



**Comparison of Common Known Reproductive Risk Factors
Associated with the Molecular Subtypes of Breast Cancer
Amongst Female Palestinians in the West Bank**

مقارنة عوامل الخطر الانجابية الشائعة المعروفة بين الأنواع الفرعية الجزيئية
لسرطان الثدي للنساء الفلسطينيات في الضفة الغربية

By: Dana Basim Mohammed Aljaber

Supervisor: Dr. Weeam Hammoudeh

Birzeit University -Palestine

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Dana Basim Mohammed Aljaber

Date of Defense: 21-6-2021

Supervisor

Dr. Weeam Hammoudeh

Committee Members

Dr. Emilia Rappocciolo

Dr. Sahar Hassan

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for the Master's in Public Health Degree from the Institute of
Community and Public Health at Birzeit University, Palestine.**



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مقارنة عوامل الخطر الإيجابية الشائعة المعروفة بين الأنواع الفرعية الجزيئية (molecular subtypes) لسرطان الثدي للنساء الفلسطينيات في الضفة الغربية

Dana Basim Mohammad Aljaber

Date of thesis defense: 21/6/2021

Thesis defense committee members:

Dr. Weeam Hammoudeh (Supervisor)

Dr. Sahar Hassan

Dr. Emilia Rappocciolo

Dedication

This thesis is dedicated...

To

My Mother

A strong Palestinian Woman, who has taught me to stay persistent, work hard, and have faith.

For the times your hands would hurt from stitching all night, to the times you would be exhausted but smile, to the times you would feel defeated but make us feel victorious I am forever grateful.

To

Dr. Victoria Seewaldt

A beautiful soul, a mentor, a leader who has reminded me of the potential I have to offer the World.

To

My People

Your strength is embodied within us all. You give me a reason to keep going and I hope to do what I can for our community now and in the future.

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Abbreviations

ER	Estrogen
PR	Progesterone
HER	Human Epidermal Growth Factor Receptor 2
DCIS	Ductal Carcinoma In situ
LCIS	Lobular Carcinoma In situ
IDC	Invasive Ductal Carcinoma
ILC	Invasive Lobular Carcinoma
BCSS	Breast Cancer Severity Score
HRT	Hormonal Replacement Therapy
IVF	In Vitro Fertilization
OC	Oral Contraceptives
BMI	Body Mass Index
TNM	Tumor, Lymph Node, Metastasis
OR	Odds Ratio
CI	Confidence Interval
PMC	Palestinian Medical Complex
MOH	Ministry of Health
PCBS	Palestinian Central Bureau of Statistics

Abstract

Background:

Breast cancer is the leading cause of cancer-related death amongst the female Palestinian population. The first step in preventing future cases of breast cancer is to properly understand certain factors that may contribute to increasing ones' risk for breast cancer. Common known risk Factors for breast cancer can be divided into environmental, demographic, lifestyle/behavioral, genetic, socioeconomic, and reproductive factors.

Breast cancer is also further divided into four distinct molecular subtypes: Luminal A, Luminal B, HER2-Enriched, and Triple Negative. Differences amongst these subtypes may be seen in relation to the prognosis of these cancers and the aggression of the cancers themselves. Studies have shown differences among risk factors and these subtypes of breast cancer. However, no studies were done in Palestine to assist risk factors amongst these subtypes. This study aims to test if there are associations between specific reproductive risk factors, (age of menarche, age of menopause, nulliparity, age of first pregnancy, breastfeeding history, oral contraceptive use, in-vitro fertilization, and estrogen or hormone therapy use), and breast cancer subtypes and to compare these associations between subtypes.

Methodology:

This study was a cross-sectional study. Breast cancer patients seeking treatment from three public hospitals in the West Bank with known receptor status were included. Data collection started in February 2020 and ended in March 2021. Information

regarding clinicopathological features (such as receptor status, tumor grade, and clinical TNM) were taken from the EHR's of the patients. Patients were asked short questions regarding common known reproductive risk factors for breast cancer to complete a reproductive risk profile, after their visits to the oncology clinics and after attaining verbal consent. Chi-squared and One-way ANOVA were used to find differences amongst risk factors and the breast cancer subtypes. A multinomial regression analysis was done to get an odds ratio and find associations amongst the breast cancer subtypes and the reproductive risk factors. All analyses were done at a p-value equal to or less than 0.05.

Results

Out of a total of four hundred and two patients, 131 had Luminal A, 181 had Luminal B, 60 had HER2-Enriched subtype, and 30 had the triple-negative subtype with the most prevalent subtype being Luminal B. The mean age of the study sample was 48.29 ± 11.37 years. The majority of patients had a high grade, a positive lymph node status, and large tumors ($>2\text{cm}$). Two hundred and twenty-five patients (59.5% of the total sample) also had a positive ki-67 status ($\geq 15\%$). One major finding of this investigation was that the more aggressive subtypes of breast cancer (HER2-Enriched/ Triple Negative) were associated with a younger age at diagnosis compared to the Luminal A subtype (OR 2.69 95% CI 1.14-6.34: $p=0.023$) and (OR 3.31 95% CI 1.06-10.31: $p=0.039$). Moreover, low parity (< 5 children) was found to reduce the risk of developing the HER2-Enriched subtype compared to the Luminal A subtype. There were no significant differences between risk of breast cancer subtypes and early age of menarche, late age of menopause, IVF history, HRT history, high BMI,

late age at first full-term pregnancy, postmenopausal status, use of oral contraceptives, never-breastfeeding, and family history.

Conclusion:

This study aimed at comparing associations of common known reproductive risk factors amongst the molecular subtypes of breast cancer-specific to the Palestinian population. The findings of this study suggest the potential impact of other risk factors such as environmental factors that may contribute to the development of breast cancer regardless of subtype at younger ages. Future investigations are needed to explore other potential risk factors of the more aggressive breast cancers in the West Bank. The clinical-pathological characteristics of our sample indicate a later detection of breast cancer amongst the population suggesting the need to further improve screening programs for selectivity of at-risk females regardless of age. Further studies should look into the impact of high parity on the development of the more aggressive breast cancer subtypes.

ملخص

مقدمة:

بالحديث عن الوفيات المرتبطة بمرض السرطان، يعد سرطان الثدي العامل الأكثر تسبباً بموت الإناث في المجتمع الفلسطيني، وللحد من أي إصاباتٍ مستقبليةٍ به، ينبغي أولاً أن نتعمق في فهم بعض العوامل التي من شأنها زيادة خطر الإصابة بسرطان الثدي، والتي يمكن تقسيمها إلى عوامل بيئية، سكانية، سلوكية، جينية او وراثية ، اجتماعية- اقتصادية، وعوامل أخرى تناسلية. من حيث النوع، يقسم سرطان الثدي إلى أربع مجموعات فرعية، سرطان الثدي لومينال أ (Luminal A) ، سرطان الثدي لومينال ب (Luminal B) ، أورام إيجابية لبروتين HER2 (HER2-Enriched)، وأورام الثدي القاعدية – سرطان الثدي ثلاثي السلبية (Triple Negative) ، ويتعلق الاختلاف بين هذه المجموعات الأربع بتشخيص الورم ومدى فتكه وعدوانيته. بينما أثبتت بعض الدراسات تفاوتاً في علاقة أبرز عوامل الخطر بالإصابة بهذه المجموعات السرطانية، لكن في فلسطين، لم تُجرَ حتى الآن أي دراساتٍ لتحديد أهم عوامل الخطر المسببة للإصابة بأنواع سرطان الثدي المذكورة آنفاً. لذا، تسعى هذه الدراسة الى لدراسة إذا ما كان هناك أي رابط بين عددٍ من عوامل الخطر، ومنها (سن الحيض، سن انقطاع الطمث، العقم، السن عند الولادة الأولى، الرضاعة الطبيعية، تناول حبوب منع الحمل، التلقيح الصناعي، تلقي علاجات هرمونية) وبين الأنواع الفرعية لسرطان الثدي، ومقارنة تلك الروابط بين المجموعات الأربع المذكورة.

منهجية البحث:

شملت هذه الدراسة عدة مجموعات، إذ ضمت عينة البحث عدداً من مريضات السرطان المعلومة حالة مستقبلات الهرمون لديهن، واللاتي يتلقين علاجاتٍ في أي من ثلاثةٍ من مستشفيات الضفة الغربية الحكومية. أما عملية جمع المعلومات، فقد بدأت في شباط من عام 2020، وانتهت في شهر آذار من عام 2021. يذكر أن المعلومات المتعلقة بالسجلات الإكلينيكية لكل حالة، كحالة مستقبلات الهرمون، وحجم الورم، ومرحلة السرطان)، قد أخذت من السجلات الطبية الإلكترونية للمريضات. من ناحيةٍ أخرى، وجهت للمريضات أسئلةً مقتضبةً حول العامل التناسلي المسبب لمرض سرطان الثدي لتكوين موجزٍ كاملٍ لعامل الخطر التناسلي المسبب لهذا المرض، وذلك بعد إتمام زيارتهن لعيادة الأورام وبعد الحصول على موافقتهن الشفهية لمشاركة تلك الإجابات. لمقارنة مدى ارتباط عوامل الخطر المسببة لسرطان بالأنواع الفرعية الأربعة لمرض سرطان الثدي، تم اعتماد اختبار مربع كاي وتحليل التباين الأحادي، في حين تم اعتماد تحليل الانحدار المتعدد لاحتماب نسبة أرجحية الإصابة والربط بين الأنواع الفرعية لسرطان الثدي وعوامل الخطر التناسلية، ينبغي الإشارة إلى أن جميع التحليلات قد أجريت وفقاً للقيمة الاحتمالية المساوية أو التي تقل عن 0.05.

النتائج:

من إجمالي 402 مريضة، تبين أن 131 منهن يعانين من لومينال أ (Luminal A) ، بينما تعاني 181 منهن من لومينال ب (Luminal B)، وأن 60 منهن يعانين من أورام إيجابية لبروتين HER2 (HER2-Enriched)، بينما تعاني 30 منهن من الثدي القاعدية – سرطان الثدي ثلاثي السلبية (Triple Negative) ، ليكون بذلك النوع الفرعي لومينال ب الأكثر انتشاراً بين العينة التي ترواحت أعمارها بين 48.29 ± 11.37 عاماً. كذلك الحال، كانت غالبية المريضات في مرحلة متقدمة من سرطان الثدي، وكُنَّ يعانين من أورام لمفاوية خبيثة ذات حجم كبير يزيد عن 2 سم. ومن خلال الدراسة، تبين أن 59.5% من إجمالي العينة كان مؤشر سرعة انقسام الخلايا السرطانية (ki-67) لديها إيجابياً ($\leq 15\%$). ومن أهم ما توصل إليه هذا البحث أن أكثر الأنواع الفرعية لسرطان الثدي عدوانيةً، وهي أورام إيجابية لبروتين HER2 وأورام الثدي القاعدية – ثلاثية السلبية، تستهدف السن الأصغر من الإناث اللاتي يخضعن للتشخيص، مقارنةً بالنوع الفرعي لومينال أ، (OR 2.69 95% CI 1.14-6.34:) و ($p=0.023$) و ($OR\ 3.31\ 95\% \ CI\ 1.06-10.31; \ p=0.039$). علاوةً على ذلك، تبين أنه كلما قل عد الولادات (< 5 أطفال)، كلما قل خطر الإصابة بأورام إيجابية لبروتين HER2، مقارنةً بالنوع الفرعي لومينال أ. بينما بينت الدراسة عدم وجود أي علاقة بين خطر الإصابة بأي من المجموعات الفرعية الأربع لسرطان الثدي وبين كلٍ من العوامل الآتية: سن البلوغ المبكر، سن انقطاع الطمث المتأخرة، التلقيح الصناعي، العلاج الهرموني، ارتفاع مؤشاة كتلة الجسم، الحمل الأول في سن متأخرة، مرحلة ما بعد انقطاع الطمث، تناول حبوب منع الحمل، عدم المرور بتجربة الرضاعة الطبيعية، والتاريخ المرضي للعائلة).

خاتمة:

هدفت هذه الدراسة إلى مقارنة مدى تسبب عوامل الخطر التناسلية بالإصابة بالأنواع الفرعية الأربع لسرطان الثدي في المجتمع الفلسطيني. وتشير نتائج الدراسة إلى احتمالية مساهمة عوامل خطر أخرى، كالعوامل البيئية، في زيادة خطر الإصابة بسرطان الثدي بأنواعه الفرعية المذكورة في سن صغيرة، لذا هناك حاجة لإجراء دراساتٍ في المستقبل لاكتشاف عوامل الخطر المحتملة للتسبب بأنواع سرطان الثدي الأشد فتكاً وعدوانيةً في الضفة الغربية. إذ تشير الصفات الإكلينيكية للعينة أن أورام الثدي المكتشفة عادةً ما تكتشف في مرحلة متقدمة من المرض، ما يدعو للعمل على تحسين برامج الفحص للنساء المهددات بالإصابة بسرطان الثدي، بغض النظر عن أعمارهن. كما أن من شأن الدراسات المستقبلية دراسة أثر تعدد الولادات على تطوير أورام ثدي ذات درجة عدوانية عالية.

Introduction

Noncommunicable diseases are heavily infiltrating both the developing and developed world (1). Amongst this global encumbrance of NCD's comes cancer. According to the World Health Organization, in 2018, 9.6 million deaths were accounted to be caused by cancer, and 70% of the cancer deaths were from low to middle-income countries (2). Out of the global 9.6 million deaths due to cancer, breast cancer accounted for 2.09 million deaths in the year 2018 alone (globally). In Palestine, breast cancer is considered the highest-ranked cancer in terms of mortality and morbidity amongst the female population (3).

However, many public health interventions have been done to help prevent the onset of breast cancer on a global scale. Amongst the most important types of intervention, is risk assessment (4). Identifying "at-risk" populations and associating specific factors that contribute to the development of breast cancer has been seen to help with prevention (4–6). Risk factors for breast cancer can be generally divided into socioeconomic, reproductive, environmental, behavioral, and genomic risk factors (5). However, among these factors, reproductive risk is known to be highly associated with the onset of breast cancer due to the influences these risks have on the hormonal cycles of the female body (7–9). These risks include the age of first pregnancy, age of menarche, age of menopause, oral contraceptive use, use of hormone replacement therapies, nulliparity, and breastfeeding status (10,11).

Reproductive risk factors have been heavily studied and association have been found between non-parity, young age of menarche, late age of menopause, lack of breastfeeding, and increased risk of breast cancer development (in both Arab and Non-Arab countries) (5,6,12). In a retrospective review, younger age of menarche was seen to increase the risk associated with breast cancer development, and a later age of menopause was seen to also increase risk (13).

Many studies support the rise of breast cancer risk seen with the use of specific oral contraceptives and intentional hormone therapy such as estrogen (14,15). Moreover, reviews found strong associations between full-term pregnancies before the age of thirty and a reduced risk of breast cancer (14–16). Additionally, women who breastfed between 1-2 years were seen to have a slightly reduced risk of developing breast cancer.

With the increase in research on breast cancer, a very significant aspect has recently started to gain attention. This aspect is the classification of breast cancer into molecular subtypes. There are four main molecular subtypes linked to breast cancer: luminal A, luminal B, HER2-Enriched, and Triple Negative/basal-like (17). Additionally, amongst the molecular subtypes of breast cancer, (Luminal A, Luminal B, HER2-Enriched, Triple Negative), survival rates had differed significantly. Luminal A is the most common subtype of breast cancer (18,19). This subtype is either estrogen receptor or progesterone receptor positive which thus associates it with better prognosis and reduced mortality in many studies due to overall less aggressiveness compared to -ER/-PR cancers (2). A study done in the United States (New Jersey, USA) sought to look for breast cancer subtypes with the highest mortality rate. This study found that patients with triple-negative breast cancer had the lowest survival rates and that patients with Luminal A breast cancer had the highest survival rates (20); thus, further supporting the evidence provided by previous research on cancer survivorship and subtypes (21–23).

Therefore, these classifications range from the least aggressive form of breast cancer (Luminal A) to the most aggressive form (triple-negative). Furthermore, some studies compared the risk factors associated with breast cancer formation amongst the molecular subtypes of cancer in a specific population. One study found that there was indeed heterogeneity of risk factors amongst the different subtypes of breast cancer (24). It found that obesity significantly

increased the risk of forming triple-negative breast cancer and that the lack of breastfeeding and late age of pregnancy increased the risk of forming luminal A/B breast cancer. On the other hand, a study done in Iran found that lack of breastfeeding and nulliparity was associated more with the development of HER2 enriched/ Triple-negative breast cancers (amongst the population) (25); therefore, making these factors linked to poorer prognosis and survival rates amongst the patients studied in the research. Moreover, a systematic review and meta-analysis done also showed the differences found amongst subtypes for reproductive risk factors by indicating that non-parity and older age increased the risk of Luminal A and B subtype formation in different populations (11).

Additionally, several studies performed in the developed world show that with an increase in socioeconomic status comes a higher risk of breast cancer development (26). However, studies have shown that among breast cancer patients, women of lower socioeconomic status and education were found to develop more aggressive subtypes of breast cancer with a poorer prognosis (27–29). Patients with lower socioeconomic status also detected their cancer at a later stage (29). Moreover, a review done on social determinants of breast cancer found that there was documented disparities in breast cancer survival by race, education, poverty, and health insurance access; additionally, they found that poverty and lack of education were associated with more aggressive forms of breast cancer such as triple-negative breast cancer and later stage of cancer at initial diagnosis (29).

In Palestine, breast cancer accounts for 34% of cancer cases among the population (30). One study gathered death registries issued in the years 1999 to 2009 (West Bank) and found that the most common cancer deaths for females were due to breast cancer (3). More importantly, it found that breast cancer mortality was highest amongst women in the southern region of the

West Bank indicating a potential risk factor amongst this subgroup. Additionally, in Gaza, one study was done to associate risk factors for breast cancer and it identified late pregnancy, high BMI, hypertension, and diabetes as risk factors for all breast cancer subtypes (31). However, there is a gap in knowledge in Palestinian research when it comes to proper identifications of risk factors within the population for breast cancer. No studies have yet been done in Palestine to further investigate breast cancer risk factor associations amongst the molecular subtypes. Hence, limiting the understanding and knowledge of factors that may contribute to more aggressive subtypes of breast cancer with poorer prognosis and survivorship. This study will aim to compare common known reproductive risk factors amongst the different subtypes of breast cancer and to identify the risk factors associated more with aggressive subtypes of breast cancer specific to the Palestinian population.

Problem Statement

When it comes to identifying gaps in breast cancer research, there is a strong emphasis on the overall lack of general information on molecular subtypes and risk factors (32). As a result, there are fewer evidence-based interventions in medicine regarding subtypes (32). Also, the lack of evidence creates a barrier in proper treatment assessments for patients and closes the field to developing better community-based preventative strategies for breast cancer. This signifies the public health standing in concern to recognize and implement measures for breast cancer treatment and prevention relative to subtyping. To properly understand the dynamics of prevention towards breast cancer, increased understanding and comprehension of the molecular subtypes/biomarkers of breast cancer progression and sufficient knowledge of risk associations (reproductive, behavioral, etc.) with breast cancer subtypes is of importance (19,32). The following study is aimed at enhancing our knowledge of breast cancer subtypes and associated

reproductive risk factors for each subtype in order to decrease the gap in the proper identification of risk, exposure, and routes of exposure for the Palestinian population.

