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Effect of sex-hormone levels, sex, body mass index and other host factors on human craniofacial bone regeneration with bioactive tricalcium phosphate grafts

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ABSTRACT

Little is known regarding the associations between sex-hormone levels, sex, body mass index (BMI), age, other host factors and biomaterial stimulated bone regeneration in the human craniofacial skeleton. The aim of this study was to elucidate the associations between these factors and bone formation after sinus floor augmentation procedures (SFA) utilizing a bioactive tricalcium phosphate (TCP) bone grafting material. We conducted a prospective study in a human population in which 60 male and 60 female participants underwent SFA and dental implant placement using a staged approach. BMI as well as levels of serum estradiol (E2), total testosterone (TT), and the free androgen index (FAI) were measured by radioimmunoassay and electrochemoluminescent-immunoassay. At implant placement, 6 months after SFA, bone biopsy specimens were harvested for hard tissue histology, the amount of bone formation was evaluated by histomorphometry and immunohistochemical analysis of osteogenic marker expression. The Wilcoxon rank-sum *U* test, Spearman correlations and linear regression analysis were used to explore the association between bone formation and BMI, hormonal and other host factors. BMI and log E2 were significantly positively associated with bone formation in male individuals ($p < 0.05$). Histomorphometry revealed trends toward greater bone formation and osteogenic marker expression with non-smokers compared to smokers. In male patients, higher E2 levels and higher BMI enhanced TCP stimulated craniofacial i.e. intramembranous bone repair.

1. Introduction

Although the number of bone regenerative procedures performed in the craniofacial domain utilizing bioactive calcium phosphate based bone grafting materials has steadily increased over the last two decades, our knowledge regarding the effect of patient-individual parameters such as sex, age, BMI, endocrinological and life-style related factors on craniofacial bone regeneration and formation is extremely scarce [1,2]. Expanding this knowledge base, however, is of paramount importance, with respect to tailoring and optimizing treatment regimes and the selection of appropriate biomaterials for individual patients in view of pursuing an individualized orofacial medicine. Bioactive calcium phosphate-based ceramics and glasses are known to stimulate bone cell function and bone tissue formation [3,4]. Therefore, elucidating how patient host factors, which are *known*

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to influence biological processes that regulate bone formation, remodeling and homeostasis, affect these biomaterial-mediated effects on bone formation is of considerable interest with respect to the clinical use of bioactive calcium phosphate bone grafting materials. A slightly greater body of knowledge regarding the role of these host factors on bone repair and regeneration exists in orthopedics, and specifically with respect to osteoporosis related fractures [5–10]. In this context, it is noteworthy that during embryological skeletal development the flat bones of the craniofacial skeleton are formed by intramembranous ossification [11], while the long bones of the extremities are formed by endochondral ossification [12,13]. Animal studies on fracture repair have shown that, while endochondral and intramembranous bone repair have common features, unique differences exist with respect to cellular and molecular factors and mechanisms which regulate both [13,14]. In addition, histologic evaluation and histomorphometric assessment are powerful tools for studies on bone repair and regeneration. Accurate histological measurements of the repair process are crucial to validate therapeutic efficacy and address questions about cellular and tissue level responses to biomaterials during repair and regeneration. These measurements can be integrated with radiological, biomechanical and molecular data, thereby providing a comprehensive set of outcome information that corroborate each other. Hence, integration of morphological outcome measurements with radiological and or biomechanical data provides significantly more compelling information than either one alone [15].

