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Up-regulated miR- 205 as a Novel Serum Biomarker for Prognosis of Epithelial Ovarian Cancer

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Abstract—*Background: Among the eleven most common cancers, ovarian cancer is the fifth leading cause of deaths after lung, breast, colorectal and pancreatic cancer. A large number of females die from disease each year due to late diagnosis and resistant to conventional treatment. New therapies and biomarkers are needed to overcome chemo-resistance as well as early diagnosis of epithelial ovarian cancer. Currently microRNAs are being explored as signature for early detection of various types of cancer. However little information is available on expression and correlation of microRNA in ovarian cancer. Expression level of miR-205 in epithelial ovarian carcinoma has been evaluated to find out the role as a potential prognostic biomarker.*

Methods: *Present study has been done to investigate the expression of miR-205 in the serum of sixty epithelial ovarian cancer patients and sixty their age matched healthy women by TaqMan PCR microRNA assay. The correlation of miR-205 with clinicopathological factors of ovarian cancer was analysed statistically.*

Results: *The results showed 3.15 fold increase in miR-205 expression of ovarian cancer patients. Fold change value of miR-205 in advanced stage was 5.75 higher than early stage. Increased serum level of miR-205 in ovarian cancer patients shown significant correlation with advanced FIGO stage and histological sub types of epithelial ovarian cancer.*

Conclusions: *Present study revealed up regulated expression of miR-205 in the serum of epithelial ovarian cancer patients. This could be explored and validated as good prognostic biomarker for ovarian cancer.*

Keywords: *Prognostic biomarker, microRNA-205, epithelial ovarian cancer, up-regulation.*

Introduction

Ovarian cancer accounts more death than any other cancer of female reproductive system. Among the eleven most common cancer of women, ovarian cancer ranks fifth in cancer related death among women. Incidence rate of ovarian cancer varies according to geographical pattern. Cancer incidence and mortality are rapidly growing worldwide with highest incidence rate in North America, central and Eastern Europe [1]. American cancer society statistics report states that united state have estimated 22530 new cases and 13980 deaths due to ovarian cancer in current year [2]. As per data of National Cancer Institute (NCI), a woman without a family history of ovarian cancer has 1 in 55 lifetime chance and this can increase up to 10 times with hereditary conditions [2]. There are more than 30 different types of ovarian cancer depending on the origin of cell types however, surface epithelium, germ cells and stromal cells tumor are more common. Among these epithelial ovarian cancer is most frequent i.e. 98%. Its subtype high grade serous carcinoma HGSC has highest mortality rate. Several factors including age, smoking, oral contraceptives, fertility treatment, being overweight and other menstrual problems have been reported with the risk of epithelial ovarian cancer. With regard to other possible risk factors nulliparity/low parity, menopausal hormone therapy and family history of breast/ ovarian cancer among first degree relatives have been shown to be associated with increase in ovarian cancer [3].

Currently diagnosis of ovarian cancer is based upon pelvic examination, ultrasound and tumor biomarkers. However, invisible symptoms, weak invasiveness and resistant to chemotherapy in ovarian cancer patients are associated with poor prognosis [4]. Various serum and plasma biomarkers like CA 125, HE4, Mesothelin, Kallikreins, and ALDH1 etc. have been proposed with higher sensitivity with malignant stage but less sensitivity and specificity at early stage diagnosis. Therefore there is a need of signature biomarkers with higher specificity and sensitivity to improve the survival in ovarian cancer patients. Currently microRNAs are being explored as signature for early detection of ovarian cancer [5]. Micro

RNAs are strongly linked to the engine room of cancer that affects nodal points in cell cycle regression, genome integrity, stress responses, apoptosis and metastasis [6]. Transcriptional profiling data evidenced that microRNA expression profiling may successfully classify different tumor types more reliably than mRNA profiling. MiRNAs have the ability to function as oncogenes or tumor suppressors. Characteristic miRNA signatures have been revealed in a variety of human cancers [7, 8]. Profiling and expression analysis of microRNA has a promising future in optimizing the personalize treatment of ovarian cancer with positive outcome. However little information is currently available on expression of microRNA in ovarian cancer. In the present study expression level of miR-205 in epithelial ovarian carcinoma has been evaluated to find out the role as a potential diagnostic biomarker.

Materials and Methods

Sixty patients with epithelial ovarian cancer and sixtyage matched healthy controls from Department of gynecology and obstetrics, PGIMS, Rohtak in the period from February, 2016 to July, 2018 were analysed for the study. Ethical approval was obtained from IHEC (Institutional human ethical committee) and all the blood samples were taken afterinformed consent of patients and controls. A specialized questionnaire has been developed to record detailed information especially regarding history of ovarian cancer, age, demographic, reproductive variables, and history of other diseases, any surgical procedures, smoking habits, exposure to oral contraceptives and other risk factors. Ovarian cancer patients were identified with the help of clinicians using International Federation of Obstetrics and Gynecology staging system to assess clinical stage of the disease.

