarian cancer: Ovarian cancer is neither a common nor a rare disease. It is the fifth most common cause of cancer death in women worldwide and is the leading cause of death from gynaecological cancer in the western countries [1]. In 2004, about 25,580 women was diagnosed with ovarian cancer (OC) in United States and 16,090 (62%) died from the disease [2]. This owes mainly to presentation with advanced-stage disease (International Federation of Gynecology and Obstetrics [FIGO] stage III–IV) due to late symptoms and the lack of effective screening for early disease, as well as increasing chemoresistance along with tumor progression.

In Malaysia, ovarian cancer is the fourth leading cause of cancer related deaths and the fifth commonest cause of cancer in women. According to the National Cancer Registry (NCR) 2007, about 658 cases of ovarian cancer diagnosed in 2007 accounted for 6.5% of all female cancer cases (i.e. gynaecological and breast cancers). Out of 426 cases reported, 52% were diagnosed at stage III and IV that is characterized by intraperitoneal spread and distant metastasis. According to the World Health Organization (WHO), ovarian cancer are classified based on histological types, because it is an important surrogate for genetic, prognostic, and, increasingly, predictive information about the response to treatment [3]. This classification categorizes ovarian cancer with regards to their derivation from coelomic surface epithelial cells, germ cells and mesenchyme (the stroma and the sex cord). The malignant epithelial tumor, is the most common group, accounting for 90% of total ovarian cancer. The four most common subtypes of OC are serous, endometrioid, clear cell and mucinous [4]. Among the OC, ovarian serous carcinoma (OSC) is the most common subtypes.

General clinical features: Ovarian cancer generally affects women in postmenopausal state with a peak between 55 and 65 years old. At the time of diagnosis, three quarters of patients have locally advanced (FIGO Stage III) or disseminated disease that is characterized by diffuse intraperitoneal spread and/or ascites. FIGO Stage III tumors characteristically metastasize widely within the abdominal cavity, as evidenced by the presence of both solid nodules on the peritoneum and malignant ascites. The symptoms of ovarian carcinoma is non-specific and similar to those seen in other benign condition. This is the possible reason for the delayed seeking of medical attention. The early symptoms include abdominal distension (bloating, increased abdominal size), pelvic and/or abdominal pain, problems with eating (loss of appetite, feeling full quickly), and frequent urination [5]. Based on the study done by Brain, et al. [5], the most well-identified symptoms of ovarian cancer were post-menopausal bleeding (87.4%), and persistent pelvic (79.0%) and abdominal (85.0%) pain. Symptoms associated with eating difficulties and changes in bladder/bowel habits were identified by less than half the sample.

The Human Epididymis 4 (HE4) gene and its significance: HE4 was first identified in the epithelium of the distal epididymis. HE4 is a glycoprotein overexpressed in epithelial ovarian cancer, especially serous and endometrioid adenocarcinoma. It is associated with cancer cell adhesion, migration and tumor growth and serves as a valuable prognostic factor for the overall survival in patients with epithelial
An extensive study was done in Boston and Grossly, the malignant serous tumour. In view of the high incidence of OC in 35 (9.2%) cases with LGSC and 346 (91.8%) with high-grade serous classified into two groups according to MD Anderson two-tier system: suggests that LGSC represents a minority within the group of invasive Cancer Institute's Surveillance Epidemiology and End Result [SEER]) genetic predisposition to ovarian cancer [9].

