<table>
<thead>
<tr>
<th>Affiliation</th>
<th>Associate Professor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Dr. Nasser Al-Daghri</td>
</tr>
<tr>
<td>Project's Title</td>
<td>Circulating Endotoxin as a Contributing Inflammatory Mediator in Osteoporosis Risk and its Association with Vitamin D Deficiency</td>
</tr>
</tbody>
</table>

**Brief Introduction:**

Osteoporosis represents a significant global health problem affecting more than 200 million people worldwide. According to World Health Organization they estimated that hip fractures will increase from 600 thousand in 1990 to over 3 million in Asia by year 2020. Osteoporotic risk arises from changes in estrogen and vitamin D levels, but other factors such as diet, ethnic background, calcium levels, smoking status, exercise levels, and chronic inflammation, which affects bone metabolism, may also have a major impact. The disease is not gender-specific, as one in three women and one in five men are at risk.

Particularly within the Kingdom, osteoporosis is as high as 44.5% in Saudi women and 33.2% in Saudi men. Furthermore, as the population is now living longer, it is vital to understand how to reduce the burden of this disease through new advances in early predictive biomarker analysis as current technology relies on expensive screening that is not economically practical for widespread screening and can only detect bone density of more than 40%.

Current studies of osteoporotic bone loss due to chronic inflammation have focused on dental bacterial infection, wound/bone bacterial infection, or sepsis, as these infections increase the innate immune response and can promote bone loss, but are not often identified as a common cause of osteoporosis. Similarly, low levels of bacterial cell wall fragments known as lipopolysaccharide, also called endotoxin, derived from the gut are higher in subjects with metabolic disease as the gut is leakier in these subjects. In essence, the endotoxin transverses the gut mucosa and can enter the blood circulation at low levels, causing an inflammatory response in tissues such as fat, liver, and muscle to produce pro-inflammatory cytokines. Osteoporotic subjects have heightened levels of pro-inflammatory cytokines, which promote bone loss, a problem potentially exacerbated by vitamin D deficiency. Reduced estrogen levels enhance the effect of endotoxin on bone loss in mice. Therefore, we hypothesize that the level of gut-derived bacteria, or endotoxin, is increased in patients with osteoporosis, affecting bone turnover and leading to bone loss. Endotoxin may also contribute to the chronic pro-inflammatory cytokine release observed in osteoporotic subjects and impacted by vitamin D deficiency. These current studies will utilize the current infrastructure and well established clinical teams to undertake these studies which has been utilized to develop the BRP.

Therefore, the aims of this study are to:

1. Evaluate circulating...
endotoxin levels in patients with and without osteoporosis and examine how this is associated with hormonal status, weight, and other important biochemical and anthropometric factors that increase osteoporotic risk;

(2) assess the relationship between endotoxin and elevated pro-inflammatory markers in osteoporosis; and (3) assess the effect of osteoporosis treatment on vitamin D levels and the impact on chronic inflammatory status, particularly endotoxin and inflammatory factors. These studies will use cutting-edge molecular biology techniques and new methodology to identify osteoporosis disease mechanisms, as well as inform the development of biomarkers for evaluating osteoporotic risk and improve healthcare delivery without compromising on standards.

Normal RON is expressed in macrophages following acute inflammation, where activation by its ligand macrophage stimulating protein (MSP) downregulates inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2), and blocks NF-κB activation. MSP/RON signaling also activates and regulates the balance between classical (M1) and alternative (M2) macrophages. Almost all tumors are infiltrated by a large number of tumor associated macrophages (TAMs), which perform several M2-associated pro-tumoral functions, including angiogenesis promotion, matrix remodeling and immune suppression. The presence of high nitric oxide levels (product of iNOS), prostaglandin E2 (product of COX-2), and multiple inflammatory cytokines (products of NF-κB activation) in the tumor microenvironment imply the absence of MSP/RON signaling in TAMs. We hypothesize that cancer cells may block MSP/RON signaling in TAMs by secreting N-terminally truncated RON isoforms into the tumor microenvironment, where they can bind both MSP ligand and RON receptor and lead to alternatively activated TAMs, which in turn aid the tumors by creating a highly inflammatory microenvironment that is consistent with an important hallmark of cancer.