Blood sampling and RNA extraction

Blood samples were collected in red vacutainer tube from patients as well as from healthy controls and centrifuged at 4500 rpm for 15 min at 4ºC. Serum samples were taken for total RNA extraction from sixtyepithelial ovarian cancer patients and sixtyage matched healthy control. MirVana microRNA isolation kit was usedto isolate total RNA from serum according to the manufacturer's instruction. Purity and concentration of RNA was checked byMyspec spectrophotometer. The extracted RNA was stored at -80 ºC until used.

cDNA synthesis and Real time PCR

Reverse transcription reaction was performed to prepare cDNA from isolated RNA samples and small nucleolar RNAU_6 as endogenous control by using TaqMan reverse transcription kit (Applied biosystem). Real time PCR of all cDNA of miR-205 and U_6snRNA was performed in triplicate.PCR master mix (No Amperase UNG) and nuclease free water were added to prepare reaction mixture. Carefully transferred 20x TaqMan microRNA assay (specific for microRNA 205 and U_6)and RT product from the reaction tube of specific microRNA. Mixed gently and centrifuged to bring solution to the bottom. Run the RT-PCR with conditions 10 min at 95 ºC for enzyme activation and 40 cycles for denaturation with 15 seconds at 95 ºC and annealing for 60 seconds at 60 ºC. The relative expression of microRNA 205 and U_6 snRNA was calculated using the equation $2^{-\Delta\Delta Ct}$ where $\Delta\Delta Ct = (Ct_{miR\text{-}205} - Ct_{U6})$ and ΔCt value negatively correlated with the serum level of microRNA.

Statistical analysis

Statistical analysis was conducted using the SPSS 17.0 software. Paired sample t- test and independent sample t-testwere used to assess miR-205 with respect to clinicopathological parameters. Expression of microRNA in both early and advanced stage ovarian cancer was analyzed using ANOVA and Mann-Whitney test. P value less than 0.05 was considered statistically significant.

Results

Clinicopathological information was obtained from 60 each of ovarian cancer patients and control. There were 39 patients in age group above 50 years and 21 in age group below 50 years. Forty controls weretaken with age group above 50 years and twentybelow age group 50. There were 23 patients diagnosed with early stage (FIGO I and II) and 37 in advanced stage (FIGO III and IV).Thirty eight(sixty three percent) cases were from rural background and rest twenty two (thirty six percent)from urban. There were 41 cases with confirmed serous ovarian carcinoma, 11 with mucinous and 8 with endometrioid ovarian carcinoma of the ovary (Table 1).

Table 1: Risk factors and clinicopathological details of control and ovarian cancer patients

Parameters	Control (n=60)	Patients (n=60)
Agenumber (%) number (%)		
≤ 50 years	20(33.34)	21 (35.0)
>50 years	40(66.67)	39 (65.0)
Smoking		
Yes	8 (13.34)	7 (11.67)
No	52 (86.67)	53(88.34)
Status		
Rural	42 (70.0)	38 (63.34)
Urban	18 (30.0)	22 (36.67)
Tumor staging		
FIGO I/II	-	23 (38.34)
FIGO III/IV	-	37 (61.67)
Histological Diagnosis		
Serous	-	41 (68.34)
Mucinous	-	11 (18.34)
Endometrioid	-	8 (13.34)

Expression difference between miRNAs with clinical characteristics of ovarian cancer patients was calculated by threshold cycle using equation $\Delta Ct = (Ct_{miR\text{-}205} - Ct_{U6})$. Independent t-test was used to compare clinical factors with micro RNA levels. Statistical analysis of both miRNAs

expression with respect to age group, smoking, living status, family history, histological subtypes and stages was done. Result showed that age, smoking, family history and living status have no influence on microRNA expression rates. FIGO stages and histological subtypes showed significant correlation with expression of microRNA 205. P value was 0.019 for miRNA-205in stages indicated significant differences between micro RNA expression and stages of ovarian cancer. P value was found 0.034 for miR-205 in histological subtypes also reported a significant observation (Table 2).