Fallopian tube that closely resembled ovarian HGSC in women with a intraepithelial carcinoma (STIC)” and occult invasive HGSC in the tubal intraepithelial carcinoma (TICs), later designated as “serous tubal Later on, a group of Dutch investigators in 2001 was first described tumour is the ovarian surface epithelium or cortical inclusion cysts. molecular genetic features [8]. Conventionally, the origin of serous diseases that can be classified based on distinctive morphologic and homogeneous disease worldwide. It is now recognized as a group of different subtypes [7]. Currently, OSC is no longer considered to be a disease with major cell types of ovarian carcinoma have undergo major shift. For several other exposures, including parity and body mass index, but smoking status. There was some evidence of heterogeneity by subtype including age, duration of breastfeeding, duration of estrogen use, and amount of psammoma bodies (Figure 4). Also present is extensive necrosis with variable bizarre form. Numerous mitoses are present which supported evidence There is usually associated with serous borderline component. Cytomorphologically, there is mild to moderate nuclear atypia with in small nest, micropapillae rather than macropapillae configuration. The individual usually unilocular, containing clear to viscous fluid (Rosai, 2011) (Figure 2). comparison, the better differentiated tumors consist of cystic masses, tend to be solid and invasive, with areas of necrosis and hemorrhage. In cases, but rarely contains KRAS or BRAF mutations. [12]. In contrast to LGSC, HGSC harbors TP53 mutations in >95% of cases, but rarely contains KRAS or BRAF mutations.

Histopathology of OSC: Grossly, the malignant serous tumour tend to be solid and invasive, with areas of necrosis and hemorrhage. In comparison, the better differentiated tumors consist of cystic masses, usually unicollular, containing clear to viscous fluid (Rosai, 2011) (Figure 2). According to WHO, 4th edition, LGSC tumor cells are arranged in small nest, micropapillae rather than macrocystic stroma. There is usually associated with serous borderline component. Cytomorphologically, there is mild to moderate nuclear atypia with single prominent nucleolus. Mitoses are scatteredly present and usually count at <2-3/10 hpf. The additional feature of LGSC is the presence of many psammoma bodies. Necrosis is rarely identified (Figure 3).

In HGSC, the tumor has high grade features arranged in solid mass with slit-like spaces, papillary, glandular and cribriform. The individual cell shows marked nuclear pleomorphism, large nuclei and some are in bizarre form. Numerous mitoses are present which supported evidence of rapid growing tumor. Also present is extensive necrosis with variable amount of psammoma bodies (Figure 4).

Risk factors of OSC: An extensive study was done in Boston and revealed that there is a significant heterogeneity across the serous invasive, endometrioid, and mucinous subtypes of OC. All subtypes show association with both reproductive and non reproductive exposures, including age, duration of breastfeeding, duration of estrogen use, and smoking status. There was some evidence of heterogeneity by subtype for several other exposures, including parity and body mass index, but these differences were not statistically significant [13]. In Malaysian ovarian cancer and also an early indicator of the recurrence of ovarian cancer [6]. Previous reports showed that HE4 used in conjunction with CA 125 improves specificity for ovarian cancer [2].

Problem statement: In view of the high incidence of OC in Malaysia, the demographic data and different grades of OC, especially OSC needs to be further investigated. Based on previous studies, identifying 2 different grading of the ovarian serous carcinoma (OSC) is very crucial and important because of their difference in pathogenesis, molecular levels, treatment choice as well as the outcome. The significant difference of HE4 expression in LGSC and HGSC was first identified by Zhu et al. Thus, this study is done to evaluate the HE4 expression trend in different grades of OSC, and to determine the significant difference in the stratifying the grades. Thus, in future, this HE4 immunomarker has potential to be used to grade OSC especially in cases where the histomorphological features are equivocal.

Objectives

General: To determine HE4 tissue expression in OSC

Specific objectives:
1. To determine the frequency of different grades of OSC.
2. To determine the clinicopathological parameters in different grades of OSC.
3. To correlate HE4 expression with clinicopathological parameters.
4. To determine and correlate HE4 expression in LGSC and HGSC.

Null hypothesis
• There is no association of prevalence among different grades of OSC.
• There is no association of demographic characteristics among different grades of ovarian serous carcinoma (OSC)
• There is no association of HE4 expression among LGSC and HGSC.