Significance of Study

Dynamically, developing the knowledge of interactions and associated factors of diseases explicit to a population generates a means to better understanding the current situation of the population. Thus, this study aims to add to the literature of breast cancer molecular subtypes from a public health perspective by developing epidemiological understandings of these subtypes specific to Palestine. Hence, it will establish a means to shrinking the gap in knowledge for breast cancer and its molecular subtypes; therefore, it will make a route to better comprehending the prognosis of these subtypes and identifying ‘at risk’ subpopulations in the Palestinian context. Additionally, this identification of ‘at risk’ subpopulations will help understand factors contributing to more aggressive forms of breast cancer such as the triple-negative subtype, which is known to have poorer prognosis and survivorship (33). This study will contribute to confronting issues on certain risk aspects (reproductive) and aid in the development of evidence-based interventions for stakeholders and (overall) policy-makers concerned with the arising issue. Likewise, it will help generate the rudiments to improving already implemented awareness programs for breast cancer in Palestine by identifying potential known reproductive risk factors that may be associated more with subtypes of poorer prognosis/survival rates.

Research Questions

Primary Research Questions

- Which common reproductive breast cancer risk factors, (young age of menarche, late age of menopause, late age of first full-term pregnancy, lack of breastfeeding, hormone replacement therapy, oral contraceptive use, and in-vitro fertilization), are significantly associated with the different molecular subtypes of breast cancer amongst the female sample population?
- Which of the known reproductive risk factors of breast cancer are associated more with aggressive subtypes in the female sample population?

Secondary Research Questions

- Are obesity and older age as a risk factor of breast cancer, associated more with aggressive subtypes in the female sample population of breast cancer?
- What are the associations between histological diagnosis, (ILC, IDC, DCIS, etc.), and the molecular subtypes of breast cancer amongst the sample population?
- What medical history as a risk factor of breast cancer, (history of diabetes, cardiovascular diseases, or hypertension), is associated more with aggressive subtypes in the female sample population of breast cancer?

- What are the associations between cancer characteristics, (stage, grade, TNM), and the molecular subtypes of breast cancer in the female sample population of breast cancer?

Research Hypothesis

Based on previous similar studies done (on a global scale), the researcher hypothesizes that:

- Breast cancer patients (in the West Bank) with less aggressive subtypes (Luminal A & B) of breast cancer will have a statistically significant positive association with the common risk factor of older age.
- Breast cancer patients (in the West Bank) with more aggressive subtypes (Triple Negative & HER2-Enriched) of breast cancer will have a statistically significant positive association with common reproductive risk factors (young age of menarche, late age of menopause, late age of first full-term pregnancy, lack of breastfeeding, hormone replacement therapy, oral contraceptive use, and in-vitro fertilization).
- Breast cancer patients (in the West Bank) with more aggressive forms of breast cancer (Triple Negative & HER2-Enriched) will have a statistically significant positive association with the modifiable risk factor of obesity.
- Breast cancer patients (in the West Bank) with less aggressive subtypes of breast cancer (Luminal A & B) will have a statistically significant positive association with the reproductive risk factor (nulliparity).
- Breast cancer patients (in the West Bank) with more aggressive subtypes of breast cancer will have a statistically significant positive association with increased tumor size and the number of positive nodes (according to the clinical TNM stage).

- Breast cancer patients (in the West Bank) with more aggressive breast cancer subtypes will have statistically significant positive associations with patients who have a family history of cancer.
- Breast cancer patients (in the West Bank) with more aggressive breast cancer subtypes will have statistically significant positive associations with a medical history of diabetes/hypertension/Cardiovascular diseases.

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Chapter 1: Literature Review

1.1 Epidemiology of Breast Cancer

The most common cancer worldwide in women is breast cancer affecting not only females of the developed world but also of the developing world (9,34). In the United States, it was estimated that nearly 270,000 cases of breast cancer were newly diagnosed in 2019 (35). Although breast cancer is thought to be a disease of high-income countries, roughly 50% of breast cancer cases (globally) and more than 50% of deaths due to breast cancer were shown to be from middle to low-income countries (34). Additionally, incidence rates vary significantly from developed to developing regions. For example, according to the WHO the incidence rate of breast cancer in Eastern Africa is 19.3 per 100,000 women compared to 89.7 per 100,000 women in Western Europe (36). However, despite the differences in incidence rates, survival rates are lower in middle to low-income countries when compared to high-income countries. Many epidemiological studies have suggested that the lower survival rates seen in developing countries may be due to women presenting with a later stage of breast cancer once diagnosed (37,38). This is believed to be caused by different barriers such as a lack of awareness/knowledge programs, lack of effective screening programs, inadequate access to primary health care facilities (especially within rural areas), lack of sufficient treatment plans, and cultural barriers (which may affect treatment-seeking behaviors) (38–40).

In Palestine, breast cancer is the most common cancer according to the health annual report published by the Palestinian ministry of health (41). It is also considered the highest-ranked cancer in mortality and morbidity amongst the female population. There was an overall 5.8% increase of newly diagnosed cancer cases in 2018 compared to 2017. Moreover, in the female population, 34% of all reported cancer cases were between the ages of 15-64 in the year

2018. Based on the health annual report, breast cancer was seen to be 14.2% of all cancer cases with an incidence rate of 16.7 per 100,000 of the population. Furthermore, breast cancer accounted for 27.6% of all female cancer cases with an incidence rate of 33.4 per 100,000 of the female population. One study done in the Gaza strip found that the five-year survival rate for women with breast cancer was at 65.1%; however, when compared to the more developed Mediterranean countries it was seen to be much lower (42). Even amongst Palestinian women living in Israel, the five-year survival rates were lower when compared to the rates of Jewish women (43). A study assessing clinical profiles of breast cancer amongst women in Jerusalem found that the five-year survival rates for Palestinian women were at 58% compared to Jewish women which were found to be more than 70% (44). This may be due to different genetic variations amongst the sub-population of Palestinians in Israel and or the associations of different risk factors such as socioeconomic factors (underserved neighborhoods/lack of access to care), environmental factors (such as different pollutants relative to area and/or certain mental stressors), etc. (45).

As a further matter, in understanding and predicting breast cancer morbidity rates, it is also good to note certain demographic changes. Palestinian women's fertility is indeed expected to decline with a current fertility rate of 3.7 in the West Bank and 4.5 in Gaza (41). This will make way for a demographic modification in which the number of women aged 60 and over will double by the year 2050. Since breast cancer is an age-dependent disease and the risk of having breast cancer increases with age, a 135% rise in breast cancer cases is expected by the year 2040 in Palestine (46).

1.2 The Female Breast & Development

When it comes to identifying factors, which may trigger the onset of breast cancer, recent studies have suggested that during puberty the breast tissues are more susceptible to triggers that may lead to breast cancer in the future (47). This is considered a “window of vulnerability”, in which a female’s breast, undergoing development, is at a higher risk of exposure to certain environmental and hormonal stressors which may cause molecular damage; hence, increasing her risk of breast cancer formation (47,48).

A female’s breast, once fully mature, is composed of lobules which are where milk is produced. From the nipple branches out a wired system of milk ducts that connect to those milk-producing lobules (49). All of which are enclosed by connective and adipose (fat) tissue. Surrounding the tissues is a series of lymph vessels and nodes which aid in collecting excess fluid released by cells and filtering it back to the body. Furthermore, lymph nodes & vessels are crucial in understanding the spread of breast cancer and the different stages of cancer itself (50).

During puberty, a woman’s breast is still maturing, the ducts start elongating and primitive structures of lobules form. It is important to note that puberty for a girl does not start with menarche but begins when the breast starts to form, which is roughly 9 to 11 years of age (51). For full maturation of the breast, it takes on average 4 to 5 years after puberty starts and menarche occurs during this time (52). However, these changes are considered rapid changes on a cellular level that start to occur with the hormonal changes of puberty. This creates the susceptibility or “the window of vulnerability”. This same “window” in many studies was also noted during pregnancy, fetal growth, implantation, and aging, especially during perimenopause and post-menopause (47).

Two key hormones are important for breast maturation, estrogen, and progesterone. These hormones help in promoting growth and cell proliferation of the breast (53,54). Many epidemiological and experimental evidence has suggested estrogen to be an important risk factor in the onset of breast cancer (53,55,56). Additionally, one study done on breast cancer cells found that estrogen and progesterone together promoted cancer cell growth more when compared to these hormones acting alone on the cells (57). During a woman's reproductive years (including premenopause), estrogen and progesterone are secreted to get the female ready for pregnancy each month. This pattern of hormone fluctuation is natural and during menopause/postmenopause the blood estrogen hormone level should normally decrease (58,59). Moreover, several studies have implicated that postmenopausal women with high levels of blood estrogen were at twice the risk of developing breast cancer when compared to postmenopausal women with low blood estrogen (60). Other studies have shown that a younger age of menarche, (an earlier age of exposure to estrogen), increased the risk of having breast cancer (61). Additionally, studies implicated that the higher levels of estrogen during pregnancy decreased one's risk of getting breast cancer (56). This suggests that the influence of estrogen on the breast is indeed age-dependent and that factors that influence the hormonal cycles of the female body, (during the "vulnerable stages" of a female's life), are associated with increased breast cancer risk, such as reproductive risk factors. Reproductive risk factors for breast cancer are discussed in more detail later on.

1.3 Characteristics of Breast Cancer

With fully trying to comprehend breast cancer, considerations towards specific tumor & cancer characteristics are ideal in identifying cancer severity and patient prognosis (62). These descriptive characteristics help predict how cancer is most likely to behave and the treatments

needed for patients (63). Moreover, several characteristics ,such as cancer grade and tumor size, do indeed predict poorer prognosis and survival rates amongst breast cancer patients (22,64,65).

1.3.1 Histological Classifications-Types of Breast Carcinomas

Most commonly, a histological classification will be used to identify the location of cancer in the breast, the morphology of the tumor, and the type of cells that are affected (66). Histologically classifying breast cancer helps categorize whether cancer is invasive/infiltrating or noninvasive. Noninvasive breast carcinomas are considered to be “in situ”, meaning that they still haven’t spread to the surrounding tissues of the specified location in the breast and are contained within the lobules or ducts (67). When compared to invasive, noninvasive carcinomas have a better prognosis and lower mortality rates (68).

The most common non-invasive breast carcinoma is DCIS (ductal carcinoma in situ), which affects the milk ducts of the breast. In several studies it has been seen that DCIS may progress to invasive cancer (68); however, due to its heterogeneity and uniqueness within itself, it's considered a nonobligate precursor of invasive breast cancer. Moreover, patients with DCIS have a great prognosis and breast cancer-specific survival rate of more than 95% (69). Another less common “in situ” breast carcinoma is LCIS (lobular carcinoma in situ), which affects the lobules of the breast. Studies agree that women diagnosed with LCIS have an increased risk of forming invasive breast cancers compared to having DCIS (70). For example, one study was done using the Surveillance, Epidemiology, and End Results data (SEER), found that at a 10 year follow up the incidence of invasive breast cancer after LCIS was 7.1%, with risk distributed equally amongst both breast regardless of which breast had LCIS (71). Hence, since LCIS is considered a risk factor for breast cancer and not a precursor, patients with LCIS have a slightly poorer prognosis compared to women with DCIS (70).

The most common invasive breast carcinoma is IDC (invasive ductal carcinoma), which has a rough estimate incidence of 80% of all breast cancers and accounts for one-quarter of all cancers in females worldwide (72). This carcinoma affects the ducts of the breast and has different types due to its heterogeneity (73). Several studies have shown that women over 60 with IDC had better prognosis and survival rates when compared to women younger than 60 years of age with IDC (72,74). Additionally, the second major invasive carcinoma is ILC (invasive lobular carcinoma), which makes up 5%-15% of invasive breast carcinomas (75). In various studies, survival was found to be significantly better in patients with ILC compared to patients with IDC (76). However, other studies comparing survival amongst these two invasive carcinomas found no significant difference between ILC and IDC types (77,78).

1.3.2 Cancer Grading

Another characteristic found to help classify breast cancer severity (along with all cancers) is cancer grading. There are three grades to breast cancer using the Nottingham grading system, (which is the most common system used for breast cancer), and each one is based on the appearance of the cancerous cells (79). Grade I is used to define cancer cells that are shaped normally and are not rapidly growing. Grade II describes abnormally shaped cancer cells that are moderately differentiated and are growing at a faster pace than normal. Additionally, Grade III designates cancer cells that are very abnormal with no architectural structure compared to normal cells. However, cancerous cells that appear to be very undifferentiated may be assigned a grade of four. Grade IV describes cancer cells that are the most abnormally structured and have a very rapid growth rate compared to the other grades (66,72). Several epidemiological studies have implicated a poorer prognosis with higher-grade breast cancers compared to lower-grade breast cancers (80). A study done using the SEER data found that the 10-year survival rate of breast

cancer patients decreased progressively as the size of the tumor and the number of lymph nodes involved increased for each grade level (81). Moreover, it suggested that this same grading of breast cancer was an important prognostic factor regardless of the tumor size and the number of involved lymph nodes (82). Another cohort study done in the Netherlands found that the BCSS (breast cancer severity score) was found to be worse in grades II and III breast cancers with a hazard ratio of 1.92 and 2.14 (respectively) at a 95% confidence interval (83). One study done in Gaza viewing patient cases from 2005-2014, noted an increased death risk for breast cancer patients with an increased grade level (42). Hence, agreeing with other studies that higher-grade breast cancers do have decreased survival rates and an increased risk of mortality compared to lower-grade breast cancers (78).

1.3.3 Stages of Cancer & The TNM Cancer Staging System

One of the most important characteristics in knowing the location and the aggressive degree of cancer is staging (84). The most common staging group used by healthcare professionals globally is the I-IV cancer stage system. However, these stages are derived from another staging system (TNM system) which focuses on adding more descriptive detail for classifying cancer (85). The letter “T” usually followed by a number (from 0-4), describes the tumor size and its growth into nearby tissue with a larger number representing larger size. Studies have shown that the larger the tumor size the poorer the prognosis was for patients with breast cancer (78,86,87). The letter “N”, also followed by a number (from 0-3), indicates the number of lymph nodes affected by cancer. The higher the number of lymph nodes infected by cancer, the higher the number assigned to the “N”. Many epidemiological studies have implicated poorer prognosis and lower survival rates with breast cancer patients having a positive node cancer status with survival rates decreasing in patients as lymph node status “N”

increased (88–91). Additionally, studies have indicated that the presence of a positive lymph node status in breast cancer patients increased the risk of local and distant recurrence; hence, increasing the risk of mortality (50,92). Lastly, the “M”, followed by either 0 or 1, describes metastasis (the spread of cancer to other parts of the body). Breast cancer patients with cancer metastasis (M1) have been seen in many studies to have poorer treatment outcomes and a higher risk of mortality due to cancer (78,81,93).

Furthermore, a patient's T, N, and M numbers are used to group cancer into stages I-IV. Stage I cancers imply cancer that has a small tumor size and hasn't spread to nearby tissue. Breast cancers of stage I have better survival outcomes and prognosis (81,87). Stage II and III cancers indicate a larger tumor size when compared to stage I and cancers that have spread to nearby lymph nodes. Stage IV cancers are cancers that have metastasized and have spread to other regions of the body. Regardless of the T/N status, cancer with M1 status is considered stage IV. Many studies have shown the worst prognosis and survival rates with stage IV breast cancer patients compared to patients with Stage I, II, & III (93,94).

1.4 Breast Cancer and Reproductive Risk Factors

Amongst the many risk factors associated with breast cancer (e.g. environmental, socioeconomic, genomic), reproductive risk factors have been found to strongly influence the onset of breast cancers due to the effects of these factors on the hormonal cycles of the female body (95). As mentioned earlier, fluctuations of certain hormones may help in the promotion and development of breast cancer; moreover, these same fluctuations, occurring during the stages of a women's life in which she may be placed in “a window of vulnerability”, may further promote the onset of breast cancer (47,56).

1.4.1 Age at First Pregnancy & Parity

The findings of several case-control studies indicated that a nulliparous woman had a higher risk of developing breast cancer compared to a parous woman (61). Moreover, in some studies, it was shown that multiparity was associated with a decreased risk of breast cancer development (11,61). In a cohort study, nulliparity was also seen to be associated with a higher grade of breast cancer and larger tumors (96). However, in one case-control study done in Iran it compared 168 breast cancer cases to over 500 age-matched controls and looked at reproductive risk (97). It found that having more than five full-term pregnancies increased the risk of the female developing breast cancer which is in agreement with other studies that showed a potential dual effect of parity on the risk of breast cancer (11,98,99). This dual effect is suggested to be due to the age-dependency of breast cancer and the role of estrogen on the female breast during the pregnancy “window of susceptibility” (53,57,100). Additionally, a cohort study conducted on African-American women found high parity to be associated with an increased risk of breast cancer formation amongst women younger than 45 years of age (101). However, there was a decreased risk of developing breast cancer with high parity in women older than 45 years of age. Therefore, adding to studies indicating the age-dependent effect of estrogen and its importance in breast cancer formation (53,57,100,102).

Furthermore, it has been seen that the first age of a woman’s first pregnancy heavily influences her breast cancer risk (103). These associations have been noted since the 1970s in one study done by MacMahon which showed a 60% decreased risk of developing breast cancer in women whose age of first birth was 18 years or younger (95). Current studies still support this claim; however, the age of first full-term pregnancy with a protective effect towards breast cancer is now considered being below 30 years of age compared to 18 years of age (11,103,104). In one prospective cohort including over 17,000 women, it was indicated that a late age of first

childbirth (more than 30 years of age) was indeed a risk factor for breast cancer (103). Other studies also indicated that a late age of first full-term pregnancy was seen to be associated with poorer prognosis and overall survival (15,61,95). A case-control study done in the Gaza Strip (including 105 breast cancer patients), indicated that women with a later age of first full-term pregnancy (over 35 years of age) were more likely to have an increased risk of breast cancer development (odds ratio [OR], 11.56; 95% [CI], 1.64–81.35) (105).

1.4.2 Age of Menarche & Age of Menopause

As mentioned previously, during puberty and menopause a woman's breast tissues are more sensitive to hormonal stressors such as increased levels of estrogen (59). In many epidemiological studies, it was found that a younger age of menarche increased a woman's risk of developing breast cancer (16,95,106). Experimental studies have suggested that a younger age of menarche increases risk due to the longer exposure of estrogen during vulnerable stages of breast development and a female's reproductive years (7,47,56). Additionally, several epidemiological studies implicated an increased risk of developing breast cancer with a later age of menopause (55 years or older) (13,61,95,97,106). As noted before, high blood estrogen in premenopausal and postmenopausal women may increase a female's chance of developing breast cancer; thus, suggesting that a later age of menopause due to higher estrogen levels and exposure may be the reason why there is an increased risk of breast cancer formation with a late age of menopause (7,56).

