SERUM AND TISSUE OSTEOPONTIN IN PATIENTS WITH KELOIDS

Thesis

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Christina Hany Riad

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List of Abbreviations

Abb.	Full term
AK	*Actinic Keratosis*
BCC	*Basal cell carcinoma*
BD	*Behcet disease*
BSP-1	*Sialoprotein I*
CPB	*Carboxypeptidase B*
CTGF	*Connective tissue growth factor*
DCs	*Denderitic cells*
DNA	*Deoxyribonucleic acid*
ECM	*Extracellular matrix*
EGF	*Epidermal growth factor*
ETA-1	*Early T-lymphocyte activation*
FGF	*Fibroblast growth factor*
HLA	*Human leucocytic antigen*
IFN	*Interferon*
IGF	*Insulin-like growth factor*
IL	*Interleukin*
iNOS	*Inducible nitiric oxide synthetase*
iOPN	*Intracellular osteopontin*
MCP	*Monocyte chemoattractant protein*
MMP	*Matrix metalloproteinase*
OPN AS ODN	*Osteopontin antisense oligodeoxynucleotides*
OPN	*Osteopontin*
OPN-FL	*Full length osteopontin*
OPN-R	*Thromin cleaved osteopontin*
OPNs	*Secreted form of osteopontin*
PAI	*Plasminogen activator inhibitor*
PDGF	*Platelet derived growth factor*
POSAS	*Patient and observer scar assessment scale*
PV	*Pemphigus vulgaris*

List of Abbreviations (Cont...)

Abb.	Full term
RGD	*Arginine-Glycine-Aspartic acid*
SCC	*Squamous cell carcinoma*
SLE	*Systemic lupus erythematosus*
SPP1	*Secreted phosphoprotein 1*
SSc	*Systemic sclerosis*
TGF	*Transforming growth factor*
Th	*T helper*
TIMP	*Tissue inhibitor of matrix metalloproteinases*
TNF	*Tumor necrosis factor*
uPA	*Urokinase plasminogen activator*
UV	*Ultraviolet*
VDRE	*Vitamin D response element*
VEGF	*Vascular endothelial growth factor*
VSS	*Vancouver scoring system*

INTRODUCTION

A keloid is an abnormal fibrous tissue outgrowth which extends beyond the borders of the wound. It does not usually regress spontaneously and possesses high chances of recurrence after excision. Keloids grow faster and become raised and thickened within 3 to 4 weeks ***(Uzair et al., 2015)***. They can occur anywhere on the body but the most common sites are the sternum, shoulders, earlobes, and cheeks ***(Juckett and Hartman-Adams, 2009).***

Keloids are only found in humans and occur in 1% to 16% of the population and tend to be familial. People with darker pigmentation are more likely to develop keloids. It is thought that men and women are equally affected. However, women present more frequently than men, possibly due to cosmetic concerns. The average age at onset is 10 to 30 years. People older than 65 years rarely develop keloids. There is a lack of population-based studies, and much of the epidemiology remains anecdotal rather than scientific ***(Cavalié et al., 2015).***

Excessive keloids form as a result of aberrations of physiologic wound healing and may develop following any insult to the deep dermis, including burn injury, lacerations, abrasions, surgery, piercings and vaccinations. By causing pruritus, pain and contractures, excessive scarring can dramatically affect a patient's quality of life, both physically and psychologically ***(Gauglitz et al., 2013)***. They may also

occur after acne formation or chickenpox infection and sometimes keloids can be formed spontaneously in sternal area in susceptible individuals ***(Monarca et al., 2012).***

Most keloids develop within 3 months of the injury but some may occur up to 1 year after skin trauma. There are several theories of keloid etiology, most of which are related to fibroblast dysfunction. Keloid fibroblasts, when compared with fibroblasts isolated from a normal wound, overproduce type I procollagen and express higher levels of certain growth factors including vascular endothelial growth factor (VEGF), transforming growth factor (TGF) β1 and β2, and platelet-derived growth factor (PDGF). In addition, these cells have lower rates of apoptosis and demonstrate a down regulation of apoptosis related genes, including p53 ***(Chike-Obi et al., 2009)***.

Osteopontin (OPN) is a multifunctional matricellular protein produced by a wide range of cells including osteoclasts, osteoblasts, T cells, macrophages, dendritic cells, and fibroblasts ***(Anborgh et al., 2011)*** and researches have defined a role for osteopontin in maintenance and reconfiguration of tissue integrity during inflammatory processes ***(O'Regan & Berman, 2000)***.