Literature Review

Ovarian serous carcinoma

Overview of OSC: The past two decades, the diagnostic criteria for all major cell types of ovarian carcinoma have undergo major shift. Although each of these major cell type show relatively minor changes, when reviewed cumulatively, the changes in diagnostic criteria can have impact on the definition of molecular and clinical features of the different subtypes [7]. Currently, OSC is no longer considered to be a homogeneous disease worldwide. It is now recognized as a group of diseases that can be classified based on distinctive morphologic and molecular genetic features [8]. Conventionally, the origin of serous tumour is the ovarian surface epithelium or cortical inclusion cysts. Later on, a group of Dutch investigators in 2001 was first described tubal intraepithelial carcinoma (TICs), later designated as “serous tubal intraepithelial carcinoma (STIC)” and occult invasive HGSC in the fallopian tube that closely resembled ovarian HGSC in women with a genetic predisposition to ovarian cancer [9].

Clinical epidemiology of different grades of OSC: Data taken from representative population-based cancer registries (e.g. National Cancer Institute's Surveillance Epidemiology and End Result [SEER]) suggests that LGSC represents a minority within the group of invasive serous tumors. A retrospective study was done in Peking Union Medical College Hospital from 2007 to 2010 among 381 patient. Patients were classified into two groups according to MD Anderson two-tier system: 35 (9.2%) cases with LGSC and 346 (91.8%) with high-grade serous carcinoma. A descriptive epidemiological study was done among 19,899 of OSC cases in Iran from 1990-2005. They found that, LGSC are more common among women <40 years old. Patients with LGSC had a significantly younger age at diagnosis. According to WHO, 4th edition, generally, patients with low grade serous carcinoma (LGSC) are one decade younger than high grade serous carcinoma (HGSC) with better survival outcome. LGSC is characterized by an indolent clinical course as it is usually developed from benign serous tumor or de novo [10]. In comparison, HGSC has rapid course of disease, aggressive behavior with poorer outcome.

Pathogenesis and grading system: Traditionally, the OSC has been graded based on a three-tier grading which have the same route of tumor occurrence. However, recently the two-tier grading system (also known as MDACC [M.D Anderson Cancer Center] grading system) has been accepted worldwide. Serial clinicopathologic and molecular genetic studies from The Johns Hopkins Hospital and M.D. Anderson Cancer Center have shown that a 2-tier grading system is easy to apply and reproducible. It is based on underlying molecular biologic differences between the low-grade and high-grade tumors. This latest grading system has been accepted by WHO and adapted in the latest WHO blue book, 4th edition, 2013. Low-grade serous carcinoma (invasive micropapillary serous carcinoma (MPSC)), has been hypothesized to arise from a serous cystadenoma or adenofibroma which progresses to an atypical proliferative serous tumor (APST) to non-invasive MPSC and then to invasive MPSC in a slow step-wise fashion (Figure 1). Both low-grade serous carcinoma and APST/non-invasive MPSC are characterized by mutations of the KRAS, BRAF, or ERBB2 genes, in which approximately two thirds of tumors have a mutation of one of these genes [11]. For the high grade serous carcinoma, these putative precursor lesions are detected in inclusions in the ovary or ovarian surface epithelium and are characterized by tubal-type epithelium with varying degrees of cytological atypia that have been termed dysplasia/carcinoma in situ [12]. In contrast to LGSC, HGSC harbors TP53 mutations in >95% of cases, but rarely contains KRAS or BRAF mutations.

...
setting, new cases of OC diagnosed in 2007 among different ethnicity showed 57% are Malay, 34% are Chinese and 8.2% are among Indian (NCR, 2007). Several other studies show parity and oral contraceptive use were inversely associated with all 4 major subtypes which are serous, mucinous, endometrioid and clear cell. A data from previous study also revealed that parity is a strong protective factor against OC and the level of protection increases with the number of childbirths [14].

**Prognosis of OC:** The data analysis from previous study revealed that there is significant difference of disease recurrence (P=0.028) after median follow up of 36 month, with 68.6% (n=24) and 83.5% (n=289) for those of LGSC and HGSC respectively. It was noted that LGSC were more likely to be confined to the ovary at the time of diagnosis. The rate ratio of advanced to early disease was 1.9 for LGSC, whereas it was 10.2 for HGSC. Optimal cytoreductive surgery has a more important

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**Figure 1:** Pathogenesis of LGSC and HGSC.