1.4.3 Oral Contraceptive Use & Hormone Replacement Therapies

Since the 1930's exogenous hormones as drugs were used to help in alleviating postmenopausal symptoms and by the 1950s the first hormonal birth control pill (oral contraceptive) was introduced to the public (95). Experimental studies implicated the role of the

exogenous sex hormones on the stimulation of breast tumor stem cells along with their role in endocrine disruption (leading to carcinogenesis) (107). In several epidemiological studies, the use of oral contraceptives (OC) during a women's reproductive years increased her risk of developing breast cancer (6,95,108,109). The pooling of data from 54 epidemiological studies, noted a 7% increase in the relative risk of breast cancer in women who had ever used OC compared to women who had never used OC's (110). Moreover, a meta-analysis of 15 studies found a positive correlation between the use of oral contraceptives and an increase in the risk of developing breast cancer (111). Another meta-analysis taking into account 10 studies, found that there was a significant linear relationship between the age of a women's first oral contraceptive use and breast cancer risk; hence, adding onto studies suggesting the age-dependent effect of estrogen on breast cancer development (109). In Palestine, the use of oral contraceptives has increased over the past years and the most reported use was found in women 30-34 years of age, women living in refugee camps, and women with lower than average economic status (112).

Another form of exogenous hormonal exposure for a female is hormone replacement therapy (HRT). These therapies are used to either manage and balance a women's irregular menstrual cycle mostly for pregnancy or to help promote menopause while lessening menopausal symptoms (113,114). HRT's usually help in the management of hormonal imbalances for estrogen and progesterone (the two key hormones in a woman's reproductive life years). The use of hormone replacement therapy has been significantly associated with breast cancer development in many studies (115). A systematic review and meta-analysis done by Wang and colleagues found that the use of HRT was positively associated with an elevated risk of breast cancer (116). The study also noted that women who received estrogen & progesterone HRT's were at a higher risk of developing ILC and LCIS compared to non-users of HRT'S. Further

studies support the findings of the review and note that prolonged exposure to the issued exogenous hormones during HRT may be the reason for the risk associations observed (56,115).

1.4.4 Breastfeeding History

In addition to the reproductive risk factors for breast cancer discussed, one, in particular, has been given much more attention since the 1920s. Lana-Claypon published a case-control study using around 500 breast cancer patients on the associated risks for breast cancer. Since lactation (back then) was suspected to irritate the breast, Lana came about a rather unexpected observation that lack of breastfeeding was positively associated with an increased risk of developing breast cancer (95). Studies today now support this same association and ever-breastfeeding has been seen to have a protective effect against breast cancer (5,7,61,97,111). A review including 32 epidemiological studies, found that the risk of developing breast cancer was increased by 14% amongst parous women who had never breastfed compared to parous women who had ever breastfed (117). Moreover, an earlier systematic review, (which took 47 studies from 30 different countries), found that the relative risk for breast cancer was lowered by 4.3% for every 12 months of breastfeeding (118). Also, it noted that the relative risk of having breast cancer was reduced by 7% when breastfeeding for each birth independently amongst parous women. In many experimental studies, it was suggested that the protective effect of breastfeeding towards breast cancer is due to the reduced lifetime exposure to the hormone estrogen during lactation (119,120). In Palestine, breastfeeding is considered common practice with 96.7% of infants (from 0-6 months) breastfed according to the 2009 PCBS- child statistics series (121).

1.4.5 Additional Risk Factors of Breast Cancer

The true understanding of breast cancer development and increased risk of entails multiple factors. These risk factors can be categorized into environmental, genomic, socioeconomic, behavioral, reproductive, and even some diseases acting as a risk for breast cancer development (6,122). Some risk factors are modifiable and can be addressed by the individual. A few modifiable behavioral risk factors, which have been strongly associated to increase the risk of breast cancer are high alcohol consumption, positive smoking status, fatty diets, and lack of exercise (6). Behavioral risks have heavily been studied with the rise of non-communicable diseases and for breast cancer, many studies indicate a dense relationship between the two. Several studies linked tobacco smoking to a higher risk of breast cancer development (123). One study furthered the analysis by linking tobacco smoking behavior to the formation of the less aggressive breast cancers (124). Moreover, a review done on obesity and breast cancer showed that higher BMI was linked to a higher risk of cancer development (125). Additionally, in a study it was noted that African-American women were more likely to be obese in comparison to non-Hispanic whites and more likely to develop aggressive breast cancers; hence, indicating genetic variations, racial disparities, and unique lifestyle interactions with the onset of aggressive breast cancers (126). These factors are of significant value for the public health sphere due to their modifiability and role in prevention strategies for populations (2,127).

Additionally, many studies have made associations between patients previously having diabetes Mellitus and forming breast cancer. A meta-analysis found that there was indeed an increased risk of developing breast cancer if having diabetes (type 2 diabetes/and gestational diabetes) (128). Hence, supporting other studies which suggested diabetes to be an independent risk factor for breast cancer (129,130). Similarly, an increased risk was noted in many studies

with other diseases such as hypertension (131). Furthermore, studies have made a positive association with a family history of breast cancer and cancer development (31,98,106). Also, other studies have linked different family cancer histories to breast cancer. A prospective cohort done on African-American women found that breast cancer risk was associated with a family history of leukemia and colon cancer (132). Moreover, other studies also found an increased risk for developing breast cancer amongst postmenopausal women with a first-degree relative who had been diagnosed with prostate cancer (133,134).

1.5 Molecular Subtypes of Breast Cancer

Looking into breast oncological treatments, physicians and health care providers formulate treatment plans based on stage, grade, and receptor status. Receptor status refers to the molecular nature of the cell and whether a cancer cell is positive or negative for interacting with a certain hormone or protein through these receptors on the cell's surface (62). If the cancerous cells are considered positive for a certain receptor, more targeted treatment therapies (with better patient responses) can be issued to the patient (135).

In breast cancer, three main receptors are tested for positivity in the cancer cells ER, PR, and Her2 receptors (65,136). Naturally, the ER receptor is a protein that binds to the hormone estrogen. Once bonded, a cascade of signals is sent through the cell, eventually influencing cell growth and proliferation. Similarly, the PR receptor binds to the hormone PR and when bonded promotes cell growth. The Her2 receptor binds to the protein Her2 and helps the cell repair, grow, and proliferate (64). Normally, these hormone receptors make way for breast development and breast changes which naturally occur during puberty, pregnancy, lactation, and menopause. However, cancerous breast cells may also rely on these receptors to promote cell growth and in

most cancers, there may be overexpression of the genes coding for these receptors. Furthermore, if cancerous cells test positive for such receptors oncologists can target these cancer cells with drugs that block the activation of the receptors; thus, creating a means to slow the growth and proliferation of cancer, (making it more manageable in treating) (78,135). The more manageable the cancer is in treatment, the better the prognosis of the patient and the overall survival.

Moreover, for breast cancer, another protein is tested for to understand the rate of cell growth. Ki67 is a naturally occurring protein that is usually found in high concentrations right when a cell is ready to divide into new cells (137). When cancer cells test positive for this protein, it indicates that the cancerous cells are growing at a rapid rate. This protein, however, is not as easily targeted in cancer treatment compared to the receptors; therefore, breast cancer patients with a high percentage of Ki67 are found to have a poorer prognosis than patients with none or a lower percentage of this protein (138).

Additionally, in Palestine, the receptor status and the Ki67 protein are tested for by the use of immunohistochemistry (41). IHC tests for the receptors/proteins by obtaining a percentage or score of cells amongst the cancerous cells that express them. Studies have described a good prognosis of ER/PR receptor status being at more than or equal to 15%. However, other studies argue that the treatment targeted for these receptors shows good results even with an ER/PR receptor status found to be at lower than 15% (64,139,140). Hence, in many studies, a positive IHC staining for ER/PR receptors is considered at any percentage above the 0% mark.

Furthermore, a HER2 positive receptor status is based on a score from 0 to +3. If the score is from 0 to +1 that means the HER2 status is negative, +2 means its borderline, and if the score is +3 that means the status is positive (141). In one study it was noted that breast cancer patients with a positive HER2 and ER/PR status had a greater resistance to treatment in comparison to the

patients with only ER/PR positivity; thus, suggesting an association between cancer resistivity to targeted therapies and the HER2 receptors (142). Moreover, low Ki67 may be indicated by a percentage of less than 10% and high Ki67 is considered more than or equal to 15%, several studies suggest a cut-off mark of 15% (143). Studies also noted that Ki67 of more than 10% has been seen to be associated with higher-grade cancers and poorly differentiated cancerous cells (138,143).

The receptor's status and Ki67 protein concentration are crucial in overall treatment planning for breast cancer. Moreover, cancers are classified according to these molecular factors as molecular subtypes. There are four distinct subtypes for breast cancers: luminal A, luminal B, Her2-enriched, and triple-negative.

1.5.1 Subtype Classifications & Characteristics

Luminal A breast cancer is generally hormone (estrogen/progesterone) receptor-positive and is negative with HER2 (score of 0/+1) and Ki-67 proteins (<15%) which are generally seen to promote cancer growth (21,144). Luminal B breast cancer is also (estrogen/progesterone) hormone-receptor-positive; however, it can test positive for HER2 proteins and has a high amount of Ki-67 protein ($\geq 15\%$) (145). HER2-enriched breast cancer is hormone receptor-negative for both estrogen and progesterone and has relatively high amounts of the HER2 protein making it more aggressive than the Luminal cancers (21). Triple Negative breast cancer tests negative for hormone receptor status and HER2 proteins, making it the hardest from all the four subtypes in terms of identifying targeted treatment plans (146).

Luminal A cancers have been seen to be associated more with lower grade cancers in comparison to the high grade considered subtypes HER2 & TNBC (78). Many studies have

noted better 5-year survival rates of both IDC and ILC luminal A breast cancers when compared to the other subtypes (21,23,84,147). Moreover, one study extracted data from over 29,000 breast cancer cases (from Canada) and found that luminal A was the most commonly diagnosed breast cancer (33). Furthermore, they found that luminal A patients had the greatest survival rates when compared to the other subtypes. Thus, agreeing with multiple population-based studies done in the developed world on the most common subtype with the best prognosis (22,81,147). Luminal B cancers have also been seen in many studies to show a greater prognosis than HER2 & TNBC's (81,147). However, when compared to luminal A, luminal B cancers have the worst prognosis and lower survival rates. Additionally, HER2-enriched cancers in many studies/reviews have been associated with overall poorer prognosis and lower survival rates in comparison to the luminal subtypes of breast cancer (22,33,142). While TNBC has been seen to have the worst prognosis and lowest survival rates amongst the other subtypes (23,94,148). In several studies, it was also seen that TNBC and HER2-enriched cancers were usually diagnosed at a younger age, higher grade, and later stage compared to the luminal subtypes A & B (147,149,150).

Table 1 The Classifications of the Molecular Subtypes of Breast Cancer

Subtype	Estrogen Receptor Status	Progesterone Receptor Status	HER2 Receptor Status	Ki-67 Status
Luminal A*	+	+/-	-	<15%
Luminal B*	+	+/-	+/-	≥15%
HER2-Enriched	-	-	+	Usually ≥15%**
Triple Negative	-	-	-	Usually ≥15%**

* Estrogen Receptor Positive and/or Progesterone Receptor Positive

** Could be any percentage of Ki-67

1.5.2 Heterogeneity of Risk Factors and Subtypes

The concept of exploring risk associated with more aggressive breast cancers maintains in overall important value in targeting certain risk (through interventions), attributed to poorer survival rates and prognosis. Several studies have found heterogeneity when addressing common known reproductive risk factors of breast cancers and molecular subtypes (11,25,151). A cross-sectional study done in Turkey by Fatma P. Turkoz and colleagues, taking a sample of 1184 breast cancer cases, found that nulliparity and age at first full-term pregnancy (30 years or older) increased the risk for developing luminal A/B cancers (OR 1.48 and 1.25 respectively) (24). Agreeing with other studies on the increased risk of luminal subtypes from nulliparity and late age of first full-term pregnancy (11,152,153). However, several studies found that risk from these factors was the same for each molecular subtype (139,154).

Moreover, Turkoz and colleagues found that ever breastfeeding had a protective effect for only luminal cancers when compared to the non-luminal. Nevertheless, this same study found no difference in risk between non-luminal and luminal breast cancers when it came to other reproductive risk factors such as late age of menopause, ever smoking, history of IVF, family history of cancer, and early age of menarche. Similarly, the same conclusion was made in other studies done (139,154). However, a few studies found differences in time spent breastfeeding and the protectivity against subtypes. In Northern China, a case-control study found that breastfeeding for more than 12 months lowered the risk of luminal B and triple-negative breast cancers only (155). On another note, one study found that ever having children was associated more with luminal cancers amongst the African American community and that breastfeeding reduced the risk of Her2 enriched and triple-negative cancers (102). It was also noted that African American women were more likely to be parous but less likely to breastfeed. Hence,

suggesting that breastfeeding could help reduce the risk of the more aggressive subtypes of breast cancer amongst this subpopulation.

Additionally, Turkoz noted that postmenopausal women who took HRT for more than 5 years were at increased risk for developing HER2-enriched and Luminal A cancers (24). Also, the study found an increased risk of patients who were overweight and obese with forming triple-negative breast cancers, disagreeing with a prospective cohort study that found obesity to increase the risk of hormonal breast cancers (156). Furthermore, a pooled cohort that included more than 11,500 cases in developed countries found that parity was associated with an increased risk of triple-negative breast cancer and a lowered risk of luminal cancers (157). Hence, supporting many studies on etiological heterogeneity and breast cancer formation and furthering suggestions on population-based heterogeneity of reproductive risks and molecular subtypes (11,158). This is suggested to be due to different interactions of all risk factors with dependence on the population's environment and genetics.

More importantly, a highly aggressive form of this cancer (Triple Negative) was seen as more abundant amongst social communities with lower social-economic statuses (27,28). One study done in the United States found that although Luminal A breast cancer was the most abundant form found in the US population, Triple-negative breast cancer was found as the top diagnosed breast cancer amongst the young female African- American communities (159,160). Further studies supported these associations (150,161,162). The same was also seen with more aggressive breast cancers, poorer prognosis, and lower survival rates amongst other social communities such as Latinas/ Hispanics compared to non-Hispanic whites (161,163). Furthermore, studies indicate that such differences amongst social/racial communities may be due to factors such as lack of health insurance, lack of health care access, disadvantaged

neighborhoods, crowded living, low income, and lower educational status (27,28). A study done in Israel, taking patient cases from 2002-2007, found that when compared to Jewish women Arab women were more likely to be diagnosed at a younger age, later stage, and were found to have more aggressive breast cancers associated with increased Her2 expression in the Arab women (164). Hence, indicating not only genetic variations amongst sub-populations but also, suggesting health disparities amongst such social communities

Breast cancer is a heterogenous disease varying amongst women in its constructive nature due to genetic variants and certain environmental influences. These same variations and influences affect the molecular nature of the breast cancer; hence, leading to population heterogeneity of breast cancer subtypes and overall survival outcomes dependent on subtype aggressiveness. In the Palestinian context, certain unique stressors (environmental/hormonal) and influences during puberty and other phases of “windows of susceptibility” for females may allow for an increased risk of breast cancer. Moreover, this same unique susceptibility interacting with certain risk factors may be associated with the more aggressive forms of breast cancer. This study aims to give a smaller piece to a larger picture in understanding risk factors associated with the molecular subtypes of breast cancer in Palestinian women, by focusing on common known reproductive risk factors, (along with BMI, smoking status, familial history, & medical history) and looking for the associations between the factors and each molecular subtype. Hopefully, the findings of this study will be used to target reproductive risk factors that are associated more with the aggressive molecular subtypes of breast cancer. By doing so it will give additional information needed to better help in the prevention of hormonal/non-hormonal breast cancers and generate a means to fill in the knowledge of risk factors for each subtype-specific to the West Bank female population.

Chapter 2: Methodology

2.1 Research Type and Design

This study is a cross-sectional study conducted in hospital settings in the West Bank. A cross-sectional study design was most suitable to answer the proposed research objectives because it can find measures of associations (165). Moreover, a quantitative approach was best in addressing the research objectives since this study aims to compare associations between reproductive risk factors and subtypes; hence, a sufficient sample size is needed and quantitative studies allow us to build on size while creating hypothesized relations based on statistical results. Furthermore, this study's main goal is to distinguish between positive associations of certain reproductive risk factors between aggressive molecular subtypes and less aggressive subtypes in this current point in time, making this design most appropriate.

2.2 Study Population and Sample Selection

The theoretic population for this study were female Palestinians with breast cancer. However, the target population was pinpointed to female Palestinians, (over 18 years of age), who were currently diagnosed with a known breast cancer type during the time of the study (carcinoma-in-situ included) and were residents of the West Bank. Calculated needed sample size (at 95% confidence) was roughly 350 participants. This was calculated by using the prevalence of female breast cancer patients in Palestine (0.25%) and using this to derive an estimated population size of this specific group to the West bank (considering a small sample population) (166). In order to increase robustness of the study and account for possible missing information a total of 403 participants were recruited, with a response rate of 99.8% for a total of 402 cases used in analysis.

Recruitment of participants into the study was conducted in three public hospitals found in the West Bank, Al-Hussein Governmental Hospital (located in Beit-Jala), the Palestinian Medical Complex (Ramallah), and Al-Watani Medical Hospital (in Nabulus). It is important to note that these three hospitals do cover a sizable portion of breast cancer patients in the West Bank. Overall, there are five major hospitals in the West Bank which provide cancer care, and amongst them three are governmental. The hospital in Beit Jala includes cancer patients from almost every governorate in the West Bank and has a 97% occupancy rate (including all departments), and serves almost 230,000 citizens (167,168). Al-Watani hospital (Nabulus) receives almost 300 cancer cases monthly primarily including lung cancer in males and breast cancers in females, serving almost 500,000 citizens (168,169). The Palestinian Medical Complex (PMC) recently opened an oncology department in late 2020, which provides care to people from the Ramallah and Al-Bireh governorates. These three hospitals are crucial oncology centers in the West Bank and allow for a better representative sample of breast cancer patients.

In terms of inclusion criteria, participants must have sought or were currently seeking treatment in the hospitals (treatment may be surgery or other therapies). For the proper validation of data, women who were currently diagnosed or were seeking care with and for breast cancer were considered for recruitment. The reason for this was because, in all three hospitals, the electronic health records system (Avicenna) was implemented back in 2012 and 2015; hence, not all clinical information was accessible in medical records found before 2012/2015.

For all hospitals, permission was obtained from the Ministry of Health (*see Appendix C*) and the head of oncology in each hospital's oncology department for access to their breast cancer patient's Electronic health records (EHR's) and the patients themselves. Potential participants were identified using the EHR's and the oncologists directed the principal investigator to the

patients to complete the mini interviews. After obtaining permission from the patient (verbal consent), the patients were asked several questions about the reproductive risk factors mentioned (*see Questions form in Appendix A1*), and the rest of the information was taken from the patient's health records (see EHR's form in Appendix A2).