Osteopontin promotes inflammation through the recruitment of macrophages, dendritic cells and T cell and contributes to the development of Th1 cytokine responses

(Rittling, 2011). Moreover, it regulates fibroblast behavior and myofibroblast differentiation ***(Lenga et al., 2008)***.

Expression of OPN in normal, healthy skin is low but increased during wound healing ***(Chang et al., 2008)***.

Osteopontin has a key role in the synthesis and turnover of matrix components in both human and murine models, thus it was speculated that serum OPN level might have an impact on the resulting scar after wound healing ***(Pardo et al., 2005)***.

Based on previous studies suggesting a role of OPN in fibrous tissue formation ***(Miragliotta et al., 2016)***, we hypothesize that OPN may contribute to inflammation-associated fibrosis and accordingly keloid formation in skin wounds.

AIM OF THE WORK

The aim of this study is to compare serum and tissue osteopontin levels in patients with keloids with age and sex-matched healthy controls, and to correlate their levels with the extent of keloid formation according to specific scoring systems (Vancouver Scar Scale "VSS" and Patient and Observer Scar Assessment Scale "POSAS").

Chapter 1
KELOIDS

Keloids are defined as excessive scar tissue that invade beyond the borders of the initial insult and do not regress spontaneously. They recur in 45–100% of cases following excision. This is due to the fact that the new closure is exposed to the same mechanical, immunological and biochemical forces as the original scar ***(Juckett and Hartman-Adams, 2009)***.

Areas particularly prone to keloids include the earlobes, chest, shoulders, upper back and posterior neck. Minor keloids are focally raised, pruritic scars that can occur up to one year following the initial injury. Major keloids are large, raised (>5 mm), dark red scars associated with pain and pruritus and continue to increase in size over years ***(McGoldrick et al., 2017).***

The first description of keloids concerned surgical techniques used in Egypt in 1700 BC. In 1806, Alibert used the term cheloide, derived from the Greek chele, or crab's claw, to describe the lateral growth of tissue into unaffected skin ***(Al-Attar et al., 2006)***.

Many years later, studies differentiated excessive scarring into hypertrophic and keloid scar formation. By their definition, both scar types rise above skin level, but while hypertrophic scars do not extend beyond the initial site of

injury, keloids typically project beyond the original wound margins. Nevertheless, clinical differentiation between hypertrophic scars and keloids can be problematic ***(Meenakshi et al., 2005)***.

Epidemiology of keloid:

Keloids are seen in people of all races and skin types. The prevalence of keloid formation in the general population is relatively low, with a higher incidence in persons of color. Specifically, African Americans have demonstrated an incidence of 6 to 16 percent, likely a result of underlying genetic propensity toward scarring. Furthermore, the overall incidence of hypertrophic scarring is highly raised following burn or thermal injuries ***(Kirby et al., 2016)***.

Keloids tend to affect both sexes equally, although there is a higher incidence of women presenting with keloids, possibly secondary to the cosmetic implications associated with the disfigurement ***(Roseborough et al., 2004).***

It is rare in albinos of all races. Familial predisposition, with both dominant and recessive modes of inheritance have been recognized ***(Gauglitz et al., 2011)***.

Etiology of keloid:

Several etiological factors for keloids have been proposed in the past including; the presence of foreign bodies

in the wound site, infection, tension present in the local skin environment, delayed healing, prolonged excessive inflammation and abnormal epithelial–mesenchymal interactions ***(Alonso et al., 2008)***.

Keloid growth may also be stimulated by various hormones. Results from some studies have suggested a higher incidence of keloid formation during puberty and pregnancy, with a decrease in size after menopause. Also, immunologic associations of keloids had been proposed ***(Schierle et al., 1997)***.

One study revealed a direct correlation between the incidence of keloid formation and levels of serum immunoglobulin E, and another study found a higher incidence of allergic symptoms in patients with keloids ***(Placik and Lewis, 1992)***.

Genetics of keloid:

There is a familial predisposition to keloid scarring. TGF-β has previously been implicated in keloid pathogenesis. There is an association between keloid development and polymorphisms within the TGF-β1, -β2, -β3 and TGF-β receptor genes ***(Bayat et al., 2005)***.

Keloid has an autosomal dominant inheritance pattern with an identified linkage to chromosome 2q23 and