**Figure 2:** Serous carcinoma of ovary. The tumour is predominantly solid, with necrotic and haemorrhagic areas.
effect on the survival of patients with LGSC because they received less benefit from adjuvant chemotherapy. Many retrospective studies and laboratory data have demonstrated the chemoresistant effect of LGSC. The mean overall survival for LGSC was significantly higher than for HGSC. The overall progression-free survival rate of LGSC is better than that of its high-grade counterpart. However, the risk for progression among women who are left with gross residual disease after primary cytoreductive surgery is identical to that of those with HGSC.

**Biomarkers for ovarian carcinoma**

CA 125: There is no sensitive or specific test available to screen for the disease at an early stage which has a higher cure rate. Cancer antigen 125 (CA-125) first identified in 1981 became the “gold standard” for the routine management of OC [15]. Great emphasis is placed on early detection in trying to reduce ovarian cancer mortality. CA -125 is the most widely used serum tumor marker for ovarian malignancy. It is also commonly done for the baseline evaluation before treatment and during chemotherapy to evaluate the response to treatment. Besides that, CA-125 is useful to monitor during follow-up to detect recurrences earlier than the development of clinical symptoms. However, the use of this marker as a first-line screening assay, alone or in combination with other procedures, is not recommended owing to the relatively low specificity and positive predictive value [16]. Elevation of the serum CA-125 can be seen in many cases of different physiological and pathological conditions, such as during menses, pregnancy, in endometriosis, pleural or peritoneal inflammatory diseases. One study
has been carried out at Queen's Hospital, Burton upon Trent, UK and they have found that there is high false positive rate and poor sensitivity and specificity associated with CA125. The substantial inappropriate usage of CA125 has led to results that are useless to the clinician, have cost implications, and add to patient anxiety and clinical uncertainty [17]. Thus, sensitive and specific screening test has yet to be developed.

**Discovery of HE4:** This protein was discovered to be a protease inhibitor involved in sperm maturation. It is also known as WFD2 gene because it contains two whey acid protein domains and a “four disulfide bond core” which consist of eight cysteine residues. Later in vitro studies using cloned WAP cDNA showed that these proteins have various function which include effects on cell growth and differentiation (Figure 5). Interestingly, results from several comparative genomic hybridization assays suggested that the 20q13 locus frequently exhibits chromosomal gains in various cancer types, including malignancies of the oral cavity, breast, ovary, colon, pancreas, stomach and uterus.

**Role of HE4 as a marker for ovarian cancer:** In 1999, Schummer et al. firstly described that serum HE4 is elevated in epithelial ovarian cancer. It was designated as a potential serum biomarker for ovarian cancer in 2003. It is commonly overexpressed in ovarian neoplastic tissue and increase levels have been found both in the early stage and in the recurrence of disease. Recent interest of this new epithelial ovarian carcinoma (EOC) biomarker, HE4 has been generated by the consistent demonstration of its overexpression in early stage ovarian cancer. In view of this evidence, this biomarker could help in discriminating between benign and malignant diseases. In comparison, HE4 has 95% specificity whereas CA125 shows 76.4% specificity in predicting the presence of OC with pelvic mass. In addition, it is more useful than CA125 as it rarely is positive in nonmalignant disease. Subsequent study revealed that raise serum protein is predominately limited to the serous and endometrioid subclasses. Imunohistochemical localization of HE4 also can be performed on sections from formalin-fixed, paraffin-embedded tissue by using HE4-specific antibodies.

**Materials and Methods**

**Study location & duration**

All histopathological slides of OSC samples were retrieved from the archives in the Histopathology Unit, Department of Pathology, Hospital Serdang, Selangor Darul Ehsan, Malaysia and reviewed in the Histopathology Unit. The clinical and demographic data of all the cases such as age, parity, race and histopathological report were retrieved from the Computerized Medical Record System of Hospital Serdang.

**Study design**

A descriptive study using retrospective archived histopathological material and clinical data.

