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Precision assessment of heterogeneity of lymphedema phenotype, genotypes and risk prediction

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ABSTRACT

Lymphedema following breast cancer surgery is considered to be mainly due to the mechanical injury from surgery. Recent research identified that inflammation-infection and obesity may be the important predictors for lymphedema. The purpose of this exploratory research was to prospectively examine phenotype of arm lymphedema defined by limb volume and lymphedema symptoms in relation to inflammatory genes in women treated for breast cancer. A prospective, descriptive and repeated-measure design using candidate gene association method was used to enroll 140 women at pre-surgery and followed at 4–8 weeks and 12 months post-surgery. Arm lymphedema was determined by a perometer measurement of ≥5% limb volume increase from baseline of pre-surgery. Lymphedema symptom phenotype was evaluated using a reliable and valid instrument. Saliva samples were collected for DNA extraction. Genes known for inflammation were evaluated, including lymphatic specific growth factors (VEGF-C & VEGF-D), cytokines (IL1-*a*, IL-4, IL6, IL8, IL10, & IL13), and tumor necrosis factor-*a* (TNF-*a*). No significant associations were found between arm lymphedema phenotype and any inflammatory genetic variations. IL1-*a* rs17561 was marginally associated with symptom count phenotype of ≥8 symptoms. IL-4 rs2070874 was significantly associated with phenotype of impaired limb mobility and fluid accumulation. Phenotype of fluid accumulation was significantly associated with IL6 rs1800795, IL4 rs2243250 and IL4 rs2070874. Phenotype of discomfort was significantly associated with VEGF-C rs3775203 and IL13 rs1800925. Precision assessment of heterogeneity of lymphedema phenotype and understanding the biological mechanism of each phenotype through the exploration of inherited genetic susceptibility is essential for finding a cure. Further exploration of investigative intervention in the context of genotype and gene expressions would advance our understanding of heterogeneity of lymphedema phenotype.

Introduction

Lymphedema, an abnormal accumulation of lymph fluid in the ipsilateral body area or upper limb, remains an ongoing major health problem affecting more than 40% of 3.1 million breast cancer survivors in the United States [1–3]. The experience of lymphedema has been linked to clinically relevant and detrimental outcomes, such as disability and psychological distress, both of which are known risk factors for breast cancer survivors' poor quality of life (QOL) and survivorship [4–7]. While mechanical injury from cancer treatment (e.g. surgery, lymph node procedure, radiation) is considered the main contributor to the risk of lymphedema, research has also found that inflammation-infection and higher body mass index (BMI>30) are the main predictors of lymphedema [8–10]. It remains puzzling that up to 23% of survivors who only had lumpectomy with sentinel lymph node biopsy (SLNB) of 1 or 2 lymph nodes removed have developed

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lymphedema, while some survivors who had mastectomy with more than 10 lymph nodes removed have not [11–14]. It is possible that genetic variations may be one of the important factors that influence breast cancer survivors' responses to the inflammatory processes and vulnerability to lymphedema, including responses to trauma (surgery and radiation) and triggering factors (infection, burns, minor injuries, higher BMI or obesity). Single nucleic polymorphisms (SNPs) known to influence gene expression and therefore protein levels of inflammatory cytokines and lymphatic specific growth factors have been identified from the non-lymphedema and lymphedema literature [15–24].

Precision assessment of lymphedema phenotype among breast cancer survivors remains a huge challenge in research and clinical practice. Traditionally, lymphedema has been diagnosed by healthcare providers' observations of swelling. Research focus has been on measuring limb girth, limb volume or limb size to evaluate phenotype of arm swelling (hereafter arm lymphedema) with arbitrarily defined criteria of >2-cm increase in limb girth, >200-mL limb volume, or >5% limb volume and bioimpedance ratio [2,6,25,26]. Yet, lymphedema phenotypes may include symptoms related to lymph fluid accumulation (hereafter, lymphedema symptoms), including arm swelling, breast swelling, chest wall swelling, heaviness, firmness, tightness, stiffness, pain, aching, soreness, tenderness, numbness, burning, stabbing, tingling, arm fatigue, arm weakness, and limited movement in shoulder, arm, elbow, wrist and fingers [25,26]. More importantly, lymphedema symptom phenotype may indicate an early stage of lymphedema in which changes cannot be detected by current objective measures of limb volume [25–29]. There is a critical need to understand heterogeneity of lymphedema phenotype in relation to inflammatory genetic variations to advance precision assessment of lymphedema phenotype and related biological mechanism. The purpose of this exploratory research was to prospectively examine phenotype of arm lymphedema and lymphedema symptoms in relation to inflammatory genes in breast cancer survivors.

