عنوان المشروع باللغة العربية- **Title of the proposed project in Arabic**	**تحديد مؤشر بيولوجي جزيئي للتنبؤ المبكر بسرطانات الثدي و المبيض في حاملات ميثلة الجين بي ار سي ون.**
Title of the proposed project in Eenglish	Identification of a molecular biomarker for early prediction of breast and ovarian cancers in cancer-free *BRCA1*-methylated carriers.
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التخصص الدقيق للمشرف الرئيس- **Specialty of PI**	**Nucleic acid Immunology**
المشرف المساعد **Co-PI**	**Nisreen Mohammed Al-Moghrabi**
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور)- **Expected time in month to finish**	**12 months**

Abstract:

Early- onset breast cancer is the most common malignancy and cause of death in Saudi Arabia. Clinical data have shown that most patients present to the clinics with advanced stage tumors. Although ovarian cancer is less common than breast cancer among Saudi women, it causes more deaths than any other cancer of the female reproductive system. This is a consequence of absence of signs or symptoms associated with early stage disease. Our group has previously demonstrated that methylated *BRCAI* promoter in white blood cells is linked to an elevated risk of developing breast and ovarian cancer in the population (1). Additionally, we have found that 10% of cancer-free females carry methylated *BRCA1* promoter in their peripheral white blood cells (WBC) (1, 2). It is reported that some microRNAs act as oncogenes in certain cancers and as tumor suppressors in other cancer types or vice versa due to their targets and mechanisms of action. For example, miR-155 was found to function as tumor suppressor in ovarian cancer and as oncogene in breast cancer (3-5). Here, we propose to investigate the possible use of microRNAs as biomarkers for early prediction of breast or ovarian cancers in cancer-free females carrying methylated *BRCAI* promoter in their WBC. Blood samples from 20 breast cancer patients, 20 ovarian cancer patients and 100 cancer-free females, will be collected and analyzed for the presence of methylated *BRCAI* promoter in the WBC using methylation-specific PCR. MicroRNAs primary transcript levels will be measured in WBC from the patients and cancer-free females carrying methylated BRCAI promoter and in the unmethylated cancer-free female controls. Additionally, the Stem loop RT-PCR assay will be used to quantify the expression levels of mature microRNA in the plasma of the patients, carriers and in the unmethylated cancer-free female control.

Hypothesis of the proposal:

This proposal is about investigating the possible use of microRNA, as biomarker for the early prediction of breast or ovarian cancer predisposition in methylated BRCA1 promoter cancer-free female carriers.

Specific objectives:

1. Study the expression levels of microRNA (miR-155) in white blood cells (precursor form) and in plasma (mature circulating form) in breast cancer patient.

2. Study the expression levels of microRNA (miR-155) in white blood cells (precursor form) and in plasma (mature circulating form) in ovarian cancer patient.

3. Study the expression levels of microRNA(miR-155) in white blood cells (precursor form) and in plasma (mature circulating form) in cancer-free females harboring methylated BRCA1 promoter in their WBC and compare it to that in the BRCA1 methylated breast and ovarian cancer patients.

Methodology & Major Techniques to be used:

The study will involve the collection of peripheral blood(Ten mls fresh blood in 2 EDTA blood collection tube), from 20 breast cancer patients and 20 ovarian cancer patients visiting the oncology clinics at the hospital of the King Faisal specialist hospital and research center. Peripheral blood will be also collected from 100 cancer-free females. The collected blood will be processed immediately in order to isolate white blood cells and plasma. DNA will then be isolated from the white blood cells to be used in the methylation specific PCR assay for studying the methylation status of the BRCAI promoter in the patients and cancer-free controls. In order to study the expression levels of the microRNA in white blood cell, RNA will be isolated from the white blood cells. CDNA will be then synthesized from the RNA and will be used in real time PCR. Additionally, circulating microRNAs will be isolated from plasma and will be used to measure the expression level of the mature circulating form of the microRNA using Stem-loop RT-PCR assay.

Major Techniques:

1. Bisulfite conversion: using EpiTect Bisulfite Kit .

2. Methylation specific PCR Modified DNA will be amplified with published PCR primers for BRCA1 .

3. Real-time RT-PCR.

4. Stem-loop RT-PCR assay.

Availability of Samples	**Yes**
Availability of chemicals	**Yes**
Availability of Instruments	**Yes**
Ethical Approval	**Yes**

References:

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3. Wang, F., Zheng, Z., Guo, J. and Ding, X. (2010) Correlation and quantitation of microRNA aberrant expression in tissues and sera from patients with breast tumor. *Gynecol Oncol*, **119**, 586-593.
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