**Sampling**

**Study population:** Archived histopathological ovarian tissue with serous carcinoma.

**Sampling population:** All pathologically diagnosed OSC in which the total hysterectomy with bilateral salpingo-oophorectomy or oophorectomy specimen has been sent for histopathological examination and has been reported in Hospital Serdang.

**Sampling frame:** A list of all OSC cases that were sent to the Histopathology Unit in Hospital Serdang from the years 2006 to 2013.

**Sampling method:** Universal sampling: The specimen number, of all the archived OSC tissue slides were obtained from 2006 until 2013. The specimen numbers were taken from the Computerized Laboratory Information Management System of Hospital Serdang. The clinical data were obtained from the Hospital Serdang Laboratory Information System (LIS). The clinicopathological variables obtained from the histopathological and surgical data are the grading of OSC (LGSC and HGSC), age, ethnic group and parity. The slides for all OSC cases were retrieved from the archives of Histopathology Unit, Pathology Department, Hospital Serdang. Cases that does not fulfil the selection eligibility were omitted. Then, one slide with high tumour density was selected and paraffin block section was done for immunohistochemical stains of HE4. Finally, scoring was done using Twice-scoring method.

**Figure 5:** Illustration of the region on chromosome 20q13 that harbors the WAP gene cluster, including HE4.
**Sampling size:** The sample size calculation is based on formula by Kirkwood (2003) Based on this formula:

\[ n = \frac{4(p)(1-p)}{a^2} \]

where:

- \( n \) = sample size
- \( p \) = prevalence of HE4 in OSC (0.933) (Based on the previous similar published data by Zhu et al. [15])
- \( a \) = margin of error (0.05)
- \( n = 104.16 \)

However, due to limited cases of ovarian serous carcinoma in Hospital Serdang with addition of exclusion criteria, the total number of cases retrieved is 71 cases.

**Selection eligibility**

**Inclusion criteria:** All pathologically diagnosed as primary ovarian serous carcinoma in Hospital Serdang from 1st January 2006 until 31 December 2013.

**Exclusion criteria:**
- a. Tissue blocks which were lost or unavailable
- b. Insufficient tissue material for sampling
- c. Cases that the diagnosis was in doubt.

**Data collection**

**Data collection technique:** The demographic data and the histopathological analysis were retrieved from the Computerized Medical Record System of Hospital Serdang from the year 2006 to 2013. These data were then manually tabulated into an Excel Spreadsheet which was later used in the data analyses.

**Data processing & statistical analysis**

**Antibody and immunohistochemical analysis**

**Antibody:** The immunohistochemical (IHC) stain used was Anti-HE4, rabbit polyclonal to HE4 with dilution of 1:40. Manufactured by Abcam, Biotech Company a with code number ab24480.

**Immunohistochemical protocol:** Immunohistochemical staining of OSC specimen was performed to detect HE4 protein expression as follows:-

1. The paraffin embedded tissues were sliced into 3 μm thickness.
2. The slides were then deparaffinized by two cycles of xylene for 3 minutes of each.
3. The rehydration sessions were then performed using decreasing concentration of alcohol (ethanol) of 100% (twice), 80%, 90% and twice of 100% for another 3 minutes.
4. The slides were then placed under running tap water for 3 minutes which were subsequently treated by citrate buffer for target retrieval within pH of 9 in a 98 degrees Celsius water bath for 40 minutes.
5. The slides were then left to incubate within the citrate buffer to cool down in the room temperature for 20 minutes.
6. Thereafter, the slides were transferred to the transfer slides.
7. Then the slides were put under running tap water before transferring them to the sequenza.
8. Subsequently, the slides were then incubated in hydrogen peroxide (H202) blocking agent for 10 minutes, then washed three times with Tris-Buffered Saline (TBS) and blocked with UV blocking agent to reduce the unnecessary background staining.
9. The primary antibody (HE4) was then diluted within a dilution of 1:40 with the antibody diluents for 1 hour.
10. These were washed again with TBS solution three times and further incubated in the second antibody Dako REALTM EnVisionTM / Horse Radish Peroxidase, Rabbit/Mouse, for the next 30 minutes.
11. They were washed again with TBS solution and soaked with the Deamine Benzidine (DAB) chromogen with the substrate buffer for another 7 minutes.
12. After running again with tap water, the slides were subjected to counter staining with haematoxylin for 30 seconds, ran again with tap water and underwent a dehydration step, with increasing concentration of alcohol (ethanol) of 80%, 90% and twice of 100% for a period of 3 minutes each.
13. Xylene was then applied twice for 3 minutes and finally mounted with mounting medium DPX (BDH 361254D).