All data was collected by the principal investigator in the presence of the medical oncologists assigned or other health care workers assigned by the oncologist (Al-Watani-Dr. Mahmoud Nassora/ Beit Jala-Dr. Ahmad Kara'a/ PMC-Dr Mohammed Manasra) to supervise as per MOH protocol. Dr. Mahmoud Nassora is an attending medical oncologist at Al-Watani and runs the Oncology clinics for the hospital. His main role is to manage patient cases in Oncology including outpatients and inpatients. Dr. Ahmad Kara'a is an attending oncologist and the head of the Oncology department at Al-Hussein hospital; moreover, he manages patient oncology cases (outpatient & inpatient) and supervises other oncologists at the hospital. During the time at the Al-Hussein hospital, the majority of the patients' records were seen under the supervision of Dr. Suhar Qatana who was assigned by Dr. Ahmad Kara'a. Dr. Moahmmmed Manasra is in charge of the new oncology department of the PMC and oversees the treatments being provided to most cancer patients at the hospital. During the time at PMC, all patient EHR's were seen under the supervision of the head nurse in oncology assigned by Dr. Mohammed Manasra. The medical oncologists were assigned by the hospital's administration to help with and oversee data collection. They were responsible for providing access to patient files and in assuring that the data collected was in line with MOH guidelines.

2.3 Subject Criteria

<u>INCLUSION CRITERIA</u>	<u>EXCLUSION CRITERIA</u>
- Female	- Male with Breast Cancer
- <i>All Women 18 Years and Older</i>	- <i>Younger than 18 years</i>
- Is a patient of the assigned medical oncologist in each hospital	- Is not a patient for the assigned medical oncologist in each hospital
<ul style="list-style-type: none"> - Currently Seeking Treatment (Surgery/Therapies) in the Participating Hospitals - Diagnosed with Breast Cancer of any type (In situ included) 	- Is not Diagnosed with Breast Cancer at All
- <i>Mentally able -has the ability to fully comprehend, reason, recognize, and Understand the Study procedure/purpose and can Give Verbal Consent on their Own</i>	- <i>Mentally Unable- does not have the ability to fully comprehend, reason, recognize, and Understand the Study procedure/purpose and is unable to Give Verbal Consent on their Own</i>
<ul style="list-style-type: none"> - Known Receptor Status on Pre-op Biopsy - Or Known Receptor Status on Pathological Specimen 	- Do Not Have Data on Known Receptor Status on Pre-op Biopsy and or Do Not Have data on known Receptor Status for Pathological Specimen
- Willing to Give Verbal Consent Before Participation	- Unwilling to Give Verbal Consent Before Participation

2.4 Data Collection

2.4.1 Identifying Participants

The identification of potential participants who met the inclusion criteria for recruitment was assessed using the proposed hospitals Electronic Health Records prior to their oncology visits on the same day. It is important to note that all female patients 18 years of age or older (regardless of how old) were considered for the study, as long as they fulfilled the other aspects of the inclusion criteria. Participant recruitment took into consideration all women groups regardless of factors such as age, marital status, parity, and menopausal status (as long as they meet all inclusion criteria).

The identification of participants was done under the supervision of each assigned medical oncologist or the healthcare worker assigned by the head oncologists. They made sure any patient identifiers were coded and only the information on the EHR forms was taken from the records. The current breast cancer patients who were seeking treatment at the time of the study in the hospitals (mentioned above) were viewed and the electronic health records form (made specific to this study *see Appendix A2*) was filled out by the investigator. Any recognizable patient identifiers were coded by the investigator and the EHR forms were kept securely and disposed of once data was fully inputted for analysis.

2.4.2 Data Collected by EHR's

Data extraction from the records was done by accessing history reports, pathological reports, clinical notes, and medical report forms. Again, this was done under the supervision of the assigned health care worker and each patient was given a coded ID only identifiable to the investigator. The EHR data questions were derived from several studies addressing breast cancer subtypes, treatments and outcomes, including “Global-Surg3” data collection forms

(12,33,81,170). Questions were removed and added to better match the data needed for analysis. All EHR paper forms, (filled out by the investigator), were kept securely and at the responsibility of the investigator. Forms were then shredded and disposed of appropriately after all data was inputted for analysis.

2.4.3 Data Collected from Participants

In order to complete the full reproductive risk profiles of each patient, missing data from the health records were retrieved from the patients themselves. Therefore, patients were asked several questions during their scheduled visits to the hospitals (*see Appendix A1*). This questionnaire tool was derived from the “Gail Model risk assessment tool” ,(taken from the National Cancer Institute), used to identify females at risk for breast cancer development (171,172). This tool has been used by many physicians in clinics to better understand a women’s risk for breast cancer and has been seen to show validated predications (173).

The assigned medical oncologist asked the patients beforehand if they were willing to answer a few questions and briefly explained the study to them. The oncologist was sure to ask the patients after they had completed their clinical follow ups with the doctor. It was noted to the patients that **participation in the study would not affect in any way the care being provided to them at the hospital**. When a verbal consent was given to the oncologist, the oncologist then allowed the investigator to further ask for consent from the patient. When a patient informed the oncologist that they did not wish to participate in the study, the investigator did not contact them after their clinical visits. Permission was requested at the end of the visit in order to reduce any potential sense of obligation or worries about access to care. Only one patient refused to participate in the study and made note of it to the oncologist; therefore, the principal investigator did not approach her after her clinical visit.

Once permission was given to the investigator by the oncologist, the investigator approached the patient after their clinical visit or before their chemotherapy treatment and then proceeded to ask once more for verbal consent. **Please see verbal consent script in Appendix A3.** It was made sure that the participants fully understood the research, meaning that the patient was able to comprehend the purpose and procedure of the research. If the patient showed full comprehension of the study and if verbal consent was given by the patient to the investigator, the participant was then taken to an empty room (close to the clinics) and several questions were asked by the principal investigator to the patient directly. The interviews were short and did not take more than 10 minutes. At times if there were no available rooms, then the patient would be asked the questions in the waiting room area or in the day care center where they waited for treatment.

2.4.4 Safety Protocol & Restrictions due to Covid-19

Since participants were breast cancer patients it was crucial that certain safety measures were implemented, taking into consideration the current concern for *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) and other infectious diseases. The principal investigator therefore, was wearing a mask at all times during the hospital visits and followed MOH recommendations (such as wearing disposable protective shirts/gowns and gloves during visits). Data collection was put on pause during the months of March 2020 to May 2020 in compliance to the student hospital restrictions set by the ministry of health with a total of 50 cases collected before March 2020. Then data collection continued from late May till early July with a total of 80 cases collected. Collection was further put on pause due to the restrictions implemented from August till early September. Afterwards, approval was obtained from the MOH to further

continue data collection. Data collection was then carried out from mid-September till late March 2021 with a total of 272 cases collected.

2.5 Ethical Considerations

Approval from the Ministry of Health was obtained to access the medical health records of the breast cancer patients and verbal approval from the stated hospital's oncology departments was also obtained for clinical visits. Approval from the MOH was obtained twice. Once, at the start of the study and after “covid-19” restrictions were placed by the Ministry. Along with this, ethical approval was obtained by the Institute of Community and Public Health Ethics Committee Board at Birzeit University (*see appendix A4*). All information taken from the health records was held confidentially. For example, any written or digital patient identifiers was coded and all information taken from both the EHR's and the patient was kept under a coded label only identifiable to the researcher. The patient themselves were identified for the mini-interviews by the oncologist and not by any written patient identifiers. Since some questions were asked to the patients, verbal informed consent was obtained from the patients after they were told of the study by their medical oncologist.

The participants were also notified that they can leave the study even after confirmation and that they are free to answer or not answer the questions asked. The participants were also asked if they had any concerns or questions regarding the study before giving verbal consent. It was made sure that the participants understand fully what was to be done and **that if they did not wish to participate no interferences would be made with their care at that specific hospital**. Moreover, it was noted that the information taken from them was to be kept confidential and any identifiable information would not be issued in any dissertations or publications. Data collected from the health records and the patients was directly coded (de-

identifiable information) and entered into a data file; therefore, any forms collected from the investigator were disposed of properly after completion of analysis. Moreover, this study aims to ensure the quality of the overall data with respect to the confidentiality and anonymity of the respondents.

2.6 Study Measures & Variables

2.6.1 Dependent Variables

The dependent variables in this study were the breast cancer molecular subtypes which were categorized into luminal A, luminal B, HER2-Enriched, and triple negative. All 402 participants were grouped into one of these categories; hence, all participants had known receptor status and some ki-67 levels to classify them into such. Patients with a positive ER or PR receptor status with low levels of KI-67 were grouped into the luminal A category. Participants with a positive ER, PR, and or HER2 status with high levels of ki-67 (more than or equal to 15%) were placed into the luminal B category. Moreover, patients with a negative ER/PR status and a positive HER2 status were grouped into the HER-Enriched category (regardless of ki-67 levels). Lastly, participants with negative ER/PR/ & HER2 status were placed into the triple negative category (regardless of ki-67 levels).

2.6.2 Independent Variables

The independent variables of this study were mainly the known reproductive risk factors: age of menarche, age of menopause, nulliparity, age of first pregnancy, breastfeeding history, oral contraceptive use, in-vitro fertilization, and estrogen or hormone therapy use. Age of menarche was taken as a continuous variable; however, was then grouped into two categories of less than or equal to 12 years and more than 12 years. The same was done with age of menopause with one category being less than 55 years of age and the other being more than 55 years of age. The

conversion of the continuous variables into grouped categorical variables was further done with age of diagnosis, BMI, age of first pregnancy, and oral contraceptive duration.

Medical risk factors were also amongst the independent variables of this study with a focus on comorbidities diagnosed before the breast cancer diagnosis: type II diabetes, hypertension, and cardiovascular diseases. These variables were grouped into yes or no categories with “yes” meaning that the patient was diagnosed with one of these disease types prior to their diagnosis of breast cancer. Furthermore, tumor characteristics such as tumor size, stage of cancer, cancer grade, clinical N status, and breast cancer type were independent variables used to further characterize the subtypes. Lastly, family history of cancer was also accounted for with a “yes” meaning a positive family history of any cancer for 1st and or 2nd degree relatives.

2.7 Statistical Analysis

The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0. Differences arising amongst the molecular subtypes with respect to the known common reproductive breast cancer risk factors were assessed by using Pearson Chi-square analysis for categorical variables and a One-Way Analysis of Variance (for continuous variables). This was done after means with standard deviations and frequencies (for categorical variables) were accounted for. Furthermore, the subtypes were divided into four major groups (Triple Negative, HER2-Enriched, Luminal A, and Luminal B). Hence, a multinomial regression analysis was done for selective variables after differences were accounted for using one of the bivariate tests listed above. Results for this analysis were recorded as an odds ratio with one of the categories for the independent variables acting as a reference. For the dependent variables luminal A was used as a reference category for luminal B, HER2-Enriched, and triple negative

subtypes to obtain an odds ratio. For luminal A, luminal B was used as the reference category. All appropriate analyses were carried out with a two-sided level of **0.05** (*p value*<0.05) and an overall 95% confidence interval.

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Chapter 3: Results

3.1 Baseline Characteristics

Amongst the participants, a total of 45% (n=181) had luminal B, 32.6% (n=131) had luminal A, 14.9% (n=60) had HER2-Enriched cancer, and 7.5% (n=30) had triple negative breast cancer. From all the participants (N=402), 75.1% (n=302) had a positive ER receptor status, 66.7% (n=268) had a positive PR receptor status, and 36.3% (n=146) had a positive HER2 receptor status. Moreover, 63.9% (n=257) had both an ER/PR positive receptor status and 15.9% of the participants had a triple positive receptor status (n=64).

Table 2 Basic demographics of all participants.

Basic Demographics		n	%
Age N=402	<40 years	92	22.9
	≥40 years	310	77.1
Governorates N=402	Northern Governorates	123	30.6
	Southern & Central Governorates	279	69.4
Hospital N=402	Al-Hussan Hospital	252	62.7
	Al-Watani Hospital	124	30.8
	Palestinian Medical Complex	26	6.5
Marital Status N=402	Single	41	10.2
	Married	317	78.9
	Widowed	31	7.7
	Divorced	13	3.2
Smoking Status N=402	Former Smoker	18	4.5
	Current Smoker	64	15.9
	Never Smoker	320	79.6
Family History N=402	No	147	36.6
	Yes	255	63.4

Ki-67 status was missing for 24 participants; however, from 378 patients 56% had a positive Ki-67 status ($\geq 15\%$) and 38.1% had a negative Ki-67 status ($< 15\%$). The majority of patients (35.8%) were diagnosed with stage II cancer at the time of their first diagnosis and 32% were first diagnosed with stage III breast cancer. From 358 participants, 48% had grade III breast cancer, 34.1% had grade II breast cancer, and only 1.0% had grade I breast cancer.

Table 3 Baseline characteristics of cancer for all participants of the study

Characteristics		n	%
Breast Cancer Type <i>N=402</i>	IDC	361	89.8
	ILC	30	7.5
	In Situ/Others	11	2.7
Cancer Grade <i>N=358</i>	I	4	1.0
	II	137	34.1
	III	193	48.0
	Other*	24	6.0
Tumor Size <i>N=396</i>	≤ 2 cm	116	29.3
	> 2 cm	280	70.7
Stage of Cancer <i>N=397</i>	I	99	24.9
	II	144	36.3
	III	132	33.2
	IV	22	5.5
Lymph Nodes Status <i>N=395</i>	No LN metastasis	107	27.1
	Movable Involved Axil LN	154	39.0
	Fixed Involved Axil LN	106	26.8
	Cancer in Internal M LN	28	7.1
Ki-67 Status <i>N=378</i>	$< 15\%$ (-)	153	40.5
	$\geq 15\%$ (+)	225	59.5

* Cases that were between different grades such as II/III and I/II and did not have a fixed grade.

Table 4 Basic descriptive of Reproductive Risk Factors for all participants.

Reproductive Factor		n	%
Age of Menarche N=401	≤12	105	26.2
	>12	296	73.8
Menopausal Status N=402	Premenopausal	259	64.4
	Postmenopausal	143	35.6
Age of Menopause N=143	<55	122	85.3
	≥55	21	14.7
Age at 1st Full Term Pregnancy N=338	<30	314	92.9
	≥30	24	7.1
Parity N=402	Nulliparous	64	15.9
	Low Parity	139	34.6
	High Parity	199	49.5
Breast Feeding History N=402	No	78	19.4
	Yes	324	80.6
HRT History N=402	No	316	78.6
	Yes	86	21.4
IVF History N=402	No	370	92.0
	Yes	32	8.0

3.2 Descriptive Statistics & Bivariate Analysis

A statistically significant difference, (by use of Pearson Chi-Square), was found amongst the subtype groups ($p < 0.05$) and the breast cancer type, grade, and lymph node status (clinical N). There was no statistical difference found amongst the subtypes for tumor size (≤ 2 cm or > 2 cm) nor for the stage of cancer. A summary of the bivariate analyses for the cancer characteristics and subtypes may be seen in table 3.

3.2.1 Age

The mean age of the participants with the luminal A subtype was 50.77 ± 11.53 , luminal B was 47.42 ± 11.16 , HER2-Enriched 46.05 ± 10.83 , and triple negative was 47.20 ± 11.67 ($p = 0.019$). Hence, to further investigate a possible association between age at diagnosis and breast cancer subtypes, age was divided into two groups (< 40 years and ≥ 40 years old).

3.2.2 Age of Menarche & Age of Menopause

The mean age of menarche was 13.46 ± 1.42 with a total of 26.2% of patients having an early age of menarche (≤ 12 years). The majority of patients were considered premenopausal and 35.6% ($n = 143$) were postmenopausal. Amongst the postmenopausal women 85.3% reached menopause before 55 years of age and 5.2% had a late age of menopause (≥ 55 years old). There were no statistically significant differences between subtypes and early age of menarche nor late age of menopause.

Table 5 Baseline characteristics of the molecular subtypes in the sample population.

Characteristics	N	Luminal A		Luminal B		HER2-Enriched		Triple Negative		Total	Test Statistic		
		N	%	n	%	n	%	n	%		χ^2	P-Value	
No. (% of Total)	402	131	32.6	181	45.0	60	14.9	30	7.5	402			
Breast Cancer Type	402												
IDC		115	31.9	159	44.0	58	16.1	29	8.0	361	13.108	0.041	
ILC		15	50.0	15	50.0	0	0.0	0	0.0	30			
In Situ/Others		1	9.1	7	63.6	2	18.2	1	9.1	11			
Cancer Grade	358												
I		3	75.0	1	25.0	0	0.0	0	0.0	4	74.029	< 0.001	
II		78	56.9	43	31.4	13	9.5	3	2.2	137			
III		29	15.0	104	53.9	38	19.7	22	11.4	193			
Other		5	20.8	14	58.3	2	8.3	3	12.5	24			
Tumor Size	396												
≤2 cm		42	36.2	50	43.1	20	17.2	4	3.4	116	5.197	0.158	
>2 cm		85	30.4	129	46.1	40	14.3	26	9.3	280			
Stage of Cancer	397												
I		42	42.4	41	41.4	13	13.1	3	3.0	99	11.002	0.276	
II		43	29.9	70	48.6	19	13.2	12	8.3	144			
III		37	28.0	58	43.9	24	18.2	13	9.8	132			
IV		5	22.7	11	50.0	4	18.2	2	9.1	22			
Lymph Nodes Status	395												
No LN metastasis		45	42.1	45	42.1	13	12.1	4	3.7	107	22.116	0.009	
Movable Involved Axil LN		48	31.2	74	48.1	21	13.6	11	7.1	154			
Fixed Involved Axil LN		25	23.6	43	40.6	23	21.7	15	14.2	106			
Cancer in Internal M LN		9	32.1	16	57.1	3	10.7	0	0.0	28			

3.2.3 Age of First Pregnancy, Parity, & Breast-Feeding History

The mean age of first full term pregnancy was 21.92 ± 4.75 with a range from (14-41). Age of first full-term pregnancy was further grouped into two categories of <30 years of age and ≥ 30 years of age. There was no significant difference found between the breast cancer subtypes and age of first full-term pregnancy. The average number of total births was 4.43 ± 3.03 . From the participants, 19.4% ($n=78$) never breastfed and 80.6% ($n=324$) had breastfed with an average duration 51.46 ± 46.32 months. There was no statistically significant difference found between subtypes and ever-breastfeeding nor breastfeeding duration.

Nulliparous women made up 15.9% ($n=64$) of the sample and parous women made up 84.1% ($n=338$). Parous women were further grouped into two categories of high parity (≥ 5 full-term births) and low parity (<5 full-term births). From amongst the parous women 49.5% ($n=199$) were considered to have high parity and 34.6% ($n=139$) to have low parity. There was a statistically significant difference found amongst the molecular subtypes of breast cancer and the parity status of the participants.

