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RANKL AS A PROGNOSTIC MARKER IN PATIENTS WITH BREAST CANCER

Thesis

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Abstract

Bone is a common site for metastasis in breast cancer; approximately ٧٥٪ of women with advanced breast cancer will develop bone metastases. Bone metastases associated with breast cancer are predominately osteolytic. Receptor activator of nuclear factor-κB ligand (RANKL) is a key mediator in the vicious cycle of bone destruction in metastatic cancer. Within the bone microenvironment, factors secreted by tumor cells stimulate stromal cells and osteoblasts to express and secrete RANKL, which binds to RANK on the surface of precursor and mature osteoclasts. RANKL is a critical mediator of osteoclast differentiation, function, and survival.

The present study aimed to assess the relevance of RANKL to other prognostic factors in breast cancer patients with and without bone metastases.

The study was conducted on eighty females who were divided into four groups: group I (n=٢٠) healthy females as a control group, group II (n=٢٠) non-metastatic breast cancer patients, group III a (n=٢٠) breast cancer patients with bone metastases not receiving Bisphosphonates treatment And group III b (n=٢٠) breast cancer patients with bone metastases and were receiving Bisphosphonates treatment

All participants were subjected to a thorough clinical assessment, and estimation of blood levels of fasting glucose, ALT, AST, alkaline phosphatase, urea, creatinine, bilirubin, and RANKL.

The serum levels of RANKL showed a significant increase in breast cancer patients (with and without bone metastasis) compared to the control group. Also, a significant increase in RANKL level was found in breast cancer patients with bone metastasis without Bisphosphonates treatment compared to those with Bisphosphonates treatment. Also, a significantly positive correlation was found between RANKL and tumor size. This means that, RANKL may be used as a prognostic marker in breast cancer patients.

Key words: Breast cancer- RANKL- Bone metastasis.

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List of abbreviations

BRCA١	Breast cancer gene ١
BRCA٢	Breast cancer gene ٢
NBR٢	Near breast cancer gene ٢
PUFAs	Polyunsaturated fatty acids
SHBG	Sex hormone binding globulin
HRT	Hormone replacement therapy
DCIS	Ductal carcinoma in situ
LCIS	Lobular carcinoma in situ
ER	Estrogen receptor
PR	Progesterone receptor
FNAC	Fine Needle Aspiration and Cytology
VAB	Vacuum-assisted breast biopsy
ROS	Reactive oxygen species
ECM	Extracellular Matrix
BBB	Blood-brain barrier
p٥٣	Tumor protein ٥٣
PI٣K/AKT	Phosphatidyl Inositol-٣-kinase
RANKL	The receptor activator for nuclear factor κβ ligand
OPG	Osteoprotegerin
MEK/RAS	Mitogen-activated protein kinase-ERK kinase)
TRAIL	Tumour necrosis factor related, apoptosis inducing ligand
ERK	Extracellular-signal-regulated kinase
RANK	The receptor activator for nuclear factor κβ
TNF	Tumour necrosis factor
DDH	Death domain homologous

TRAFs	TNF receptor associated factors
[NFAT] c١	Calcineurin/nuclear factor of activated T cells
Grb-٢	Growth factor receptor binding protein-٢
MITF	Microphthalmia transcription factor
PTH	Parathyroid hormone
TGF-β	Transforming growth factor-β
M-CSF	Macrophage- colony stimulating factor
OCPs	Osteoclast precursors
TGFa	Transforming growth factor a
VEGF	Vascular endothelial growth factor
PTHrP	Parathyroid hormone related peptide
IL	Interleukin
mRNA	Messenger RNA
BP	Bisphosphonates
sRANKL	Soluble RANKL
NF-κB	Nuclear factor-κB
ODF	Osteoclast differentiation factor
ODAR	Osteoclast differentiation and activation receptor
OPGL	OPG ligand
OCIF	Osteoclastogenesis inhibitory factor
SOFA	Stromal osteoclast-forming activity
TRANCE	TNF-related activation-induced cytokine
TNFSF	TNF superfamily
TR-١	TNF receptor-like molecule ١
FDCR	Follicular dendritic receptor
ALP	Alkaline phosphatase
FBS	Fasting blood sugar
AST	Aspartate transaminase
ALT	Alanine transaminase

ELISA	Enzyme Linked Immuno- Sorbent Assay technique
ESR	Erythrocyte Sedimentation Rate
CRP	C- Reactive protein
TNM	Tumour-Lymph node-Metastasis
ECM	Extracellular Matrix
HIV	Human Immunodeficiency virus
ZOL	Zoledronic acid

Introduction

Breast cancer is the third most common cancer in the world, and in ٦٥٪ to ٧٥٪ of patients, with progressive disease, bone metastases are common **(Lipton, ٢٠٠٦).**