Material and Methods

Ethical consideration

This study was approved by the Institutional Review Board of the study institute in the metropolitan area of New York.

Research design

We employed a prospective, descriptive and repeated-measure design using candidate gene association method that enabled phase-specific monitoring of lymphedema phenotypes prior to surgery (baseline), at 4–8 weeks and 12 months post-surgery.

Procedures

Researchers were trained for obtaining informed consent and collecting data. Protection of human subjects was ensured by following the guidelines set forth by the Institutional Review Board and successful recruitment procedures used in our prior studies [25,28,30]. Written consent to the study was obtained. Procedures for using the perometer and bioimpedance device [25,28,30] as well as collecting saliva samples were followed as recommended by the manufacturers.

Study participants

Between December 2011 and April 2014, we enrolled 140 women at pre-surgery baseline and followed the participants at 4–8 weeks and 12 months post-surgery. Study participants were over 21 years or older, had a first time diagnosis of breast cancer (Stage I–III), and were scheduled for surgical treatment of lumpectomy or mastectomy, including SLNB, lymph node dissection or axillary lymph node dissection and neoadjuvant or adjuvant therapy [8–10]; Women were excluded if they had [1] prior history of lymphedema and breast cancer [2]; renal or heart failure, cardiac pacemaker or defibrillator, artificial limbs or pregnancy.

Phenotype measures

Demographic & clinical information

Demographic and clinical information were collected: breast cancer treatment, stage of disease, cancer location, type of adjuvant therapy and treatment complications [4,25,27].

Height & BMI

Height was measured to the nearest 0.1 cm with a portable stadiometer without shoes. An electrical bioimpedance device (InBody 520, Biospace Co., Ltd) was used to measure weight and BMI [28]. The device assesses weight and automatically calculates BMI using the formula: weight (kg)/height (m^2).

Infra-red perometer measurement

Perometry 350S was performed on each arm. A 3-dimensional limb image was generated and limb volume was calculated for each participant. This optoelectronic method has a standard deviation of 8.9 ml (arm), less than 0.5% of limb volume with repeated measuring [29,30].

Lymphedema symptom assessment

The Lymphedema and Breast Cancer Symptom Experience Index is a valid and reliable self-report instrument to assess symptoms related to lymph fluid accumulation [25,27,31].

DNA data collection

Selection of genes and polymorphisms (SNPs)

Genes were selected for this exploratory investigation based on functional implications from previous publications Table 1. These inflammatory genes include lymphatic specific growth factors (VEGF-C & VEGF-D), cytokines (IL1-*a*, IL-4, IL6, IL8, IL10, & IL13) and tumor necrosis factor-*a* (TNF-*a*). Functional SNPs known for inflammation and lymphatic specific growth factors were selected for the genes where these types of functional SNPs have been documented. VEGF-C and VEGF-D do not have any known functional SNPs. Therefore these genes were evaluated using tagging SNPs (tSNP) that cover the variability in the entire gene as well as the 5′ flanking promoter regions. The criteria used for selection of tSNPs were based on a MAF cutoff of 20%, R^2 cutoff of 80% using Pairwise Tagger for a Caucasian population (http://hapmap.ncbi.nlm.nih.gov/) [32].

Sample collection and DNA extraction

Saliva sample collections were conducted using the *Oragene* DNA self-collection kit from DNA Genotek Corporation. DNA were extracted using the protocol and reagents for extraction supplied with the *Oragene* kit.

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