3.2.4 Oral Contraceptive Use

Amongst the participants, 41% ($n=165$) of women had a history of ever using oral contraceptives and 59% ($n=237$) reported to never have used oral contraceptives (OC). The mean duration of contraceptive use was 10.54 months. Oral contraceptive duration was further grouped into four categories no/never used, less than 2 years of use, equal to and greater than 2 years of use- 5 years of use, and more than or equal to 5 years of use. One hundred and six patients (26.4%) reported to have used oral contraceptives for less than 2 years, and 25 (6.2%) reported to have used OC for more than or equal to five years. No statically significant difference was found between the subtypes and history of oral contraceptive use.

Table 6 Mean comparisons of several risk factors amongst the molecular subtypes

Variable		Luminal A (n=131)	Luminal B (n=181)	HER2-Enriched (n=60)	Triple Negative (n=30)	Total (n=402)	p Value ^a
Age at Diagnosis		50.77 ± 11.53	47.42 ± 11.16	46.05 ± 10.83	47.20 ± 11.67	48.29 ± 11.37	0.019
Numbered BMI		27.85 ± 4.38	29.21 ± 5.56	28.72 ± 5.19	28.50 ± 4.94	28.64 ± 5.12	0.144
Number of Births		4.34 ± 2.92	4.10 ± 3.21	5.33 ± 2.78	4.93 ± 2.59	4.43 ± 3.03	0.039
Number of Miscarriages		0.85 ± 1.43	0.93 ± 1.40	0.33 ± 0.542	1.07 ± 1.70	0.83 ± 1.36	0.018
		Luminal A	Luminal B	HER2-Enriched	Triple Negative	Total	p Value
Age of Menarche	N=401	13.60 ± 1.42	13.41 ± 1.43	13.55 ± 1.31	13.00 ± 1.60	13.46 ± 1.42	0.190
Age of Menopause	N=143	48.69 ± 6.33	49.19 ± 5.50	50.71 ± 3.97	50.00 ± 4.55	49.25 ± 5.59	0.588
Age of 1 st FT	N=338	21.90 ± 4.87	22.24 ± 4.83	21.27 ± 4.43	21.67 ± 4.57	21.92 ± 4.75	0.618
Pregnancy							

BMI, Body-Mass Index; FT, full-term Pregnancy

^a Mean Comparisons were done using One-way ANOVA

3.2.5 Hormone Replacement Therapy & In Vitro Fertilization

The majority of women 78.6% (n=316) had a history of never using HRT's. While 21.4% (n=86) had a history of using HRT. The mean duration of HRT use was 4.52±15.38 months. There was no significant difference found between HRT use and breast cancer subtypes and there was no significant increased risk found amongst the subtypes. Three hundred and seventy participants (92%) had reported no history of using IVF and 32 (8.0%) had reported a history of IVF use.

Table 7 Differences of risk factors amongst the molecular subtypes of breast cancer by univariate analysis. The table is continued on the next two pages.

Risk Factor	N	Luminal A		Luminal B		HER2-Enriched		Triple Negative		Total	Test Statistic	
		N	%	n	%	n	%	n	%		χ^2	P-Value
Age at Diagnosis	402											
< 40 yrs		21	22.8	44	47.8	19	20.7	8	8.7	92	6.561	0.087
≥ 40 yrs		110	35.5	137	44.2	41	13.2	22	7.1	310		
Age of Menarche	401											
≤12 yrs		28	26.7	53	50.5	14	13.3	10	9.5	105	3.604	0.308
>12 yrs		103	34.8	127	42.9	46	15.5	20	6.8	296		
Menopausal Status	402											
Premenopausal		77	29.6	122	47.1	43	16.6	17	6.6	259	4.684	0.196
Postmenopausal		54	37.8	59	41.3	17	11.9	13	9.1	143		
Age of Menopause	143											
<55 yrs		46	37.7	50	41.0	14	11.5	12	9.8	122	0.642	0.887
≥55 yrs		8	38.1	9	42.9	3	14.3	1	4.8	21		
BMI	402											
Normal/Under Weight		38	38.4	41	41.4	14	14.1	6	6.1	99	4.440	0.617
Overweight		54	34.4	70	44.6	21	13.4	12	7.6	157		

Obese		39	26.7	70	47.9	25	17.1	12	8.2	146		
Oral Contraceptive Use	402											
No/Never Used		74	31.2	114	48.1	36	15.2	13	5.5	237	8.130	0.521
< 2yrs		35	33.0	45	42.5	14	13.2	12	11.3	106		
≥ 2yrs- 5 yrs		15	44.1	11	32.4	6	17.6	2	5.9	34		
≥ 5yrs		7	28.0	11	44.0	4	16.0	3	12.0	25		
Oral Contraceptive Use	402											
No		74	31.2	114	48.1	36	15.2	13	5.5	237	4.596	0.204
Yes		57	34.5	67	40.6	24	14.5	17	10.3	165		
Breast-Feeding	402											
No		27	34.6	41	52.6	6	7.7	4	5.1	78	5.443	0.142
Yes		104	32.1	140	43.2	54	16.7	26	8.0	324		
HRT Use	402											
No		106	33.5	135	42.7	53	16.8	22	7.0	316	6.027	0.110
Yes		25	29.1	46	53.5	7	8.1	8	9.3	86		
IVF History	402											
No		123	33.2	159	43.0	59	15.9	29	7.8	316	9.081	0.028
Yes		8	25.0	22	68.8	1	3.1	1	3.1	86		

Age 1st FT Pregnancy	338											
< 30 yrs		101	32.2	136	43.3	52	16.6	25	8.0	314		
≥ 30 yrs		8	33.3	10	41.7	4	16.7	2	8.3	24	0.027	0.999
Parity	402											
Nulliparous		22	34.3	35	54.7	4	6.2	3	4.7	64		
Low Parity		42	30.2	72	51.8	17	12.2	8	5.8	139	15.011	0.020
High Parity		67	33.7	74	37.2	39	19.6	19	9.5	199		
Type II Diabetes Status	402											
No		118	33.5	156	44.3	53	15.1	25	83.3	352		
Yes		13	26.0	25	50.0	7	17	5	16.7	50	1.600	0.659
Hypertension Status	402											
No		88	30.0	137	46.8	47	16.0	21	7.2	293		
Yes		43	39.4	44	40.4	13	11.9	9	8.3	109	3.909	0.271
Family History of Cancer	402											
No		50	34.0	63	42.9	25	17.0	9	6.1	147		
Yes		81	31.8	118	46.3	35	13.7	21	8.2	255	1.617	0.656

3.2.6 Smoking & Body Mass Index

From amongst the participants, 79.6% reported to be non-smokers at the time of diagnosis, 15.9% were current smokers, and 4.5% were former smokers at the time of diagnosis. There was no significant difference found amongst smoking status and the molecular subtypes of breast cancer.

The mean BMI calculated for all participants was 28.64 ± 5.12 ; hence, the majority of the participants 39.1% (n=157) were overweight. Ninety-nine patients (24.6%) were categorized into a normal and underweight category and 146 women (36.3) were grouped into the obese category (based on calculated BMI). Moreover, amongst the postmenopausal participants, 49% were obese and 38.5% were overweight. There was no statistically significant difference found amongst BMI and the molecular subtypes of breast cancer.

3.2.7 Hypertension, Diabetes, & Family History

Amongst the participants, 27.1% (n=109) had a positive hypertension status prior to diagnosis, 1.7% had a known CVD status prior to diagnosis, and 12.4% had positive type II diabetes status prior to diagnosis. There was no statistically significant difference observed amongst these diseases and the molecular subtypes of breast cancer.

Two hundred and fifty-five participants (63.4%) had a family history of any cancer (1st and 2nd degree relatives). While, 36.6% (n=147) reported to have no family history of any cancer. Out of the participants, 28.8% reported to have a family history of breast cancer, 9.2% reported to have a family history of leukemia, 15.8% reported to have a family history of colon cancer, and 7.9% reported to have a family history of prostate cancer. However, the association of family history of any cancer did not differ significantly across the molecular subtypes of breast cancer.

3.3 Multinomial Regression Analysis

Multinomial regression analysis was conducted to test for associations between risk factors and breast cancer subtypes. The risk factors included in the regression analysis were age of menarche, age at diagnosis, menopausal status, breastfeeding history, oral contraceptive use, history of HRT, history of IVF, and parity status. These variables were included due to the statistical associations found between the variables amongst the subtypes in the bivariate analysis. The multinomial regression was run with Luminal A as the reference category for Luminal B, HER2-Enriched, and triple negative subtypes. Luminal B was used as the reference category for Luminal A (*refer to table 6*).

The younger age at diagnosis of less than 40 years was found to be associated with increased risk of the HER2-Enriched subtypes (OR 2.69 95% CI 1.14-6.34: $p=0.023$) and triple negative subtypes (OR 3.31 95% CI 1.06-10.31: $p=0.039$). Premenopausal status was associated with a decreased risk of having luminal A with reference to Luminal B (OR 0.82 95% CI 0.49-1.37: $p=0.442$) and an increased risk of having HER2-Enriched/Luminal B cancers; however, these associations were found to be insignificant ($p > 0.05$). Moreover, there were no significant increased risk associations found amongst never breast feeders and the subtypes ($p > 0.05$). Low parity was associated with reduced risk of HER2-Enriched breast cancer (OR 0.45 95% CI 0.20-0.98: $p=0.043$) compared to high parity and Luminal A breast cancers. There was no statistically significant increased risk observed with IVF nor HRT use amongst the subtypes.

Table 8 The odds ratios and 95% confidence Intervals for risk factors and breast cancer subtypes. The reference category was luminal A and for Luminal A it was referenced back to Luminal B. The table is continued on the next page.

Risk Factor	Luminal A (n=131)		Luminal B (n=181)		HER2-Enriched (n=60)		Triple Negative (n=30)	
	OR (95% CI) ^a	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Age of Menarche	1.09 (0.93-1.29)	0.271	0.91 (0.78-1.07)	0.271	0.98 (0.77-1.23)	0.841	0.71 (0.53-0.97)	0.029*
Age at Diagnosis								
< 40 yrs	0.73 (0.38-1.39)	0.333	1.38 (0.72-2.63)	0.333	2.69 (1.14-6.34)	0.023*	3.31 (1.06-10.31)	0.039*
≥ 40 yrs	1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)	
Number of Miscarriages	0.94 (0.80-1.12)	0.473	1.07 (0.90-1.27)	0.473	0.54 (0.36-0.81)	0.003*	1.09 (0.82-1.45)	0.568
Menopausal Status								
Premenopausal	0.82 (0.49-1.37)	0.442	1.22 (0.73-2.05)	0.442	1.40 (0.66-2.98)	0.387	0.60 (0.23-1.54)	0.286
Postmenopausal	1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)	
Breast-Feeding								
No	1.22 (0.35-4.26)	0.759	0.82 (0.24-2.88)	0.759	1.09 (0.19-6.29)	0.927	0.93 (0.10-8.87)	0.953
Yes	1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)	
Oral Contraceptive Use								
No	0.78 (0.48-1.26)	0.302	1.29 (0.80-2.09)	0.302	1.27 (0.65-2.46)	0.485	0.65 (0.28-1.50)	0.310
Yes	1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)	
HRT Use no. (%)								
No	1.19 (0.64-2.23)	0.584	0.84 (0.45-1.57)	0.584	1.22 (0.45-3.32)	0.698	0.62 (0.22-1.70)	0.348
Yes	1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)	

OR, odds ratio; CI, Confidence Interval; HRT, Hormone replacement Therapy

^a The OR's and 95% CI were found by comparing luminal B, triple negative, HER2-enriched subtypes to luminal A. The OR's and 95% CI for luminal A was found by comparing luminal A to Luminal B.

IVF History no. (%)								
No	1.81 (0.71-4.62)	0.218	0.55 (0.22-1.42)	0.218	2.53 (0.27-24.07)	0.419	2.10 (0.22-20.01)	0.527
Yes	1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)	
Parity no. (%)								
Nulliparous	0.72 (0.18-2.89)	0.642	1.39 (0.35-5.60)	0.642	0.16 (0.02-1.31)	0.088	0.56 (0.03-6.22)	0.561
Low Parity	0.77 (0.45-1.34)	0.359	1.29 (0.75-2.23)	0.359	0.45 (0.20-0.98)	0.043*	0.55 (0.20-1.48)	0.235
High Parity	1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)		1 (<i>ref</i>)	

OR, odds ratio; CI, Confidence Interval; IVF, In vitro fertilization

^a. The OR's and 95% CI were found by comparing luminal B, triple negative, HER2-enriched subtypes to luminal A. The OR's and 95% CI for luminal A was found by comparing luminal A to Luminal B.

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Chapter 4: Discussion

For this study, we aimed to investigate and compare the associations of common known reproductive risk factors amongst the four molecular subtypes of breast cancer, amidst a sample of 402 female breast cancer patients who sought care in three public hospitals in the West Bank. Moreover, this study looked into characterizing the molecular subtypes based on prognostic factors such as cancer grade. Exploring risk and understanding associations is important in developing stronger methods of prevention in regards to the hormonal and non-hormonal breast cancer subtypes. Likewise, it helps create a direction to further future investigations towards understanding the epidemiology of breast cancer, exploring elements relating to poorer prognoses of breast cancer, and finding a means to better control for such elements in the West Bank. One major finding of this investigation was that the more aggressive subtypes of breast cancer (HER2-Enriched/ Triple Negative) were associated with a younger age at diagnosis compared to the Luminal A subtype. Additionally, the most prevalent breast cancer subtype of our population was Luminal B with the majority of the participants having a high grade, large tumor size ($> 2\text{cm}$), positive lymph node status, and high levels of Ki-67 ($\geq 15\%$). Moreover, lower parity (<5 children) was found to lower the risk of developing the HER2-Enriched subtype compared to higher parity. Hence, indicating that higher parity increases the risk of developing the HER2-Enriched subtype compared to the Luminal A subtype. *(These findings are further discussed in their relevant categories below).*

4.1 Cancer Characteristics

From amongst the sample, Luminal B breast cancer was the most prevalent of the diagnosed subtypes with Luminal A, HER2-Enriched, and triple-negative following respectively. This does not compare to the predominant subtype on an international level in which Luminal A

is the main diagnosed subtype (25,33,144,155,157). One study done in Saudi Arabia had Luminal A as the most prevalent subtype and HER2-Enriched as the least common subtype (174). Another study done in Eastern Morocco also had Luminal A as the most prevalent subtype. One study done in Colombia, found Luminal B breast cancers to be the most prevalent in the population and found that this prevalence was independent of the genetic ancestry of the participants (175). Hence, compared to the majority of studies, the difference in the distribution of subtypes between Luminal A & Luminal B may suggest other factors besides genetics such as social disparities/ environmental risks affecting the epigenetics of the population and contributing to a higher distribution of a more aggressive subtype (compared to Luminal A). Furthermore, having a higher prevalence of the luminal B subtype is indicative of a poorer prognosis for the population since luminal B cancers are more aggressive and have more limitation in treatment strategies compared to Luminal A (18,176,177).

Moreover, amongst the sample, the most frequent histological grade was grade III making up 48% (n=193) of the sample. For the luminal A subtype, grade II was most frequent; however, for luminal B, HER2-Enriched, and the triple-negative subtype, grade III was the most common. This differs from several studies in which the majority of the total sample would be of grade II (22,83,139,142). The grade of cancer is an important prognostic factor since it looks into the morphology of the cancer cells, with grade I cancers indicating better prognosis (slow-growing) compared to the other grades (79). A study done in Indonesia found an association of grade I cancers to the luminal A subtype which is supported by other studies (144,178,179); however, this was not the case in this study. Grade I cancers made up only 1% (n=4) of the total sample with the majority of grade I cancers being from the Luminal A subtype but the majority of the Luminal A cancers were of histological grade II. Also, women who were considered

premenopausal had a high proportion of grade III cancer (64.8%) compared to women who were postmenopausal (35.2%). This supports the relationship between the aggressiveness of the breast cancer subtypes and younger age at diagnosis (25,180). In addition, our results show that patients with cancer grade III were more likely to have a tumor size of more than 2 cms and had a positive lymph node status which supports studies indicating a faster growth of grade III cancers (83,138,150,181). The high grade of cancer amongst our sample may suggest a lack of early detection. Hence, indicating the need to further improve breast cancer screening programs by targeting at risk women and the need to encourage screening for premenopausal women or younger women (less than 40 years of age) in general who are more susceptible to aggressive breast cancer subtypes (81,182).

Based on the results, 70.7% (n=280) of the patients had a tumor size of more than 2 cms. This compares to other studies that also had a larger tumor size in their sample population (24,25,77,84,174). Amongst the subtypes, the majority of the cases within each subtype had a tumor size of more than 2 cm's even amidst the Luminal A subtype. This differs from other studies which associate smaller tumors with the luminal A subtype (22,144,147). A study done by Meng-Ting Chen and colleagues looked into metastatic breast cancer using the SEER's dataset and found that the majority of the sample had a tumor size of more than 2 cm (183). This is expected given that the sample was made up of patients with metastatic breast cancer (since metastasis and tumor size are indicative of one another) (184). However, for our study sample, metastatic cancer (stage IV Cancers) made up 5.5% (n=22) of the participants. The majority of participants were diagnosed with Stage II, (36.3% /n=144), and stage III, (33.2%/ n=132), cancers. Only 24.9% of participants had stage I cancers with the majority being of Luminal A subtype. For stage II, luminal B made up 48.6% and the majority of the HER2-Enriched patients

had stage III cancer. This may be associated with the higher grades of cancer amongst our sample indicating faster-growing cancers that are more aggressive than cancers of lower grades. Moreover, this suggests the need to further work on early detection screening programs which may help detect tumors of smaller sizes and hopefully early-stage cancers.

The majority of cases were diagnosed with invasive ductal carcinoma (IDC) 89.8% of the sample and 7.5% had invasive lobular carcinoma (ILC). This compares to other sample populations in the Middle East in which IDC was the most prevalent followed by ILC (22,97,174,185,186). In situ and other cancers only made up 2.7% of the total cases. This again supports the suggestion of the need to further early detection programs and perhaps the need to bettering the awareness of women of where to go for screening. In our study, ILC cancers were only distributed within the luminal subtypes. Since ILC has been seen to have a higher rate of reoccurrence compared to IDC (75,77), this distribution suggests a need to provide follow-up programs for women who have finished treatment for luminal cancers in regards to future means.

Furthermore, 73% of our study cases had a positive lymph node status with the majority having an N1 (movable axillary lymph node) status (n=154). Within the lymph node metastasis category, the Luminal B subtype had the most N3 cases (57.1%) followed by the Luminal A subtype (23.6%). Compared to the luminal subtypes the majority of the non-luminal subtypes were of N2 status instead of N1 status. This further supports the literature indicating an association between higher lymph node staging and the more aggressive subtypes (24,88,178). However, the distribution of lymph node metastasis within the luminal subtypes again suggests the lack of early detection which may be more likely to occur within these subtypes. This may be due to the smaller tumor sizes found at the start of these subtypes which may affect the self-

awareness of the women in seeking screening. Hence, this emphasizes the importance of supporting screening programs with a women's complete risk profile in mind instead of just age or genetics.