Table 2: Comparison between the relative expressions of miR-205 with clinicopathological details

MicroRNA-205		
Clinicopathological characteristics	**Mean ± SD**	**P-value**
Age (Number of patients)		
≤ 50 years (21)	4.94± 1.28	0.54
>50 years (39)	6.17 ± 2.12	
Smoking habits		
Smokers (7)	4.86 ± 1.68	0.82
Non-smokers (53)	7.95 ± 2.16	
Living status		
Rural (38)	6.22 ± 1.82	0.97
Urban (22)	4.16 ± 1.98	
FIGO Stages		
FIGO I/II (23)	2.23± 1.51	0.019*
FIGO III/IV (37)	5.75± 0.98	
Histological subtypes		
Serous (41)	6.54 ± 1.73	0.034*
Mucinous (11)	3.23 ± 0.67	
Endometrioid (8)	2.34 ± 1.52	

$\Lambda\Lambda Ct = (Ct_{miR-205} - Ct_{U6})$; data were expressed as mean of ΛCt ± standard deviation; * significant p value

Expression of miR-205 in different stages of ovarian cancer

Among the subtypes of epithelial ovarian cancer, serous ovarian carcinoma was most common and calculated fold change value in miR-205was 3.15. Fold change value calculated from RT-PCR for mucinous ovarian carcinoma was increased up to 1.67 for microRNA 205. Endometrioid carcinoma was very rarely diagnosed among other subtype and miR-205 showed 1.34 fold increased in cases (Table 3).

Table 3: Expression of miR-205 in different subtypes of epithelial ovarian cancer

Subtypes of EOC	miRNAs as targets and U6 as internal control	(ΛCT) Mean ±SD	$\Lambda\Lambda$CT	Fold change
Serous ovarian carcinoma	miR-21/U_6	1.78 ± 1.22	-2.96	3.15
Mucinous ovarian carcinoma	miR-21/U_6	1.23 ± 2.06	-1.87	1.67
Endometrioid ovarian carcinoma	miR-21/U_6	0.93 ± 1.5	-1.93	1.14

Statistical analysis by paired t-test revealed a significant difference between ovarian cancer patients and in control serum samples(Figure 1). P value from independent t-test compared with histological subtypes was found 0.003 for miRNA-205 showed significant difference(Table 4).

Table 4: MicroRNA-205 expression rates in ovarian cancer and healthy women

MicroRNA	Ovarian cancer patients (n= 80)	Control (n=80)	Paired t-test
	Mean ± SD	Mean ± SD	P value
MIR-205	2.98 ± 1.56	5.94 ± 1.9	0.003

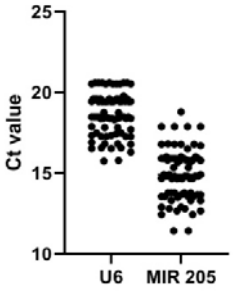

Figure 1. Relative expression of microRNA with U_6 (endogenous control)

Discussion

Most of the techniques of diagnosing ovarian cancer based upon pelvic examination, transvaginal ultrasonography, abdominal ultrasonography and biopsy for evaluating pelvic mass. Most commonly used available biomarker is cancer antigen (CA) 125. However, reported specificity and sensitivity is not upto the mark. Therefore, research investigation has been focussed towards new biomarker. Currently microRNAs are being explored as serum biomarker. These small noncoding RNA molecules are considered that functions as non-invasive biomarkers in blood. A number of microRNA has been identified in ovarian cancer development, but their molecular mechanism in cancer progression is still not well known. Several studies revealed that microRNAs have a potential as therapeutic and novel biomarker in the progression of different type of cancer (Zhang et al. 2007; Nam et al. 2008; Bandyopadhyay et al. 2010; Qu et al. 2011). In this study, it was confirmed by qPCR that miR-205 levels are significantly higher in the serum of ovarian cancer patients, and that a high level of miR-205 expression correlated with poor tumor differentiation, lymph node metastasis and increased tumor stage. Notably, patients with high serum miR-205 levels had a significantly lower survival rate than those with low expression levels, and serum miR-205 was an independent risk factor for poor prognosis. These results suggested that serum miR-205 can be used as a potential predictor of prognosis in ovarian cancer. Tumor

tissue miR-205 or serum miR-205 are associated with the development and prognosis of tumors. MiR-205 is frequently dysregulated in many cancers and acts as a tumor suppressor or an oncogene depending on cellular context [9]. In ovarian cancer, miR-205 functions as an oncogene, promoting proliferation and migration of cancer cells [10] MiR-205may be explored as probable tumour biomarker for early detection as well as in planning treatment module in ovarian cancer.

Conclusions

Present data demonstrated that the advanced stage (FIGO III/IV) had comparatively high expression of miR-205 as compared to early stage. Data analysis revealed up-regulation of miR-205in epithelial ovarian cancer patients. Future investigations are needed to understand how up regulation of miR-205 contributes to the progression of ovarian cancer. Therefore, Mir-205 may be explored as serum diagnostic biomarkers for early detection of ovarian cancer.

Conflict of interest

The authors declare that they have no conflict of interest.

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