**Controls:** For positive controls, formalin fixed sections of normal human distal epididymis has been used and stained with primary antibody (Figure 6). Negative control will be performed on similar sections omitting the primary antibody.

**Immunohistochemical interpretation:** Immunostaining results were assessed using Twice-scoring method. Five fields of region with the most dense carcinoma tissues are counted under 400x light microscope. Average positive rate and positive cells in each high power field (hpf) was calculated [15]. The percentage of immunopositive cells was scored as 0 (0%), 1 (<10%), 2 (10-50%), 3 (50-80%) and 4 (>80%). The intensity was scored as 0 (colourless), 1 (mild), 2 (moderate) and 3 (dense) (Figure 7).

Then, all the scores for intensity and for positive cells were added and the final scores were:

- < or equal 2: negative
- >2: positive
- 4-5: moderate positive
- 6-7: strong positive

The interpretations were done by two independent observers, which were the researcher and the supervisor. Interpretations were done without prior knowledge of background clinico-pathological information. Cases with discrepancy were then discussed by the two observers to reach an agreed consensus.

**Statistical analysis:** All data were tabulated and processed using SPSS 22.0TM.

\( \alpha \) Frequency analyses was used to determine the frequencies of OSC and its grades.

\( \beta \) The X\(^2\) test (Chi-square) and Fisher’s exact test analyses was used to determine the associations between HE4 expression with clinicopathological parameters and in between different grades of OSC. The P value is taken as <0.05 to consider as statistically significant.

**Study ethic**

This study has been submitted to the Medical Research Ethics Committee (MREC) and The Ethics Committee for Research of
University Putra Malaysia (JKEUPM) for review and now awaiting for the ethics approval. It was conducted according to the Malaysian Good Clinical Practice (GCP) guidelines.

The NMRR ID number is: NMRR-14-1602-21337.

Definition of terms & variables

**Dependent Variable**: Human epididymis 4 (HE4) is the gene, also known as WFDC2, encodes a protein with WAP-type four disulphide core (WFDC) domain [16].

**Independent Variables**: Maternal Age is categorized into two subcategories, which are <40 years old, and ≥ 40 years old.

Maternal Race are ethnic groups of Malaysia. The groups are categorized into Malay, Chinese, Indian and Others (NCR, 2007).

Parity is classified into nulliparous and multiparous. Nulliparous defined as women who never had pregnancy or had history pregnancies less than 20 20 weeks gestation. Multiparous defined as one or more pregnancies of at least 20 weeks gestation.

**Results**

**The frequency of different grades of OSC**

Out of the 71 cases of OSC studied from 1st January 2006 to 31st December 2013, 48 cases (67.6%) were HGSC and 23 cases (32.4%) were LGSC.

**Clinicopathological parameters in different grades of OSC**

The age at diagnosis of OSC range from 16 to 82 years old with a mean and median age of 50 (Figure 8). Age category of less than 40 years old was 23% and more than 40 years old was 77.5% which forms the majority of the cases. Among the HGSC, 45 out 48 cases (94%) were...
women above 40 years old and 3 out of 48 women (6%) were less than 40 years old. Whereas in the LGSC, 10 out of 23 cases were women above 40 years old and 13 out of 23 cases were women less than 40 years old. Thus, there is statistical significance for difference group of age with different grades of OSC ($X^2=22.51, p<0.001$).