In our study, 75.1% of the cases had a positive ER status, 66.7% had a positive PR status, and 36.3% had a positive HER2 status. Moreover, 15.9% had a triple positive receptor status and 63.9% had an ER/PR positive status. This distribution is similar to other studies in which the ER-positive status makes up the majority of the cases (24,25,33,155,157,187). We observed that the triple and double receptor-positive cases had a majority of N1 status, a histological grade of III, and a tumor size of more than 2 cms. Previous studies have shown that higher tumor grades (grade III) were observed more with either an ER- or PR- status (81,139,148,157); however, our study results show that the majority of positive ER/PR status (Luminal subtypes) also had a higher grade of cancer. From amongst our sample, 59.5% of cases in our study had a positive ki-67 status ($\geq 15\%$) with the majority of premenopausal women also having a positive ki-67 status. A study aimed to understand ki-67 as a prognostic parameter, found that higher ki-67 percentages were associated more in women younger than 30 years of age with triple-negative and the luminal B subtypes (188). In our study ki-67 percentage was categorized as either positive or negative; therefore, percentage level comparisons could not be made. Nonetheless, ki-67 status is indicative of higher grade cancers and poorer prognosis (138,189,190). Thus, indicating the potential aggressiveness of the tumors in our sample population compared to other populations. This may also indicate other factors (such as environmental or socioeconomic factors) interfering with the epigenetics of the population. Likewise, it suggests a lack of early detection; hence, the larger tumor sizes and positive lymph node status even amongst the hormone receptor-positive sample.

4.2 Reproductive Risk Factors & Molecular Subtypes

The prime objective of our study was to further investigate and compare associations of known reproductive risk factors amongst the molecular subtypes of breast cancer. These risk factors include early age of menarche, late age of menopause, use of oral contraceptives, HRT/IVF use, lack of breastfeeding, and nulliparity. In several experimental studies, it has been noted that these risk factors may increase the risk of breast cancer due to the influence the extra estrogen/progesterone hormones have on a female's body especially during her reproductive life years (7,55,191).

4.2.1 Age of Menarche & Age of Menopause

Based on the results obtained, the average age of menarche was about 13 years of age. One study done on Palestinian female university students also had an average of less than 14 years of age for menarche (192). Compared to other studies, this average age of menarche was quite similar to countries such as Turkey, Russia, and Egypt but was more than the average age of menarche for countries such as Mexico, Japan, and the United States (193). There was no significant difference in our study for early menarche and the molecular subtypes. However, reduced risk of developing triple-negative cancers compared to luminal A cancers was observed with an increase in the age of menarche (OR 0.71 95% CI 0.53-0.97: $p=0.029$). This differs from several international studies which noticed an association of early age of menarche to the development of the Luminal cancers (98,106,158).

Furthermore, the majority of cases in our study were premenopausal (64.4% / $n=259$) and 36.6% were postmenopausal ($n=143$). A case-control study conducted in Hong Kong China had a different distribution with the majority of the patients being of postmenopausal status (194).

Several other studies had a higher percentage of postmenopausal women compared to premenopausal women in their samples, especially in the luminal subtypes (13,139). In our study, there was no statistically significant difference found amongst menopausal status and the molecular subtypes. All subtypes, however, did have more premenopausal cases compared to postmenopausal. In comparison to Western countries, this differs. Postmenopausal status in many studies was seen to be associated with an increased risk of developing Luminal A & B breast cancers (11–13,157). This may be due to the younger ages at diagnosis for our study sample compared to older ages at diagnosis for Western countries (195,196).

In our study, the majority of the postmenopausal patients (68.5%) had early menopause (< 45 years of age) and only 5.2% were considered to have a late age of menopause (≥ 55 years of age). Older age of menopause was found to be associated with the Luminal subtypes specifically the estrogen receptor-positive cancers in many studies (11–13,61). On the other hand, a study done in Turkey by Turkoz et.al found no difference in breast cancer subtype risk for a late age of menopause which is similar to other studies (24,158,197). There were no significant differences between the late age of menopause and the molecular subtypes of breast cancer in our study. In our study, the majority of patients were premenopausal and there was no difference in postmenopausal status distribution amongst the subtypes; hence, indicating a shift from studies done in Western countries that found postmenopausal status to be associated more with the luminal subtypes (157,198). This may suggest that there are other factors such as lifestyle factors or environmental factors instead of hormonal factors interfering with the development of the Luminal subtypes of breast cancer that may explain the increased prevalence of luminal B cancers in our sample population.

4.2.2 Age of First Pregnancy, Parity, & Breast-Feeding History

In terms of age at first pregnancy, in our study sample, the average age a woman underwent a full-term pregnancy was about 22 years of age, with the youngest being 14 years of age. The first full-term pregnancy has been hypothesized to have a crucial influence on a woman's mammary tissue restructuring (187,199); hence, an influence on the breast tissue of the women and the reduced risk of developing breast cancer. In a pooled cohort of nine separate studies, it was found that an older age of first full-term pregnancy was associated with an increased risk of developing Luminal A and Luminal B subtypes (157). This also supports other studies done which have found a younger age of first full-term pregnancy (less than 30 years) to be a protective factor for both Luminal subtypes (12,24,103). However, in our study, the results show no variations amongst the subtypes for the association of late age of first full-term pregnancy. This may be due to the majority of the sample undergoing a first full-term pregnancy before 30 years of age. Compared to many of the studies which found associations amongst the age of first full-term pregnancy and the luminal subtypes, our sample population still has an early age of marriage which may contribute to an earlier age of first pregnancy. Hence, there may have not been enough women with a late age of first full-term pregnancy (> 30) to better compare between the subtypes for increased risk of development.

Moreover, the average number of full-term births was about 4.4 which is a bit higher than the current 2020 fertility rate (3.9) in the Occupied Palestinian Territory (oPt), but similar to the 2015 fertility rate (4.5) in the oPt (200). The average obtained from our study is comparable to the average of many developing countries; however, it's greater than developed countries such as the US (201). In our study, parous women made up the majority of the sample (84%), with 49.5% of the parous women having high parity and 34.6% having low parity. The highest

average number of births was found amongst the HER2-Enriched subtype. There was a significant difference amongst the subtypes and parity status, with more than 60% of the non-luminal subtypes having high parity (≥ 5 children). Moreover, low parity reduced the risk of developing the HER2-Enriched subtype compared to high parity (OR 0.45 95% CI 0.20-0.98: $p=0.043$). No statistically significant associations were observed amongst the other subtypes. These results differ from what was expected since the majority of the studies find a reduced risk with an increased number of births and the development of the luminal subtypes. In one meta-analysis including 15 studies, it was found that parity reduced the risk of developing the luminal subtypes of breast cancer by 25% compared to nulliparity (11). Furthermore, a study based on two cohorts (one from Denmark and the other from Norway) found that women who had higher parity had a reduced risk of the overall development of breast cancer than women who had lower parity (202). Likewise, amongst the subtypes, parous women were found to have a reduced risk of developing Luminal A and Luminal B breast cancers (the hormonal subtypes) compared to nulliparous women (13,157). A nested case-control study done using the Norwegian Breast cancer screening program also found that women who had high parity also had a reduced risk of developing the HER2-Enriched subtype (203). Hence, in many studies, parity was found to be a protective factor against luminal breast cancer.

This difference in our results from international studies may be due to the suspected linked biological associations of parity and tumor aggression. A study conducted in northern Israel found that amongst a sample of triple-negative breast cancer patients nulliparous women had better overall survival rates compared to high parous women and that Arab-Palestinian patients within this group had higher mortality rates compared to Jewish Israelis (204). Similarly, another study found that higher parity predicted a poorer breast-cancer-specific survival and that

this association was strongest amongst the luminal subtypes making high parity a poor prognostic factor (205). Thus, high parity may play a role in increasing the aggression of breast tumors even after diagnosis. Furthermore, other risk factors may contribute to the increased risk of developing the HER2-Enriched subtype in high parous women. For example, increased stress or lower socioeconomic status found amongst Palestinian women with high parity may contribute to the increased risk of the more aggressive subtypes of breast cancer (26,28,29,164,206). Such risk factors were not recorded for this study and we cannot draw conclusions on the role of such factors (environmental contamination/social disparities/income status/etc.). Further examination is important for a complete understanding of the etiology of the breast cancer subtypes in the West Bank population especially taking into consideration the increased risk attributes related to living under military occupation.

An established protective factor for the development of breast cancer is ever breastfeeding. Studies have shown that amongst breast cancer subtypes, ever-breastfeeding reduces the risk of the development of Luminal A and Luminal B subtypes (12). Moreover, a meta-analysis study found that history of ever-breast feeding was also protective against the triple-negative subtype (207). Based on our results, 81% of participants had a history of ever-breast feeding, and no significant associations were found amongst the subtypes. This may be due to our sample population having a higher fertility rate compared to other sample populations in different studies; hence, contributing to an increase in ever-breastfeeding amongst our participants and giving a smaller size for comparison between never breastfeeders and ever breastfeeders within the subtypes. However, we may state that from observations many participants noted breastfeeding obstacles such as breast engorgement or mastitis. Several studies have found an association between increased risk of developing breast cancer and these obstacles

(117,208). Further investigation needs to be done to understand the role breastfeeding complications may have on the development of the different molecular subtypes.

4.2.3 Oral Contraceptive Use, Hormone Replacement Therapy, & In Vitro Fertilization

Based on our results, 41% of the participants had a history of ever using oral contraceptives with an average duration of 10 and a half months of use. Prolonged use of oral contraceptives (more than 5 years) has been associated with the increased risk of developing ER/PR positive breast cancer (luminal subtypes) (12,24,108,110,111,203). A case-control study done using the African American Breast cancer epidemiology and risk consortium with over 2,000 cases and over 10,000 controls, found that recent use of oral contraceptives with long durations of use before diagnosis was associated with increased risk of the luminal subtypes along with the triple-negative subtype (209). However, in this study, they found that oral contraceptive use associations were more pronounced amongst overweight/obese women which may contribute to the triple-negative subtype association, since there is increasing evidence on a potential link between obesity and the formation of the triple negative subtype (126). In our study, there were no significant differences amongst ever-users of oral contraceptives and the molecular subtypes of breast cancer nor were there any significant associations with increased risk for a particular subtype. Similarly, a cross-sectional study done in Turkey by Turkoz and colleagues also found no significant associations with increased risk and ever-users of oral contraceptives which was the result in several other studies as well (24,97).

These conflicting results amongst different studies may be due to the length of use for oral contraceptives and the time of use. As stated previously, the use of exogenous hormones such as oral contraceptives during a woman's "window of vulnerability" may interfere with the further development of breast tissue and increase her risk of breast cancer overall. Some studies

have noted that prolonged use of OC's such as 10 years or more has been associated with an increased risk of breast cancer (regardless of subtype) (203,210). Likewise, current use of OC's at the time of diagnosis was associated with increased breast cancer risk. In our study, we did not take into account whether the participants were current users of OC's prior to diagnosis and in our sample, only 6.2% of patients reported to have used OC's for more than 5 years. Therefore, future investigations should look into differentiating current OC users amongst the subtypes to better understand any potential associations.

Furthermore, a total of 21.4% of our sample had a history of HRT use with a mean duration of 4.52 months. There were no significant differences found amongst the subtypes nor between the luminal and non-luminal groups. The use of exogenous hormones especially ovarian hormones such as estrogen and progesterone have been associated with an increased risk of estrogen/progesterone receptor-positive breast cancers such as Luminal A and Luminal B (55,191). However, several studies have shown that prolonged use of HRT (> 5 years) was strongly associated with increased odds of developing Luminal A breast cancer (12,24,56,115,157). Likewise, the history of ever doing IVF has been shown to increase the risk of the Luminal subtypes (especially ER receptor-positive cancers) (12,157).

On the other hand, one study done in Tehran (Iran) found that there were no significant differences between the risk of breast cancer subtypes and the history of HRT and IVF (25). This was similar to results obtained in several different studies (155,197). In our study, 8.0% of the participants had a history of IVF. There was no significant difference amongst the increased risk of the breast cancer subtypes and history of IVF or HRT similar to the study in Iran (and several other studies) (24,97,116,155,197). Interestingly, these associations are considerably different amongst different study populations suggesting a potential role of genetic attributes in the

development of the breast cancer subtypes and exogenous hormone use (IVF/HRT/OC's).

Moreover, these differences may also suggest other risk factors besides genetics contributing to a population's increased risk of the breast cancer subtypes.

4.3 Additional Risk Factors

4.3.1 Age at Diagnosis

In our sample population, the overall average age at diagnosis was 48.29 years with the highest average age being in the luminal A subtype (50.77 years). The youngest aged participant was 22 years old and 22.9% of the total sample were less than 40 years of age. The overall younger age in our sample compares to some studies done in the Middle East and other developing countries (22,97,186,211,212). Although there is a lower incidence rate of breast cancer in Middle Eastern countries compared to the West, most of the studies have shown that Middle Eastern women have a younger age of diagnosis and the majority are premenopausal, similar to our study (22,97,213). For example, a study done in Egypt found 31.8% of their sample to be aged 40-49 and interestingly showed an increase in incidence rates of breast cancer amongst their population with an increased shift towards 50-59 years of age at diagnosis (214).

Moreover, amongst the subtypes, several studies have found older age to be a risk factor for the luminal subtypes and have shown associations between younger age and the more aggressive subtypes of breast cancer (HER2-Enriched/Triple Negative) (160,161,186,211). A case-only study done in China, had an average age at diagnosis being 52.3 years of age and amongst the subtypes, Luminal B cases had the youngest mean age at diagnosis being 49.9 years (139). Meanwhile, a study looking into the Nurses' Health Study (U.S) showed that the average age at diagnosis for all four subtypes was more than 55 years of age with Luminal A having the oldest age at diagnosis (59 years) (215). Furthermore, a cohort study found young age at

diagnosis (< 40 years) to be an independent risk factor for the development of triple-negative breast cancers (216). A study done in North Carolina found that younger age and the more aggressive breast cancer subtypes were associated more with African Americans compared to Non-Hispanic white Americans (217). In our study, young age was found to increase the risk of having HER2-Enriched (OR 2.69 95% CI 1.14-6.34: p=0.023) and triple-negative breast cancer (OR 3.31 95% CI 1.06-10.31: p=0.039). This compares to several studies which have found similar associations between younger age and the more aggressive subtypes of breast cancer (11,157).

Additionally, in several studies younger age was associated more with higher-grade tumors, higher stages of cancer, poor prognosis, and higher mortality rates compared to women over 40 (81,96,218). This may be due to young women not paying much attention to potentially having breast cancer; hence, the associated later stages and more aggressive tumors. Moreover, the younger ages at first full-term pregnancy may also contribute to our findings. In our study, the average age at full-term pregnancy was around 22 years. Studies have found that certain gene expression patterns following pregnancy were mostly attributable to the triple-negative subtype (219). Furthermore, other risk factors may contribute to the formation of the more aggressive subtypes in our sample population such as environmental factors (ex. heavy metal pollutants) especially since the majority of our sample were still in their reproductive life years; hence, the “window of vulnerability” to certain factors on the epigenetics of the female.

4.3.2 Smoking Status & Body Mass Index

Modifiable risk factors such as smoking and high body mass index have been generally associated with an increased risk of breast cancer (123). Moreover, higher BMI's have been associated more with the aggressive subtypes of breast cancer such as the triple-negative subtype

(125,156). A study looking into obesity and survival of breast cancer patients found obesity to influence tumor characteristics such as higher grade and larger tumor sizes; hence, obesity was associated with more aggressive breast cancers and with poorer survival rates (220). Another study done found that African American women were more likely to be obese compared to non-Hispanic whites and were more likely to develop the HER2-Enriched/ triple-negative subtypes (221). In our sample, the mean BMI was around 29 kg/m², meaning the majority of the patients were overweight at the time of diagnosis. Additionally, amongst the postmenopausal women, almost half were obese and amongst premenopausal women, 39.4% were overweight (29.3% obese). A case-control study done with 1256 cases, showed that amongst premenopausal women being obese/overweight increased the risk of developing the luminal subtypes along with the triple-negative subtype. It was also found that amongst postmenopausal women being overweight/obese increased the risk of developing the luminal subtypes only (222). Another study found that higher BMI's (≥ 25 kg/m²) increased the risk of developing breast cancer overall in postmenopausal women compared to normal weight (13). This association is further supported by our findings with the majority of postmenopausal women 87.5% being obese or overweight. It is believed that postmenopausal women have an increased risk of the luminal subtypes when they have an increase in BMI because estradiol concentration, (an estrogen steroid hormone), increases with increasing BMI (13). Therefore, as mentioned previously, more frequent exposure to estradiol has been seen to increase the risk of the luminal subtypes (mainly ER receptor-positive cancers) (55,57,191). However, in our study, amongst the breast cancer subtypes, there was no significant increased risk with higher BMI. Taking into consideration the overall high BMI in our sample, it is important to note that high BMI is associated with more aggression in tumors which may be an attributable factor to the relatively high grades of cancers

amongst our population. The limited number of cases with BMI within the normal range may also affect our ability to detect a statistical association.

Similarly, there was no significant difference found amongst the molecular subtypes and smoking status in our sample. In a study done by Butler and colleagues, ever smoking was found to increase the risk of the luminal subtypes of breast cancer, and once stratified for race it was found that black women had an elevated increase in risk compared to white women (124). Hence, indicating potential genetic variation, racial disparities, and unique lifestyle interactions which may further contribute to the increased risk. Although in our study the majority of participants were never-smokers, it is important to note that in Palestine the prevalence of smoking among men is higher than that among women (223). Additionally, based on our observations many of the participants would note that there were smokers in their families, mainly their husbands or sons. The influences of second-hand smoking were not taken into consideration for this study; however, for future investigations, a look into secondhand smoking status and potential associations amongst the molecular subtypes of breast cancer may better represent our population of females and the etiology of breast cancer.

4.3.3 Hypertension, Diabetes, & Family History

Based on our findings, there were no significant associations in the risk of the breast cancer subtypes and hypertension, type II diabetes, and CVD status. A prospective cohort study found that an increase in diastolic blood pressure was associated with an increased risk of developing the triple-negative subtype (224). However, there are still conflicting views on hypertension status and the risk of breast cancer development overall (224,225). On the other hand, several studies have shown increased risk of breast cancer with patients who have diabetes (226). One study found that the odds of developing triple-negative breast cancer were greater for

women who had type II diabetes compared to the luminal subtypes (227). This supports other studies which found the same associated risk of type II diabetes status and triple-negative cancer (126,150,228). It has been hypothesized that the increase in elevated insulin and insulin-like growths factor (IGF) in diabetic patients creates an oncogenic effect since IGF promotes cell proliferation (227,228). Furthermore, IGF is higher in triple-negative tumors compared to ER receptor-positive tumors (227). On the other hand, a cohort study found that history of diabetes increased the risk of developing ER receptor-positive breast tumors (229). In our study there were no significant differences amongst the breast cancer subtypes and history of diabetes discussed this may be due to the small sample size of women who were diagnosed. This suggests a need for further investigations to better clarify the impact of diabetes on overall breast cancer subtype risk.