Previous studies using lung cancer cell lines and tumor tissues have indicated the presence of numerous RON transcripts (>35 unique deletions and insertions), formed through alternative splicing, and their protein products, and several cell lines lacked the wild type RON receptor. Alternative transcripts were found to have lost the transmembrane sequence during splicing, and hence were capable of diffusing into the tumor microenvironment. Many intracellular RON isoforms were found to be phosphorylated, which indicated constitutive activation. The wide range of functions attributed to aberrantly expressed RON and the numerous transcripts identified in cancer cells imply a vastly complex role for RON in cancers.
We propose to delineate the role of all the individual RON isoforms in
tumors by sequencing all the full-length transcripts from several tumor
cell lines, by developing isoforms-specific quantification methods and by
identifying their functions both in vitro and in vivo.

Research methodology
Recruitment of Patients

Recruitment of patients to this study will be made possible by
collaboration with primary care centers throughout Riyadh and the
Biomarkers Research Program (BRP), College of Science, King Saud
University. As the BRP has been central to collection of the 16,000
subjects from the local primary care centers in Riyadh we have extensive
knowledge and logistical information on how the centres run, the staff,
as well as the families that come to the centres. We also have other co-
current studies within the primary care centres so we have an
established relationship with staff and the local community. Therefore
taking into consideration the potential for patient withdraw, as well as
other factors, we envisage that the time allocated for recruitment will be
sufficient acknowledging that challenges that may occur.

Subjects will be recruited from different sources which operate within
the Biomarkers Research Programme (BRP). Initially for Part 1-3 of the
study, patients with and without osteoporosis will be recruited within
current BRP, utilising primary care facilities and assessed by DEXA and
systemic blood marker evalution. For Part 4 the recruitment of people
with new fractures this will be recruited from the adjacent hospital to
KSU and College of Science, again where current studies within the team
are undertaken and where DEXA scans are taken. It should be stressed
that all subjects will receive appropriate medical treatment as part o this
study

Part 1: To evaluate circulating endotoxin levels in patients with and
without osteoporosis

We will first construct a cohort of randomly selected menopausal women
aged 55-79 years [El-Dessouki, 2003]. The subjects will be recruited
through primary care centers in Riyadh or through follow-up clinical
care. These subjects will allow the team to assess how endotoxin levels
correlate with inflammatory markers, hormonal status, weight, and other
important biochemical and anthropometric risk factors for osteoporosis.

1) Menopausal women with or without osteoporosis (n=200 in each
group).

2) Retrospective analysis on aged-matched men and women with and
without vitamin D deficiency (n=100 each group, aged 55-79),
subsequently treated with or without vitamin D supplementation and
bisphosphonates.

Longitudinal assessment of newly diagnosed osteoporotic patients with
or without vitamin D deficiency treated over 24 months (n=50, men and
women aged 55-79), assessed at baseline and 3, 6, 12 months and 24 months with subsequent future funding provision.

<table>
<thead>
<tr>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>The project is applicable within (months)</td>
</tr>
<tr>
<td>more than 12</td>
</tr>
</tbody>
</table>

**REFERENCES**

- Alokail MS, Al-Daghri NM, Al-Attas OS, Alkharfy KM, Sabico SB, Ullrich A. Gender-specific associations between insulin resistance, hypertension, and markers of inflammation among adult Saudis with and without diabetes mellitus type 2. Eur J Clin Invest. 2011 Sep;41(9):987-94. doi:

- Dawson-Hughes B, Mithal A, Bonjour JP, et al. IOF position statement:


- Hadjigogos K. The role of free radicals in the pathogenesis of
• Kopp A, Buechler C, Neumeier M, Weigert J, Aslanidis C, Schölmerich J,


  • Nguyen TV, Eisman JA, Kelly PJ, Sambrook PN. Risk factors for


• Paton NI, Macallan, DC, Griffin GE Pazianas M, Bone mineral density in patients with human immunodeficiency virus infection, Calcif. Tissue Int. 61 (1997), pp. 30–32.


• Seeman E. "Osteoporosis in men, the silent epidemic strikes men too" IOF; 2004.