The major ethnic group was Malay which was 56% (40/71), followed by Chinese of 22% (15/71), Indians of 18% (13/71) and Others of 4% (3/71). More than half of the HGSC cases are Malay (26/48) followed by Chinese (12/48), Indian (8/48) and Others (2/28). Among LGSC cases, the major ethnic group was also Malay (14/23) followed
by Indian (5/23), Chinese (3/23) and Others (1/23). Statistical analysis reveals that there is no significant difference of race among LGSC and HGSC with p>0.05.

The parameter of parity generally showed, most of the OSC cases occurred among multiparous women which were 52/71 cases (73.2%) and more than 50% of multiparous women were among HGSC group. Statistical results show there was no significant difference of parity among LGSC and HGSC, with X^2=1.117, p>0.05 (Table 1).

**Correlation of HE4 expression with clinicopathological parameters**

Association of HE4 expression with patient's clinicopathological parameters were shown in Table 2. All women less than 40 years old show positive HE4 staining (100%). Among women above 40 years old, 54 out of 55 cases (98%) show positive HE4 staining and only 1 out of 55 cases (2%) show negative staining to HE4. Statistically, there is no significant difference among age group and HE4 expression, X^2=0.295, p>0.05. Among different races, Malay showed 39 out of 40 cases with positive staining (98%). Chinese, Indian and Others were all showed 100% positive staining. Thus statistical analysis reveals there is no significant difference in between races and HE4 expression with Exact-Fisher=2.667 and p>0.05. All nulliparous showed positive staining to HE4. Among the multiparous women, 51 out of 52 cases (96%) showed positive staining and only 1 out of 52 cases (4%) showed negative staining. Therefore, there is no significant difference of parity with HE4 expression, X^2=0.371, p>0.05.

**HE4 expression in LGSC and HGSC**

Expression of HE4 in LGSC and HGSC tissues were shown in Table 3. In 71 cases, 99% showed positive staining. Among the HGSC, 100% showed positive staining. Whereas in LGSC, 22 out of 23 cases (96%) showed positive staining and only 1 out of 23 (4%) revealed negative staining. These results showed that there was no significant difference of HE4 expression in both grades of OSC, X^2=2.117, p>0.05.

**Discussion**

**Frequency of different grades of OSC**

Most of OSC cases in Hospital Serdang were HGSC, which comprised of 67.6% and LGSC was only 32.4%. This result is supported by many previous studies that show the incidence of HGSC cases were commoner than LGSC. In the series by Seidman et al. [7] 9% of OSC were low-grade. Another study done by a Gynaecologist from Beijing found that more 90% of OSC cases were HGSC.

**Clinicopathological parameters of different grades of OSC**

Generally, the incidence of OSC was low in women under the age of 40, but rises steeply after the fifth decade to reach a peak in the 80- to 84-year-old age group. The median age at diagnosis is currently 63 years. In this study, the age of patient at the time of diagnosis range from 16 to 82 years old with a median age of 50. Another study done by Yang et al. revealed that majority of OSC cases were those among women age in between 55-69 years old [18]. Among OSC cases, patients with LGSC are younger than those with high grade tumor, with mean ages of 45-57 years and 55-65 years respectively.

Based on NCR 2007, Malay is the majority group of women diagnosed as OC followed by Indian and Chinese. These data support the finding in our study (NCR 2007). There is no other published local study to compare the result finding of OC among different races in Malaysia.

Parity is a strong protective factor against OEC and the level of protection increases with the number of childbirths [19]. Women with more than five births had an 80% reduction in risk. This study has shown that, there is no significant difference of parity among different grades of OSC.

In this study, the HE4 expression is positive in almost all cases regardless of the grade of the OSC. Thus, this study show there is no association of HE4 expression in between LGSC and HGSC which contradict the finding by Zhu et al. There are a few possibilities. The sample size of this study is considered small, as compared to the actual sample size that is calculated based on the formula. Based on the collected cases, majority of the OSC cases in Hospital Serdang are HGSC rather than LGSC. This factor may contribute to the skewed positive result. Majority of the OSC are expected to be from HGSC group. This fact is supported by many studies. The other possibility is inadequate sampling of tumor tissue may contribute to false positive result. According to [20], extensive sampling of the tumour tissue is warranted to avoid intratumoral variability of staining [21].