Family history of cancer has been found to increase the risk of developing breast cancer in many studies (12,98). In our study, 63.4% of the participants had a family history of any cancer (1st/2nd-degree relatives). No significant difference was found between the molecular subtypes of breast cancer and family history of any cancer. A recent correlation study by Liu and colleagues also found no significant difference between the hormone receptor status of breast cancer and family history (230). However, in the study, they observed that patients who had a first-degree relative diagnosed with breast cancer showed a more advanced stage of disease and were of older age. Thus, for future measures, a closer look into clinicopathological cancer characteristics and family history should be taken into consideration.

Further, studies have shown a difference in risk amongst family history of different cancers (133,231). For example, a cohort study found associations with breast cancer risk and family history of leukemia and colon cancer amongst African-American women (132). From the

255 participants with a family history of cancer in our sample, 28.8% reported to have a family history of breast cancer and 15.8% reported to have a family history of colon cancer. In Palestine, breast cancer is the most common reported cancer followed by colon cancer this could account for the distribution the cancer reported for family history in our sample (41).

4.3.4 Potential Environmental Attributes

It is key to note that there may be other factors (such as environmental or socioeconomic factors) contributing to the risk of breast cancer subtype development. In our sample, the majority of the patients were from the Southern Governorates of the West Bank mainly the Hebron governorate (170 patients). This correlates to the higher number of residents in the Hebron governorate which makes up 15% of the total population in the oPt compared to the other governorates in the West Bank (200). The Southern governorates have been seen to be affected by several environmental risks. Environmental risk factors, such as heavy metal pollution, have been strictly identified to have a direct effect on genetic alterations and breast cancer formation (232,233). Lead, along with other metals such as Chromium, has been seen to induce oxidative stress in cells by altering the cell's epigenome (232,234,235). The alteration of oxidation is known to play a role in the development of cancers (236).

Looking into the Palestinian context, one study gathered death registries issued in the years 1999 to 2009 (West Bank) and found that the most common cancer deaths for women was due to breast cancer (3). More importantly, it found that breast cancer mortality was highest amongst women in the southern region of the West Bank. In the Southern region of the West Bank, studies have shown dense environmental pollution (30,237–242). Generally, proper waste management, efficient regulation of metal refineries, and effective control of leeching from processing sites (such as sanitary landfills) is far from reached in regions such as the South of the

West Bank. Waste is at an estimated 1.387 million tons per year for the West Bank and Gaza. Almost 42% of the waste generated in the West Bank is distributed into landfills (243).

However, even with sanitary landfills, leachate gathering systems and protective liners may be improperly managed and leeching of chemicals from these sites into soil/groundwater is possible (244). There has been a limited number of studies for environmental pollutant contamination of water, air, and soil in the West Bank. Two studies, one in the north and the other in the south of the West Bank tested well water for heavy metals (237,238). These studies found high levels of the heavy metals lead, chromium, nickel, cadmium, and aluminum. As explained previously, heavy metals and other chemicals have the ability to alter the epigenome. One study done in the Idhna district of Hebron (located by an E-waste site), took into account the workers of the E-waste graveyard and found a positive correlation between the workers of the E-waste and DNA damage (245). Hence, raising a public health concern concerning the formation of several cancers linked to improper DNA regulation and damaged DNA by environmental risk factors. Further investigations are needed to look into the potential risk associations between environmental factors and the formation of the more aggressive subtypes of breast cancer in our population.

Strengths & Limitations of Study

To our knowledge, this is the first study which looked into comparing reproductive risk factors amongst the molecular subtypes of breast cancer in the West Bank. Moreover, it is the first study done, which is not a case-control study, to divide a larger sample into the four distinct molecular subtypes of breast cancer in Palestine. Therefore, this study can help with the estimation of the prevalence of molecular subtypes of breast cancer in the occupied Palestinian Territory. Additionally, our study was the first to exam associations between clinicopathological features such as cancer grade, lymph node status, and tumor size with the molecular subtypes of breast cancer in the West Bank. Hence, making this study a good baseline for future studies investigating the molecular subtypes of breast cancer in our sample population. This study will contribute to the literature of the epidemiology of breast cancer for the occupied Palestinian Territory; thus, providing a smaller piece to a larger puzzle on risk associations amongst aggressive subtypes and making way for future investigations which may entail other risk factors (such as socioeconomic) and their associations amongst the subtypes. This study can also be used as a means of comparison for prospective studies done on the breast cancer subtypes in the occupied Palestinian Territories or other regions.

This study also has limitations. One main limitation is the study design. Since this study utilizes a cross-sectional design, it is important to note that only associations were made and that causal factors cannot be declared. Moreover, no explanation addressing any significant associations found can be made on the effects of these associations. Also, the sample taken was from patients already diagnosed or newly diagnosed with breast cancer. Thus, results obtained cannot be generalized on the female Palestinian population in the West Bank. However, to increase the external validity in this case, information collected such as height, smoking status,

weight, and comorbidities were taken from the patient's file at the time of diagnosis and questions asked to the patient were referred to prior to diagnosis. Additionally, some patients were first told of the study by the oncologist in the clinic before they were asked to participate by the principal investigator. This may have interfered with their participation in the study; however, we may note that patients were asked in the clinics by their oncologist first since there is believed to be trust between the physician and the patient therefore the patient may have felt more comfortable declining to participate to the oncologist than to the principal investigator. Also, there was missing information in the EHR's of the patients due to incomplete profiles and clinical notes. This allowed for several missing values in certain tumor characteristics amongst the sample. Furthermore, we cannot draw causal links or make conclusions between the examined risk factors and risk of breast cancer diagnosis. An alternative design for future studies which may want to conduct a study similar to this one, would be a case-control design which may help better identify outcomes with risk and better compare the risk factors with the molecular subtypes amongst diseased to healthy females.

Moreover, specific to this study, multiple variables may interfere with each other such as the possible affects between occupational risk, environmental risk (as stated above), socioeconomic risk, and genetic risk with the development of the breast cancer subtypes. Hence, the presence of these variables may affect the variable being studied for association amongst the subtypes of breast cancer; thus, altering the actual relationship between the variable and the subtypes. In this study data on environmental and occupational risk factors were not available and limited our ability to examine potential associations between these risk factors and breast cancer subtypes.

Recommendations

The following recommendations are based on the results obtained from the study and are influenced by field observations during data collection.

In the West Bank, there are several breast cancer screening programs conducted by the ministry of health for certain regions (246). Testing for breast cancer is also suggested every other year for women over 40 and every year for women over 50. However, such programs lack selectivity and hence may contribute to not providing screening to “at risk” females. One report done on mammographic screening indicated a false-positive result rate of around 85% for their sample in the West Bank (247). This may indicate the lack of screening done for women who are truly at risk for developing breast cancer. Based on our results, women were at young age at diagnosis (about 48 years), had larger tumor sizes, positive lymph node status, higher cancer grades, and later stages of cancer. This suggests the potential lack in targeting women with a true risk of developing breast cancer regardless of subtype. Therefore, measures should be taken to install a potential risk profile to the EHR's of patients such as a derivation of the Gail Risk Model (173,248). This profile should be filled out not only by oncologists but also by gynecologists and more importantly primary care physicians. Moreover, a risk score should be calculated based on the model and made available to screening facilities. Women with a high-risk score should be tested annually regardless of age. This will help focus surveillance programs to target women who are at high risk of developing breast cancer and may help reduce the aggregated clinicopathological features of the cancers. Hence, allowing for better prognosis and overall survival rates.

Additionally, at the start of planning the study methodology, we predicted that a well-established breast cancer risk profile would be found for each female in the electronic health

records. However, such a risk profile was not present nor were there any means to help in developing a risk score for the female in the health records. This raises a concern on a potential gap of information for the patients in the electronic health records.

The implementation of the EHR application (AviCenna Health Information System), in the West Bank started in late 2012 (249). Moreover, based on our observations, it seems health care professionals are still shifting towards proper adaptation of this newer technology, especially since the instructions in the system are in English (only patient/clinic names in Arabic)¹. In developed countries, it is usually mandatory that health care professionals go through training modules in ethics and more importantly in the use of the hospitals current EHR application before starting the job. Training workers on how to use the EHR's and on how to file notes has been seen to reduce the time burden spent on filling the EHR's, the felt work load of the doctor, and physician burnout (250).

Moreover, in developed countries patients are able to access their own health records or at least have a platform where they can view their own lab tests, diagnosis, and follow-up plans. There is still no such system here in Palestine which makes the patient's own records available to them. This makes it difficult for the patient to understand their own diagnosis and maintains centralization in hospitals. It also decreases the quality of care and increases the stress on the individual. There were many instances in which a patient would ask the physician about their current lab test and due to the workload, the physician would just tell them bluntly that their results are good or bad. The patients weren't able to get details on their lab results and this may

¹ Once, a nurse had me help her open up the system to the outpatient files of the oncology clinic instead of the oncology day care center. She was working in the hospital for more than 5 years, so when asked she stated that she didn't know how to use the system.

cause the lack of understanding of their disease and treatment for many of the patients interviewed.

Going back to the risk profiles, information pertaining to breast cancer risk such as parity, history of IVF, etc., was not easily found on the EHR's of the patients and for the majority, this information was even missing from clinical notes. This may be due to the lack of interdepartmental communication which can be fixed with the proper use of the EHR's. For example, the records of the patient from the oncology department can only be accessed by the department itself so if a patient was to have history of a CVD that information won't be seen on the records for the oncologist instead the patient would self-report it to them. Although an ethics issue may be in concern for sharing records across departments, it is crucial to understand that the sharing of selective records can be done within ethical guidelines, such as having a certain department file a mandatory electronic report with clearly written information on a patient's current detailed treatment plan that can be viewed on the patients record regardless of the department. It is hard to believe that the oncologists at times would not know the current medications their patient was taking due to other comorbidities just because they did not have it evidently reported on file. Sometimes even, the physician would ask the patient of their current medications and the patients wouldn't know what they were taking nor the name of the drug so the physician wouldn't be able to make a note on the records of that medication. This interferes with overall patient quality of care by enhancing the strain on the patient of being aware of what to report and raises a question of whether the patient is truly being treated with the best possible plan. This is also the case with receptor status. Some patient's receptor status wasn't found anywhere on the EHR's which is shocking since they were getting treatment. Treatment for breast cancer is revolved around the receptor status and not having that piece of information

clearly available to the oncologist is problematic especially if the physician was to develop a new treatment plan for a current breast cancer patient.

Additionally, with the increase of private fertility clinics in the West Bank and the potential risk fertility treatments have on the development of breast cancer, it is important to set certain standards and develop strict monitoring/surveillance of such clinics. Women should be made aware of the potential risk of fertility treatments and should even sign a form stating that they have understood risk. Unfortunately, many of the women interviewed who underwent fertility treatment noted that they weren't made aware of the evidence-based risk of their treatments and they were only told what to do. Therefore, for overall better patient care the MOH should look into developing rules which should be implemented into these fertility clinics such as an obligation of informing the patient before treatment of potential risk. Also, going back on our last note, proper reporting of the women's detailed treatment in a fertility clinic should be made accessible to her health care provider at a public hospital through the EHR's. Although this may be difficult, since the private establishment usually has its own form of reporting, set standards should be set for clinics to at least provide the full details of treatment to the individual where they fully explain the treatment plan to the patient.

In Palestine, there is a lack of specialized doctors when compared to Israel and other countries (251,252). The majority of specialized doctors are seen in private hospitals more when compared to public hospitals including all specialties except surgery (253). This general lack of doctors specialized creates a burden on specialized workers working in the public hospital; hence, altering the quality of services provided and financed by the Governmental Health Insurance scheme. Therefore, many referrals are done by patients mainly seeking secondary or tertiary care. In 2018, referrals made from public to other hospitals within Palestine (including

East Jerusalem), was roughly 89,000 cases (41). Also, the total cost of these referrals transferred reached a peak of over 500 million NIS. Hence, not only indicating the lack of public secondary/tertiary care hospitals but also showing the burden of the Palestinian referral system which may create for most referral a huge risk of rendered financial protection. Moreover, even with referrals, there are limitations and restrictions implemented on Palestinians (especially in Gaza) who need an out-of-state referral for better quality treatments due to the needed Israeli permits (251). As observed, the referrals or needed forms for referrals done by the clinics created stress and burdened both the doctor and patient. There should be a central region presumably in each hospital that deals just with referrals that allows the hospital to link the referral back to Ramallah instead of the patient themselves.

Related to the lack of specialized physicians, the doctor would take more time typing a clinical note for the patient than giving the patient their full attention. Everything in the clinics to the oncology daycare was rushed. Some patients would take 10 minutes in the clinic, others five, and some even would jump into the clinic for less than a minute to see if their lab results were okay. One public hospital, had a better system in reducing the workload on the oncologist compared to the other two public hospitals. However, there was still an overall lack of communication between the physician and the patient. This lack of proper patient-doctor communication has been seen to impact the overall quality of care for the patients and disconnects the physician from dealing with the patient in a more humane manner (254).

Moreover, in one public hospital the door of the clinic would be open while different patients went in to discuss their case with their physician. Patients would be standing at the door waiting for their turn to jump in. There was no patient privacy and some of the women interviewed made note of this. For example, one time, after I interviewed a patient, she asked me

of whether or not it was okay to be intimate with her husband after her treatment. I noted to her that I wasn't fully aware of her treatment plan and couldn't judge what would be best for her nor was I a physician. I told her to ask the oncologist and she laughed stating that she barely sees the oncologist even when it's her turn in the clinic, so how could she ask him such a private question. Although in this specific hospital they have been trying to control multiple patients coming at once by closing the door of the clinic, this specific hospital now has all three oncologists in one room dealing with multiple patients. This raises an issue in patient privacy and reduces the overall quality of care for the patient. Certain measures should be implemented specific to each hospital that give patients a fixed timing for their appointments even if that means opening the clinic an extra day of the week or by expanding the clinic work hours. This will contribute to bettering the quality of care provided to patients at these public hospitals.

Building on this, several women interviewed also noted being in a bad mental state and not being able to get out of it. Moreover, the women felt that they couldn't discuss it with the physician due to a lack of time in the clinic. For example, one patient told me that she felt "depressed" and alone. I advised her to talk about it with her physician but she said that she couldn't get the time to even look at her blood test with the physician. When I talked about it to one of the oncologist they said that their job was to focus on the cancer and not whether the patient was sad. This suggest a need to train the physicians on how to spot and deal with the mental health of the patient whether that means suggesting a counselor or transferring the patient to the psychiatric clinic. Additionally, based on observations, there is still a medicalized focus on treating mental health even in patients with a load of medications. Once, a patient come through the open door of the clinic and told the doctor she was excessively sad, didn't want to do anything, and hated waking up each morning (all of which are signs of depression or other

mental health concerns) (255). The oncologist just prescribed the patient with a drug for depression and didn't further the discussion nor recommend a facility she could go to seek proper mental health care. There were no programs, pamphlets, or even posters in the clinics addressing mental health in any way for the breast cancer patients. Nor was there any discussion between the oncologist and the patients with regards to their mental health. Studies have shown that bad mental health of the patient contributes to poorer prognosis and quality of life (256).

This is extremely important to breast cancer prognosis and development, especially in the Palestinian context. The occupation creates, geographical fragmentation, political instability of governance, high rates of poverty and unemployment due to a lowered economic stance, unpredictable Israeli military violations, economic & financial insecurity, food Insecurity (roughly 31.5% of Households in the oPt are food insecure) (10), etc. All of these are contributing factors to overall stress on the population and stress is considered an environmental risk factor which increases an individual's onset of diabetes, cardiovascular disease, and cancer (257–259). This also contributes to the poorer mental health of individual breast cancer patients. For example, health care accessibility amongst Palestinian towns/villages is fraught by a transitioning system of checkpoints, with 140 fixed Israeli checkpoints and over 2000 altering/temporary checkpoints (251). This not only prevents current patients from getting their prescribed treatments on time but it also prevents women from seeking care due to the issue of transportation. Taking into account the stress created by the occupation, the stress of being a mother/daughter, and the stress of getting proper care and treatment one can imagine the impact all these factors have on the patient's mental health. Therefore, better psychological support is needed for breast cancer patients and their families. This can be done by training health care providers of what to do when they see signs of distress in their patients and by creating breast

cancer social support groups that women can join to discuss the impact of their disease with others going through or have gone through the same thing.

Another resolution to most of the issues noted above would be to incorporate a multidisciplinary team approach in which each individual patient is evaluated and treatment plans are made not only by an oncologist but by a team including members from different fields. Multidisciplinary teams have been seen to reduce the workload on a physician and help create better quality of care especially for patients needing more complex care (260–262). For example, a breast cancer patient with diabetes would have her cancer treatment plan take into consideration her current diabetic status by having an oncologist, a nutritionist, and an endocrinologist aid in the development of her treatment plan. Moreover, if that same patient shows signs of a mental health issue a psychiatrist on the team would be able to assist the patient and help with furthering the patients care.

Furthermore, in the oncology day care centers, patients had to take their own blood test tubes and give them to the laboratory in the hospital. If the patient did not have an individual with them to help, the patient themselves would go down to the lab to give them their test tubes. After the effect of Covid-19, one public hospital had their oncology department moved to another location. Hence, patients doing checkups would go to the oncology clinic in one location, get their blood withdrawn from that location, send their test tubes to a lab in another location, and then go back to the first location to get their results. This was the case for patients testing for certain tumor markers, usually patients who were done with treatment and were doing a follow up. Moreover, for many patients before diagnosis they would have to test in one location and go to another for the interpretation of the results. There were also noted delays in imagery and report forms from radiologist in public hospitals. This fragmented form for treatment creates

excessive stress on patients and patient families reducing quality of care. It also allows for a delay in diagnosis and treatment; which contributes to our results since the majority of women had a higher grade, positive lymph node status, and larger tumor size.

This fragmentation can be reduced by expanding hospital facilities and allowing for patients to do all their lab testing/images in the same area. Additionally, phlebotomy should be introduced into public hospitals. A phlebotomist withdraws blood for patients and is in charge of the lab tubes sent to the labs. Certain training programs should be created for phlebotomy at the hospitals to encourage more community worker participation and to create a means to improve the quality of care for the patients by reducing the stress of the nurses, the patients, and by creating more community-centered jobs.

Considering all these aspects, it is important to implement measures towards better quality of care and measures that will help in identifying at-risk patients for breast cancer screening programs. To further summarize the above recommendations, future measures should focus on:

- I. Creating a mandatory format that is easily accessible on the EHR system that entails information pertaining to a reproductive risk profile. This will help in allowing selectivity for breast cancer screening programs to women who are at a higher risk of developing breast cancer regardless of age.***

- II. Allowing for accessibility of the EHR's to different public hospitals which are integrated with clinical notes and files from the different***

departments such as allowing for the oncology department to see certain files from the gynecology department. This will make it easier for health care professionals to obtain information from individual patients who were previously treated in a different governorate or department for different morbidities but whose information would be important for their treatment plan.

