INTRODUCTION
In women, ovarian cancer represented the fifth leading cause of death causes related with cancer. It has been estimated that the survival rate is lower than fifty percent 30%-40% only [1]. The incidence ratio of epithelial ovarian tumor is one for each 72 women [2-3], with increasing rate in developing countries. Three categories of ovarian tumors have been identified: epithelial 60%, germ cell 30%, and sex-cord stromal 8%, the beginning of malignant ovarian carcinoma in vast majority of cases 80-85%, occurs in ovarian epithelium [4-5]. Absence of symptoms make it difficult to diagnose epithelial ovarian tumors during early stages, the asymptomatic condition is due to certain factors that related to clinical and histopathological features [6]. Losing of portent lesions and their evolution, is the reason why vast majority of patients with ovarian tumors are diagnosed at advanced stages with poor prognosis [7-8]. Over the years many genes/proteins have been identified as fundamental genes in carcinogenesis as they play a key role in the development and progression of various cancer types. RAS genes and their products are among the key genes that frequently mutate in human cancers which make them difficult to be targets for cancer therapy [9]. KRAS is a member of the RAS GTPase family. This gene involved in cells proliferation and apoptosis among variety of biological processes including [10]. Genetic variation in the exons of KRAS gene may associate with protein hyperactivity. Increasing activity of protein is an oncogenic mutation that very common human cancer. This sort of mutations was detected in a variety of human cancers including 17% of lung cancer, and 14% of ovarian cancer [11]. Previous studies have been providing substantial information that clarifies the role of KRAS in ovarian physiology and pathology, one of that information that the activation of RAS genes is regulated within ovarian surface epithelial cells as well as granulose cells. This activation is definitive for initiation of luteinizing hormone pathway which is involved ovulation. As a result any genetic variation that leading to mutation or over expression in the active form of KRAS gene leads to interception of granulose cell cycle and then deteriorate follicle growth [12]. On the other hand, over expression of KRAS gene in ovarian surface epithelial cells that have deficiency with Pten gene may responsible for the development of serous epithelial adenocarcinoma. Other studies reported that the over expression of mutant KRAS increase the cells susceptibility to tumorigenesis and stimulate senescence of certain ovarian cells particularly epithelial and fibroblast cells [13]. Goal of this study is to detect if the KRAS gene and its mRNA expression levels in ovarian cancer patients had any clinical significance.

THE AIM
The goal of this study was to detect if KRAS gene and levels of had any clinical significance in the ovarian cancer by measuring levels of KRAS mRNA.
MATERIALS AND METHODS

To implement the present study, tissue sections from eighty four patients who already diagnosed with ovarian carcinoma at different stages, have been used. Tissue biopsies are obtained from certain Iraqi hospitals after the patients undergo any of the following surgeries: Total abdominal hysterectomy with bilateral salpingo oophorectomy (TAH-BSO), subtotal abdominal hysterectomy, vaginal hysterectomy, and endometrial. For a control 28 tissue sections of benign tumors were included. Tissue sections then maintained and subjected to molecular analysis. Demographic and pathological information were collected from hospitals records.

RNA EXTRACTION, REVERSE TRANSCRIPTION

Formalin fixed paraffin-embedded tissue sections were subjected to sectioning and then RNA extraction using RN easy FFPE kit for RNA purification (Qiagen – USA). Total RNA has been reversed using the reverse transcription Thermo-Script TM (Invitrogen/United States) kit. This procedure was conducted in the 50 μl reaction volume composed of 15 μl denaturized RNA,0.2 μl Random hexamere primers 3μg/μl, 5μl of 10 μlNTP Mix, 10μl of 5x cDNA synthesis buffer, 2.5μl RNase OUT (40U/μl), 2.5μl Thermo Script RT (15 units/ml), 14.8μl DEPC-treated water. The samples were then placed in a 96-pit thermal cycler, cycling at 25°C for 10 minutes, 10 minutes at 37°C, 60 minutes at 42°C, followed by 75°C for five minutes. The converted cDNA has been stored at -80°C and used to amplify Kras as a PCR template.

QUANTITATIVE REAL-TIME PCR

Real-time PCR was performed using the SYBR Green Supermix Kit (Qiagen – USA). Kras primers that described previously by Latif AH [14], were utilized in this study, for PGK1 primers were forward:

GGTCAAGGTGAAGATAATACCTAA,

And reverse:

CATTAAACTTGTTGTTGCTCTT

The Applied Biosystems 7900 equipment was used to run quantitative real-time PCR tests in duplicate. Reaction volume of 20 μl was used for PCR reaction. It was contain of SYBR Green master mix (10 μl), primer mixes (1 μl), RNase free water (5 μl0, and cDNA template (4 μl). Conditions of Real-Time PCR was as follows: stage 1: 50 °C for 2 min., stage 2: 95 °C for 10 min., stage 3: a two-step cycle process (95 °C for 15 sec. and 65 °C for 1 min.) repeated for 6 cycles, and stage 4 in a two-step cycle procedure (95°C for 15 Sec. and annealing 61°C for 1 min.) repeated for 40 cycles. The endogenous reference gene (PGK1) was used as a control gene.