Breast cancer displays a high predilection for metastasis to bone, which results in pathological bone fractures, hypercalcemia, spinal cord compression, as well as significant pain burden **(Mundy, ٢٠٠٢).** The formation of distant metastases is a multi-step process that includes local tumor migration, intravasation, survival in circulation, extravasation, and the ability to thrive in the metastatic site **(Chambers et al., ٢٠٠٢).**

Bone remodeling in adults occurs by removal of old bone (resorption) by osteoclasts, followed by new bone formation by osteoblasts. The key molecular regulators of bone resorption are receptor activator of nuclear factor- κB (RANK), RANK ligand (RANKL) and osteoprotegrin (OPG)

RANKL is a member of the tumor necrosis factor (TNF) super family, the locus for which has been traced to the ١٣q١٤ human chromosome **(Anderson et al., ١٩٩٧)**. It is produced as a ٣١٧ amino acid and exists as either a membrane-bound protein or can be cleaved to form a secreted protein that still retains activity **(Blair et al., ٢٠٠٧)**. RANKL is highly expressed in peripheral lymph nodes and bone marrow, thymus, spleen, Payer's patches, brain, heart, skin, skeletal muscle, kidney, liver, lung and mammary tissue **(Lacey et al., ١٩٩٨)**. Cells that produce RANKL include cells of osteoblastic lineage, bone marrow stromal cells, synovial cells, activated T cells, B cells, fibroblasts, endothelial cells, chondrocytes and mammary epithelial cells **(Hofbauer et al., ٢٠٠١)**.

RANKL expression is influenced by several hormones, growth factors, peptides and cytokines **(Theoleyre et al., ٢٠٠٤)**.

RANKL has an important role at various stages of osteoclast differentiation and function. The fusion of osteoclast precursors to form multinucleated cells, their differentiation into mature osteoclasts and the attachment of osteoclasts to bone and activation to resorb bone are all influenced by RANKL **(Lacey et al., ١٩٩٨)**. RANKL also inhibits osteoclast apoptosis and thereby leads to continued survival of these cells **(Lacey et al., ٢٠٠٠)**.

Ligation of RANKL to its cognate receptor RANK, which is expressed by osteoclasts and their precursors, results in the fusion, differentiation and activation of osteoclasts. OPG is a soluble decoy receptor for RANKL that can inhibit the osteoclastogenic interaction between RANKL and RANK **(Blair et al., ٢٠٠٧).**

RANKL is implicated in the pathogenesis of bone metastasis at several levels: increased bone resorption as a result of excess RANKL results in the release of growth factors that facilitate tumor cell division or survival **(Yubin et al., ٢٠٠٨).** A study by **Jones et al., ٢٠٠٦** suggested a role for RANKL as a chemo-attractant for certain cancer cells metastatic to bone. Furthermore, RANKL can stimulate both angiogenesis and endothelial cell survival **(Kim et al., ٢٠٠٣)**, suggesting a role for RANKL in supporting vascularization of bone metastases.

Bisphosphonates are important inhibitors of bone resorption widely used clinically to treat orthopedic disorders, it reduce skeletal morbidity rate by about ٢٥٪ -٤٠٪ in patients who present with breast cancer metastasized to bone **(Rosen et al., ٢٠٠٣).**

Recent evidence suggests that bisphosphonates also regulate essential signaling molecules involved in osteoclastogenesis such as RANKL. bisphosphonates also modulate OPG and preventing RANK activation **.**also it increase osteoblast proliferation and up regulate expression of genes involved in new bone formation **(Viereck et al., ٢٠٠٢)**

Objectives: (Aim of the work)

The aim of this study was to assess the relevance of RANKL to other prognostic factors in breast cancer patients with and without bone metastases.

Cancer Breast

Breast cancer is a malignant breast neoplasm which originates from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk ***(Sariego, ٢٠١٠).***

Epidemiology of the disease:

Worldwide, breast cancer is the most common invasive cancer in women. It comprises ٢٢,٩٪ of other invasive cancers in women and ١٦٪ of all female cancers ***(Powles et al., ١٩٩٨).***

The incidence of breast cancer varies greatly around the world; it is lowest in less-developed countries and greatest in the more-developed countries ***(Stewart and Kleihues, ٢٠٠٣)***

National Cancer Institute in Cairo registry reported breast cancer to represent ٣٥,١٪ of female cancers ***(Abdel-Fattah et al., ٢٠٠١).***

A recent article about breast cancer in Cairo indicates a higher than expected detection rate of ٨ per ١٠٠٠ breast cancer cases upon first screening of a target group of ٤١١٦ invited women aged ٣٥–٦٤ years living in a geographically defined area in Cairo, which suggests that many women in the community with early but palpable breast cancer fail to seek medical attention until their cancer is advanced ***(Boulos et al., ٢٠٠٥).***

Early clinical breast examination can reduce the incidence of locally advanced disease, and improve breast-conserving surgery rates ***(El-Attar, ٢٠٠٥).***

Risk factors for breast cancer