III. Allowing for patient access to their electronic health records by the potential implementation of a separate program for patients in each hospital. This will help keep the patient informed of their current treatment and may help patients stay updated with potential risks of disease that they may have.

IV. Developing training modules for current and prospective health care professionals on the use of the EHR's and the ethics of using the EHR's. Moreover, properly training physicians at screening facilities to read mammograms and other image tests.

V. Enhancing doctor-patient communications by reducing the workload on the physician and setting fixed time blocks for each patient appointment. This can be done by providing a larger number of specialized training for young physicians in secondary and tertiary care which can allow for a greater number of doctors

seeing a suitable number of patients during clinic hours. Hence, promoting better quality of care by reducing work strain and increasing the time spent with the patient.

- VI. Creating a set of standards for private fertility clinics in which the patient should be legally made aware of the potential risk of treatment and the full details for treatment. This information should also be added to a patient's reproductive risk profile on their EHR's.*
- VII. Building of community health workers to provide better accessibility of services such as the implementation of phlebotomy in public hospitals. With of course the development of a more decentralized care focus which includes overall comprehension of community and prioritization of needed specialized care.*
- VIII. Working towards a multidisciplinary team approach in the public hospitals. Which will help better a patient's treatment plan to be more focused on the individual and take into consideration all diseases the patient may have such as a breast cancer patient with coronary artery disease or all disease a patient may develop. Hence, bettering the overall quality of care and disease prognosis.*

- IX. The development of needed lab, image, and overall testing facilities in one location in which the patient can conduct all the needed test for treatment in an easily accessible manner. Hence, reducing stress and helping in the early detection of disease for better prognosis.***
- X. The strengthening of psychological support to breast cancer patients and their families by potentially developing support groups in each hospital and by training physicians of what to do when a patient is showing signs of mental distress.***
- XI. The creation of programs that work in Educating and Raising awareness of the proper use of oral contraceptives and of how to prevent or deal with certain breastfeeding complications such as mastitis for young mothers.***
- XII. The implementation of health programs that looks into creating a means to better improve fitness and weight of women in their reproductive life years. Hence, health programs installed in schools for teenagers and nutrition programs placed in hospitals. Although in our study an association couldn't be made amongst BMI and the breast cancer subtypes, the majority of the patients were overweight. Higher BMI's were seen in several studies to***

increase breast cancer risk (125,263). Therefore, preventing higher BMI's by training individuals in health programs may help reduce the risk of developing cancer along with other diseases.

***XIII. Educating* women of where to go to seek care and treatment for a potential diagnosis of breast cancer. Women should have easily accessible information of clinics, laboratories, and screening programs that they can go to. This will help in the early detection of disease and overall better prognosis.**

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Appendix

Appendix A1: Questions to be asked to Participants

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Questions to Be Asked to Participants/ Form Filled out by Investigator

1. How old were you when you first got your menstrual cycle?
 - a. 0-Not applicable (Never Menstruated)
 - b. Age in Years _____

-If answer above is A, then question 2 will be skipped.

2. Do you still get your menstrual cycle, if no what age did it stop?
 - a. 0-Not applicable
 - b. Age in Years _____
3. Are you:
 - a. Single
 - b. Married
 - c. Divorced
 - d. Widowed
4. Have you ever smoked prior to diagnosis (including Water Pipe Smoking-Hookah), if yes how often did you smoke?
 - a. No/Never
 - b. Yes-Duration _____

-If the Answer for question 3 is either B, C or D then questions 5-8 will be asked. Regardless of the answer for question 3, questions 9-11 will be asked.

5. Do you have any children, if yes how many children do you have?
 - a. 0-Not applicable
 - b. Number of Children _____
6. How old were you when you first got pregnant?
 - a. 0-Not applicable
 - b. Age in Years _____
7. Did you ever breastfeed your children, if yes roughly for all children how many weeks or months in total did you breastfeed them?
 - a. 0-Not applicable
 - b. No/Never
 - c. Yes-Duration in Weeks/Months _____
8. Have you ever done In-Vitro fertilization?
 - a. 0-Not applicable
 - b. Yes
 - c. No
9. Did you ever take any oral contraceptives, if yes roughly how long did you take them for?
 - a. No/Never
 - b. Yes-Duration in Months _____
10. Did you take any form of hormone therapy (Used to balance out female hormones and or to relieve symptoms of menopause examples include Premarin/Other Estrogens), if yes roughly how long did you do the therapy?
 - a. No/Never
 - b. Yes-Duration in Months _____
11. Did or does anybody from your family have/had any type of cancer, if yes which type of cancer did they have?
 - a. Yes-1st Degree Relative-Type: _____
 - b. Yes-2nd Degree Relative-Type: _____
 - c. Yes- 1st/2nd Degree Relatives-Types: _____/_____
 - d. No-None of the 1st or 2nd Degree Relatives

Appendix A2: Data Collection Form (EHR)

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BIRZEIT UNIVERSITY

DEPARTMENT OF COMMUNITY AND PUBLIC HEALTH

**Comparisons of Common Known
Reproductive Risk Factors Associated with
Molecular Subtypes of Breast Cancer Amongst
Female Palestinians in the West Bank**

DATA COLLECTION SHEET

Basic Information (FORM I)

Code-ID: _____
Governorate: _____
Hospital: _____
Age: _____
Marital Status: _____
Single/Married/Widowed/Divorced
Date of Birth (M/D/Y): _____
Height: _____
Weight: _____
BMI: _____
Sex: M / F / Unknown

Breast Cancer/Tumor Characteristics (FORM2)

Date of Known Diagnosis: (M/D/Y) _____

Type of Breast Cancer: (e.g. Invasive Ductal
Carcinoma): _____

Breast Density (Density Score) _____

Histology: _____

Size of Invasive tumor: _____ cm

Identified Molecular Subtype (Pathological
Base): _____

All patients

Clinical TNM: T1/T2 /T3/T4 | N0/N1/N2/N3 |

M0/M1

Essential TNM(Breast): M+ / R2 / R1/ A / L2 / L1

Receptor status pathological specimen:

0. No, not available in this hospital
1. No, but available in this hospital
2. Yes, NEGATIVE result
3. Yes, POSITIVE result
9. Unknown

ER: 0 / 1 / 2 / 3 / 9

PR: 0 / 1 / 2 / 3 / 9

Ki-67: 0 / 1 / 2 / 3 / 9

HER2: 0 / 1 / 2 / 3 / 9

Note: This form may contain sensitive information and should be kept in a secure location, then destroyed appropriately once data is inputted for analysis.

<p>Reproductive Risk Profiles (FORM3)</p> <p>Age of Menarche (Y): _____</p> <p>Age of Menopause (0-If not applicable): _____</p> <p>Age at 1st Pregnancy (0-If unknown): _____</p> <p>Number of Gravida and Para: _____</p> <p>Number of Miscarriages (By suffix): _____</p> <p>History of Breast Feeding: <u>YES/NO/UNKNOWN</u></p> <p>➤ Breastfeeding Duration (0-If unknown): _____</p> <p>History of Oral Contraceptive Use: <u>YES/NO/UNKNOWN</u></p>	<p>➤ Oral Contraceptive Use Duration (Refer to sum of months on OCs/0-If unknown or not applicable): _____</p> <p>History of Hormone Therapy: <u>YES/NO/UNKNOWN</u></p> <p>➤ Hormone therapy Duration (Refer to sum of months on HT/0-If unknown or not applicable): _____</p> <p>➤ Type of Hormone Given (0- If unknown): _____</p> <p>History of In-Vitro Fertilization: <u>YES/NO/Unknown</u></p> <p>Smoking Status: No, never/Stopped>6weeks/Yes/Unknown</p>	<p>MEDICAL HISTORY (FORM4)</p> <p>Diabetes: Status: No/ Prediabetic/Yes/ Unknown Specify: _____</p> <p>Current Treatment: Medications/ Insulin/ Diet /Other Medications if Known: _____ Other: _____</p> <p>Date of Diagnosis: (M/D/Y) _____</p> <p>Hypertension: Status: No/ Yes/ Unknown Specify: _____</p> <p>Current Treatment: Medications/ Diet/ Other Medications if Known: _____</p>
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Note: This form may contain sensitive information and should be kept in a secure location, then destroyed appropriately once data is inputted for analysis.

Other: _____	Medications if Known: _____
Date of Diagnosis: (M/D/Y) _____	Other: _____
Cardiovascular Diseases: _____	Date of Diagnosis: (M/D/Y) _____
Status: Yes/ No/ unknown	History of Cancer (Ovarian/Uterine/etc.) _____
Specify: _____	Status: Yes/ No/ unknown
Current Treatment: _____	Specify: _____
Medications/ Diet/ Other _____	Date of Diagnosis: (M/D/Y) _____
Medications if Known: _____	Other: _____
Other: _____	Known Genetic Test Done: _____
Date of Diagnosis: (M/D/Y) _____	Status: _____
Other Illnesses: _____	Yes/ No/ Unknown/ Not Done in Hospital
Status: Yes/ No/ Unknown	Known Genetic Mutations: _____
Specify: _____	Status: Yes/ No/ Unknown
Current Treatment: _____	Specify (e.g. BRCA1): _____
Medications/ Diet/ Other _____	Family History of Cancer (1 st /2 nd Degree)
	<u>YES/NO/UNKNOWN</u>

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Note: This form may contain sensitive information and should be kept in a secure location, then destroyed appropriately once data is inputted for analysis.

Appendix A3: Verbal Consent Script

“Hello, my name is Dana Aljaber. I am a graduate student at Birzeit University in the institute of Community and Public Health and I am here undertaking research that will be used in my thesis project. The purpose of this study is to determine and compare the potential reproductive risk factors for different breast cancer classifications. We intend to 1) find the associated reproductive risk factors for each different type of breast cancer and 2) better understand the risk factors for breast cancer for the female Palestinian population. You have been asked to participate in this research study because you are currently diagnosed with breast cancer and you are a Palestinian living in the West Bank.

The information you share with me will be of great value in helping me to complete this research project, the results of which could help enhance our understanding of breast cancer. There are no benefits that you directly get from taking part in this study. However, the information you give us will contribute to better understanding breast cancer specifically amongst the Palestinian population. Moreover, there are potential benefits to the future of medical, genetic, and basic scientific research for the Palestinian community.

Since this study consist of only a questionnaire, there are no direct risk to participating in this study. All answers given with the survey will be kept confidentially. I will not link your name to anything you say in the text of my thesis project or any other publications. The questionnaire will last roughly 5-7 minutes; moreover, you may choose not to answer any questions which you feel uncomfortable in answering. You may choose not to participate in this study without penalty or loss of benefits in which you are otherwise entitled and not participating will not affect your care at this hospital. At any given moment in the study, you may choose to not participate anymore without penalty. Information taken from you will be kept confidentially.

Do you have any questions about this research? Do you agree to participate?

If so, let's begin....”

*Appendix A4: Ethics Approval from the Institute of Community & Public Health at
BZU Ethics Committee board*

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Institute of Community and Public Health – Birzeit University

Ethics Review Committee Decision

Date: July 27, 2020

Applicant's Name/Principal Investigator: Dana Basim Aljaber (1185125)

Unit: The Institute of Community and Public Health

Reference No:	2020 (7 – 1)
Project Title:	Comparison of Common Known Reproductive Risk Factors Associated with the Molecular Subtypes of Breast Cancer Amongst Female Palestinians in the West Bank
Names of contributing researchers, other than the Principal Investigator/ Applicant:	Supervisor: Dr. Weeam Hammoudeh

Thank you for submitting your application for the ethics review of your research proposal. Your application was examined carefully, and discussed by the Ethics Review Committee during a meeting which took place on July 27, 2020. The following documents were reviewed:

1. Ethics Review Application Letter/Form
2. Consent Form
3. Project proposal and related documents, as revised based on the comments of the Committee
4. Study questionnaire

The ICPH-BZU Research Ethics Review Committee has approved your research proposal.

Approval is given for three years. Projects, which have not commenced within two years of original approval, must be re-submitted to the Ethics Review Committee. You must inform the Committee when the research has been completed. If you are unable to complete your research within the three year validation period, you will be required to write to the Ethics Review Committee to request an extension. You may also need to re-apply for approval by the Committee.

Any serious adverse events or significant changes which occur in connection with this study and/or which may alter its ethical considerations must be reported immediately to the Ethics Review Committee. On such an occasion, an "Amendment Form" must be submitted to the Committee for re-assessment.

Thank you
Ethics Review Committee Coordinator
Maysaa Nemer, PhD






معهد الصحة العامة والمجتمعية
Institute of Community and Public Health



**Institute of Community and Public Health – Birzeit University
Ethics Review Committee (ERC)**

Part I: To be completed by the applicant:

Date: July 7, 2020

Applicant's Name/Principal Investigator: Dana Basim Aljaber (1185125)

Unit: The Institute of Community and Public Health

Reference No:	2020 (7 – 1)
Project Title:	Comparison of Common Known Reproductive Risk Factors Associated with the Molecular Subtypes of Breast Cancer Amongst Female Palestinians in the West Bank
Names of contributing researchers, other than the Principal Investigator/ Applicant:	Supervisor: Dr. Weeam Hammoudeh

Comments:

Student master's thesis. Already have permission from the MOH. No data collection will take place until after MOH allows for research or resume.

Part II: To be completed by the (ERC)

***Decision:**

The ICPH-BZU Research Ethics Review Committee approves this study.

Decision date: July 27, 2020

Ethics Review Committee members, qualifications and signatures:

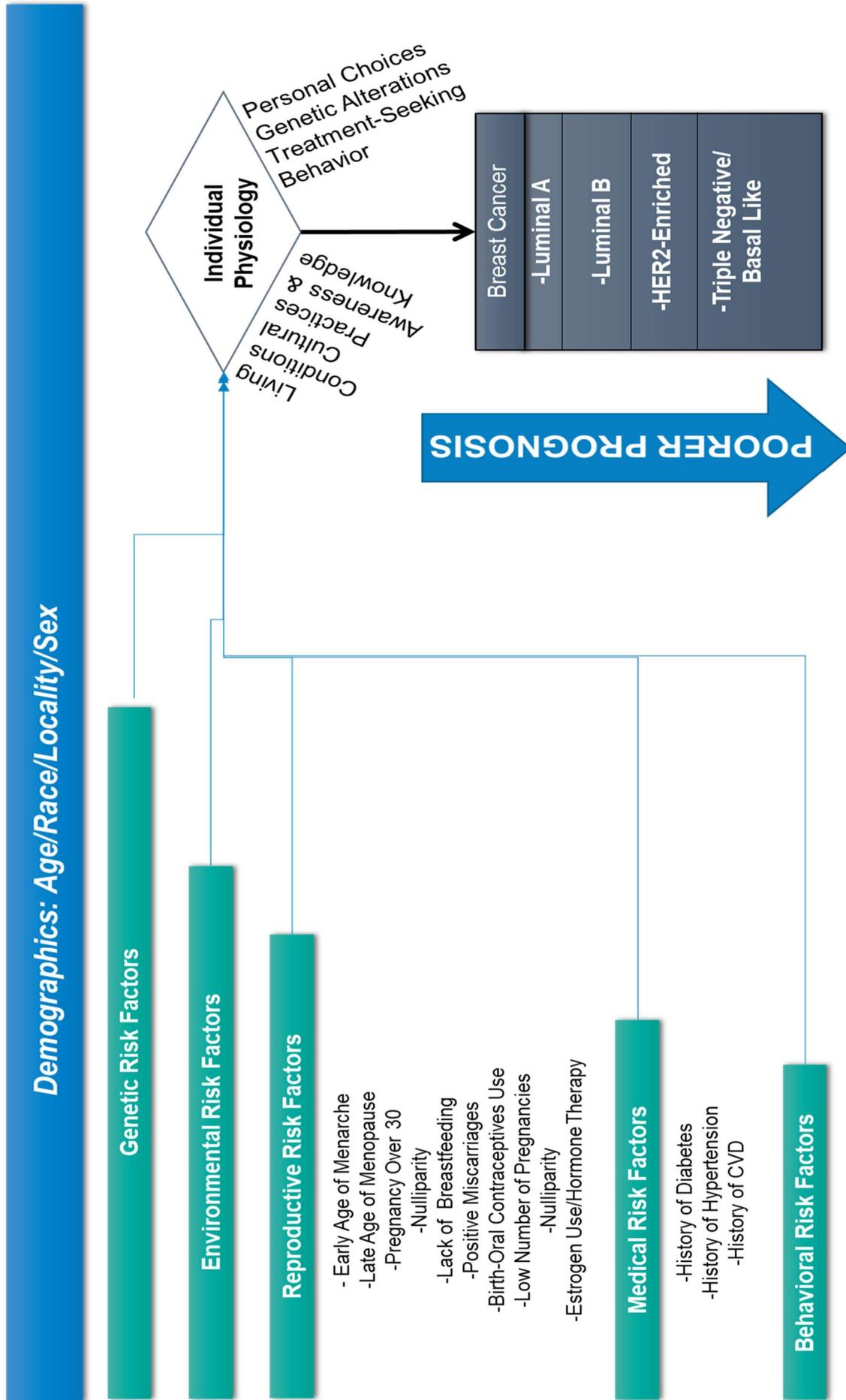
Name	Specialty	Qualifications	Signature
Maysaa Nemer	Occupational Epidemiology	PhD	
Suzan Mitwalli	Public Health	MPH	
Shiraz Nasr	Spatial Analysis	MSA	



Appendix B: Conceptual Framework

- **FIGURE 1 CONCEPTUAL FRAMEWORK OF BREAST CANCER RISK FACTORS AND BREAST CANCER DEVELOPMENT**

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Appendix C: Permission Letter from The Ministry of Health

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State of Palestine
Ministry of Health - Nablus
General Directorate of Education in Health



دولة فلسطين
وزارة الصحة - نابلس
الإدارة العامة للتعليم الصحي

Ref:
Date:.....

الرقم: ١٩٩/١٠٠/٢٠٢٠
التاريخ: ٢٠٢٠/٠٥/٠٥

الأخ مدير عام الادارة العامة للمستشفيات المحترم،،،
تحية واحترام،،،

الموضوع: تسهيل مهمة طالبة ماجستير - جامعة بيرزيت

يرجى تسهيل مهمة الطالبة: دانا جابر- ماجستير صحة عامة/ جامعة بيرزيت، في عمل بحث بعنوان: " *Comparison of Common Known Reproductive Risk Factors Associated with the Molecular Subtypes of Breast Cancer Amongst Female Palestinians in the West Bank* "، علماً بأن الدراسة بإشراف د. وئام حمودة، وذلك من خلال السماح للطالبة بالحصول على معلومات تخص البحث من ملفات المريعات عينة الدراسة (بوجود مسؤول الملفات)، بحيث لا يتم الحصول على المعلومات التعريفية للمريعات وذلك في:

- مستشفى بيت جالا الحكومي

- مستشفى الوطني/ نابلس

علماً بأنه سيتم الالتزام بمعايير البحث العلمي والحفاظ على سرية المعلومات.



نسخة: مدير برنامج ماجستير الصحة العامة والمجتمعية المحترم/ جامعة بيرزيت