DATA ANALYSIS

Results of RT-PCR analyzed using 2−ΔΔCt method [15]. Comparative standard curves were used to assess the efficiency (E) of qPCR amplification for target and reference genes, figure (1). Serial dilutions of reaction materials that included cDNA of reference sample and study primers were used for standard curve generation. Ct values against logarithmic values plotted for estimation proportional of cDNA copy numbers in the reference sample and stability and efficiency of primers. Slope and equations of effectiveness (E) were used as following:

\[ E = \frac{10^{-1/\text{slope} - 1}}{100} \]

The cycle’s threshold (Ct) is defined for each sample as the number of PCR cycles required for the fluorescence defined by the user. The Ct is inversely proportional to both the target (Kras) and reference (PGK1) genes. The level of Kras mRNA was determined by the following equations:

Logarithmic copy no. (PGK1) = \( Ct - 32.85 \) / -3.3592

Copy no. (PGK1) = \( 10^{\text{Log copy}} \)

Logarithmic copy no (Kras) = \( Ct - 37.437 \) / - 4.4513

Copy no (Kras) = \( 10^{\text{Log copy}} \)

Fold change = copy no. (Kras)/copy no. (PGK1)

STATISTICAL ANALYSIS

The SAS (2012) program for the Statistical Analysis System was employed to determine the impact on study parameters of various variables. The smallest difference – LSD (ANOVA Variation Analysis) or T-Test – has been used in this research in order to compare the means.

RESULTS

Eighty four samples with ovarian cancer and 24 samples with benign ovarian tumors that used as a control group were studied for the expression Kras. The median age was 47 years old and the patients were 14-70 years old. All samples of ovarian cancer have been negative for family history, according to the family's history. The clinical features of ovary cancer samples are listed in table (I). With respect to menopause, 36(46.1%) of the samples were premenopausal, while 42(53.9%) of these were postmenopausal. According to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system, the majority of samples 57(73%) were in stage I, while the other 21(27%) were at stage III. The samples have been divided into five clinical groups according to tumor histology types; Serous epithelial tumors 33(42.3%) samples, mucinous epithelial tumors 15(19.2%) samples Endometriod tumors 21(26.9%) samples, germ cell tumors 6(7.695%) samples and 3(3.84%) of Burner tumors. Table (II) shows that in both malignant and benign tumor Kras mRNA was expressed. In ovarian cancer samples the highest Kras mRNA levels were statistically important in comparison to benign tumor (mean ± SE: 661, 51 ± 75, 09, **P≤0.01), respectively (mean ± SE: 7, 82 ± 0.22).

The present study shows statistically no significant differences in gene expression levels with menopause and family history, while significantly associated with age groups, since the highest levels of gene expression in patients aged between 50-74 years (mean ± SE: 1445.96 ± 82.37, **P≤0.01). In correlation with the histopathological type of ovarian tumors Endometriod tumors showed statistically significant difference in the level of Kras gene expression (Mean ± SE: 2469.55 ± 137.06, **P≤0.01)
compared with other histopathological tumor type. Statistic
difference between the 57(73%) stage I samples with the highest
expression level (Mean ± SE: SE: 831.78 ± 71.55, **P≤0.01) and
the 21(27%) stage III samples (Mean ± SE: 59.45 ± 3.16) were
observed according to the tumors stage.

**DISCUSSION**

A link between RAS family genetic variation like mutation
or over expression and tumor lesions with advanced degree,
have been recorded by only a few studies. In the present
study, relative KRAS gene expression levels were evaluated
in 84 ovarian tissues obtained from patients with benign
and malignant tumors using the real-time PCR method.
Ovarian cancer samples in comparison to benign tumor.
Similarly to this result, Hong et al [16] reported that there
was almost no p21Ras expression in normal breast tissue,
but high level of expression in breast cancers. Latif [14]
also detected that the mRNA of KRAS gene was highly
expressed in samples of colorectal cancer comparing with
controls. In correlation with clinopathological parameters,
the present study shows that the relative levels of KRAS
mRNA and older age of patients were significantly asso-
ciated. Endometriod tumor histological type and primary
stage of tumor (stage I). Except for the age of patients,
Pzik et al. [17] found that none of the clinopathological
parameters they looked at (patients’ sex, age, and tumor
histological type, stage, grade, and chemotherapy treat-
ment) were significantly linked to KRAS expression and
levels of KRAS mRNA. Wan et al. [18] found significant
associations between KRAS expression and cancer patient
age, with the highest levels of KRAS mRNA found in pa-
tients aged over 56 years, which is similar to the current
study’s findings. Their findings, on the other hand, contra-
dicted the findings of the current study, as they discovered
a significant link between high levels of KRAS mRNA and
poor tumor differentiation. The current study’s findings
contradicted those of Birkeland et al. [19], who discovered
that high KRAS mRNA levels were significantly associated
with advanced FIGO stage, non-Endometriod histologi-
ical type, high grade, and lymph node metastasis. What is
interesting about the findings of the present study is that
the KRAS gene over expression increased remarkably in
the subgroup of patients over 50 years of age. Also what is
interesting the association of KRAS over expression KRAS
with early stage of ovarian cancer which is conflict with
results of most previous studies that demonstrated the
correlation between KRAS over expression and late stages