In clinical studies, dealing with biomaterial stimulated bone repair and regeneration, however, sampling bone biopsy specimens is rarely possible, as most reparative orthopedic procedures do not include a reentry phase, in which removal of newly formed bone tissue for endosseous implant placement is required. As a result, in orthopedics clinical outcomes as well as the effect of various patient individual factors on these outcomes can only be assessed by less substantive radiological data, standardized clinical pain scales and quality-of-life assessment questionnaires [16,17]. In contrast, in craniofacial surgery bone regenerative procedures exist, in which a staged approach is applied, which features a reentry procedure for dental implant placement into the newly regenerated osseous tissue after biomaterials implantation. Surgical preparation of the implant bed necessitates removal of osseous tissue, which rather than being discarded can be processed for histomorphometric assessment without violating generally accepted ethical standards for patient treatment. In addition, translation of biomaterials to the clinic requires generating knowledge regarding their potency, efficacy and performance in various patient populations and varying anatomical sites. This is of utmost importance with respect to optimizing biomaterials and treatment regimes as well as selecting the appropriate biomaterial for a given clinical situation. Thus, the current study tested the hypothesis that sex-related host factors may affect calcium phosphate stimulated bone formation. As a result, this study evaluated the effect of sex, sex-hormone concentrations, BMI and age on craniofacial bone regeneration six months after sinus floor augmentation using a β-tricalcium phosphate (TCP) bone grafting material in a cohort of 120 patients controlled for gender. To this end, the effect of these parameters on bone formation and osteogenic marker expression was examined utilizing undecalcified hard tissue histology in combination with histomorphometric and immunohistological analyses [18–20]. In addition, a number of host factors were recorded, which are either clinically known or are suspected to quite likely affect bone formation and bone cell function such as smoking, diabetes, alcohol intake and physical exercise.

2. Materials and methods

2.1. Study design

We conducted a prospective study in a human population, in which 60 male and 60 female participants underwent sinus floor augmentation procedures using a β-tricalcium phosphate (TCP) bone grafting material and dental implant placement using a staged approach. Medical history and lifestyle information was obtained by questionnaire. This was in addition to determining the BMI (Body mass index). At implant placement, 6 months after sinus floor augmentation using a β-TCP synthetic bone grafting material, bone biopsy specimens were harvested for hard tissue histology, the amount of bone formation was evaluated by histomorphometry and immunohistochemical analysis of osteogenic marker expression. Levels of serum estradiol (E2), total testosterone (TT), free testosterone, sex hormone binding globulin (SHBG), and the free androgen index (FAI) were measured by radioimmunoassay (RIA) and electrochemoluminescent-immunoassay (ECLIA).

The Freiburg Ethics Commission International approved the study (study code ZD-MA-MS-2013-1, reference number 013/1294). All participants were fully informed about the procedures, including the surgery, bone substitute materials and implants, and provided written informed consent (registry number DRKS-00007538).

2.2. Bone grafting material

A commercially available β-TCP-based bone grafting material, which consisted of pure synthetic β-TCP granules, was used in this study. The TCP granules were polygonal particles of 700–1400 μm grain size with 70% porosity (material denominated TCP) (CEROS TCP®, Mathys, Switzerland). The bulk density of the granules was 0.65 ± 0.02 g/cm^3. Fabrication and material properties of these TCP granules have been described in detail previously [20].

2.3. Participants

Participants were recruited from a single specialized center in oral implantology and maxillofacial surgery in Berlin, Germany. Inclusion criteria were partial edentulism in the post-canine region, and that sinus floor augmentation was required in order to facilitate dental implant placement in the posterior maxilla with the height of the residual alveolar crest being less than 3 mm. The width of the alveolar crest, however, had to be greater than 6 mm so as to facilitate sampling bone biopsy specimens in a safe and easy manner. In addition, patients had to be in good general and oral health without any active periodontitis. Both smokers and non-smokers were included in the study. Exclusion criteria were: Pathological changes of the Schneiderian membrane, compromised health (ASA (III or IV) – American Society of Anaesthesiology) drug abuse, including alcohol abuse, or any significant systemic disease. The study comprised a consecutive series of 120 eligible participants, 60 women (ranging in age from 30 to 79 years, mean 60.7 years) and 60 men (aged 24–80, mean 58.3), in whom sinus floor augmentation procedures were performed. All patients returned for implant placement 6 months after sinus floor augmentation. Consequently, there were no drop-outs. Patient characteristics are shown in Table 1.

2.4. Hormone measurements

Blood samples (5 ml) were collected from participants between 8 and 10 a.m. after an overnight fast. Estradiol and SHBG concentration were measured using an electrochemiluminescent-