**Association between HE4 expression and clinicopathological parameters**

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**Table 1: Clinicopathological parameters in different grades of OSC.**

<table>
<thead>
<tr>
<th>Sociodemographic data</th>
<th>N (%)</th>
<th>OSC</th>
<th>Chi Square (df)</th>
<th>p value *</th>
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<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 40 years old</td>
<td>16(23)</td>
<td>13</td>
<td>6</td>
<td>22.511</td>
</tr>
<tr>
<td>≥ 40 years old</td>
<td>55(27)</td>
<td>43</td>
<td>51</td>
<td>(1)</td>
</tr>
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<td>Race</td>
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<td></td>
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<tr>
<td>Malay</td>
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<td>14</td>
<td>26</td>
<td>3(60)</td>
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<td>Chinese</td>
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<td>12</td>
<td>25(54)</td>
</tr>
<tr>
<td>Others</td>
<td>13(18)</td>
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</tr>
<tr>
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<td>8</td>
<td>3</td>
<td>11(23)</td>
</tr>
<tr>
<td>Multiparous</td>
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<td>15</td>
<td>25</td>
<td>65(77)</td>
</tr>
</tbody>
</table>

**Table 2: Association of HE4 expression with patient’s clinicopathological parameters.**

<table>
<thead>
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<th>Dependent variables</th>
<th>n (%)</th>
<th>OSC</th>
<th>Chi square (df)</th>
<th>p value *</th>
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<tbody>
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<td>HE4 expression</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>70(99)</td>
<td>22</td>
<td>(96)</td>
<td>2.117</td>
</tr>
<tr>
<td>negative</td>
<td>1(1)</td>
<td>1</td>
<td>(4)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: HE4 expression in different grades of OSC.**
This study revealed that there is no association of clinopathological parameters with HE4 expression. One of the previous data revealed that there is no significant difference of HE4 expression with parity. They also found that there is no significant difference of HE4 expression among white and non-white ethnicity [22]. Among healthy individuals, serum HE4 was higher in older women (age>49 years) than in younger females (age<49 years).

**HE4 expression in LGSC and HGSC**

According to previous study by Devan et al. [23] MS in UMMC, 2013, they revealed that there was significant expression of HE4 immunomarker in all malignant epithelial ovarian tumors compared to benign epithelial ovarian tumors. This is similar to finding in other studies. In their study, the malignant epithelial ovarian tumors that they include are mucinous adenocarcinoma, serous carcinoma and endometrioid carcinoma. In addition, among the histologic types of ovarian cancer, OSC had the highest concentration of serum HE4 followed by mucinous adenocarcinoma, endometrioid cancer and clear cell carcinoma. Another study by Granato et al. revealed that serum HE4 marker was significantly elevated in malignant epithelial ovarian carcinoma rather than benign lesion. Extensive literature search have been done and found that most of the previous studies were related to the serum HE4 expression instead of malignant ovarian tissue expression. Thus, not much comparison can be done regarding tissue expression of HE4 in OC generally, in between this study and the previous ones [24-27].

**Summary, Conclusion & Recommendation for Future Research**

**Summary and Conclusion**

In Hospital Serdang, majority of OSC cases are of high grade type. Most of the LGSC are among <40 years old and HGSC are >40 years old [28]. There was no statistical significance of HE4 expression in different grades of OSC. There was no significant difference of race and parity of all grades of OSC. Also in our study revealed that there was no correlation of HE4 expression with age, race and parity.

**Recommendation**

For future study, we should consider the serum HE4 level in cases of OSC to correlate the serum level of HE4 with immunohistochemical expression in tissue [29-33]. The reason is because many previous studies revealed about the significant increase in serum HE4 in epithelial ovarian carcinoma, however there is not many studies interested to look for HE4 expression in ovarian tissue.

**References**