| Table I. Clincopathological characteristics of patients with ovarian carcinoma |
|-----------------------------|-----------------------------|
| **Age groups**              | **%**                      |
| 0-14 years (Children)       | 9(10.7%)                   |
| 15-24 years (Teenagers and young adults) | 3(3.57%)  |
| 25-49 years (Adults)        | 33(39.28%)                 |
| 50-74 years (Old age)       | 39(46.42%)                 |
| **State of menopause**      |                            |
| Premenopausal no. (%)       | 40(47.6%)                  |
| Postmenopausal no. (%)      | 44(52.4%)                  |
| **Family history**          |                            |
| Positive no. (%)            | 0                          |
| Negative no. (%)            | 84(100%)                   |
| **Tumor histological types**|                            |
| Serous epithelial tumors    | 34(40.47%)                 |
| Mucinous epithelial tumors  | 20(23.8%)                  |
| Endometriod tumors          | 21(25%)                    |
| Germ cell tumors            | 6(7.14%)                   |
| **FIGO stages**             |                            |
| Stage I no. (%)             | 63(75%)                    |
| Stage III no. (%)           | 21(25%)                    |
| Burner tumours              | 3(3.57%)                   |

**Fig. 1.** Standard curves for qPCR efficiency calcu-
lation of target Kras gene
of tumor. The majority of previous research has focused on the frequency of mutations in the KRAS gene. They claim that the KRAS gene is frequently mutated in various types of tumors and plays a role in cell proliferation, apoptosis, migration, and differentiation. KRAS expression has been studied for its prognostic value in a variety of cancers, but the majority of studies have found a link between KRAS gene over expression and advanced tumor stages. To our knowledge, the present study is one of the few studies that have found a link between KRAS gene over expression and tumor development in the early stages. In general, over expression of Ras genes has been linked to a poor prognosis for colon carcinoma, leading to the conclusion that Ras over expression cannot be used as a predictive factor for ovarian cancer. Further studies with a larger cohort of ovarian cancer specimens in both primary and advanced stages are needed to confirm these findings.

**REFERENCES**


**Table II.** Relative expression levels of KRAS mRNA in ovarian cancer patients, compared to clinopathological features

<table>
<thead>
<tr>
<th>KRAS gene expression</th>
<th>Tumor group</th>
<th>Mean ± SE of KRAS gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign ovarian tumors</td>
<td>7.82 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Malignant ovarian tumors</td>
<td>661.51 ± 75.09</td>
<td></td>
</tr>
<tr>
<td><strong>T-test</strong></td>
<td>47.911**</td>
<td></td>
</tr>
<tr>
<td>Age groups</td>
<td>Mean ± SE of KRAS gene</td>
<td></td>
</tr>
<tr>
<td>children age 0-14 years</td>
<td>87.46 ± 4.07</td>
<td></td>
</tr>
<tr>
<td>Teenagers and young adults aged 15-24 years</td>
<td>5.76 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>Adults aged 25-49 years</td>
<td>3.98 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Adults aged 50-74 years</td>
<td>1445.96 ± 82.37</td>
<td></td>
</tr>
<tr>
<td>LSD Value (P-value)</td>
<td>52.269** (0.0001)</td>
<td></td>
</tr>
<tr>
<td>Histological tumor type</td>
<td>Mean ± SE of KRAS gene</td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>10.48 ± 1.94</td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>36.58 ± 2.66</td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>2469.55 ± 137.06</td>
<td></td>
</tr>
<tr>
<td>Germ cell tumor</td>
<td>35.52 ± 1.83</td>
<td></td>
</tr>
<tr>
<td>Burner tumor</td>
<td>2.692 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>LSD Value (P-value)</td>
<td>23.605** (0.0001)</td>
<td></td>
</tr>
<tr>
<td>Tumor stage</td>
<td>Mean ± SE of KRAS gene</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>831.78 ± 71.55</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>59.45 ± 3.16</td>
<td></td>
</tr>
<tr>
<td>LSD Value (P-value)</td>
<td>37.205** (0.0001)</td>
<td></td>
</tr>
</tbody>
</table>


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Conflict of interest:
The Author declare no conflict of interest.

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Received: 05.11.2021
Accepted: 08.03.2022

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article