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Plasma insulin-like factor 3 (INSL3) in male patients with osteoporosis and Klinefelter's syndrome

Andleeb Hussain[1], Ramasamyiyer Swaminathan[2], Arun Sankaralingam[2], Paul V. Carroll[1], Geeta Hampson[1], Barbara M. McGowan[1,*]

[1] Department of Diabetes and Endocrinology, Guy's & St Thomas' NHS Foundation Trust
[2] Department of Chemical Pathology, Guy's & St Thomas' NHS Foundation Trust

Summary

Insulin-like factor 3 (INSL3) is a peptide hormone produced in leydig cells of the testes. Its role in the adult male is unknown but INSL3 and its receptor RXFP2 have been linked to bone cell differentiation. It is speculated that low levels of INSL3 could be responsible for low bone mineral density in patients with primary osteoporosis and Klinefelter's Syndrome.
The aim of this study was to assess plasma INSL3 in patients with osteoporosis and Klinefelter's Syndrome compared to healthy males.
Fourteen healthy males, 21 males with osteoporosis (4 primary and 17 secondary) and 4 patients with Klinefelter's Syndrome were studied. Plasma INSL3, testosterone, LH, FSH and Sex hormone-binding globulin were evaluated.
Plasma INSL3 concentrations were similar in osteoporosis patients compared to healthy controls (0.72 vs. 0.69 ng/mL, p=0.26). INSL3 was significantly higher in patients with primary osteoporosis (n=4) compared to age-matched healthy controls (n=8) (0.845 vs. 0.665 ng/mL, p=0.021). INSL3 levels in Klinefelter's Syndrome patients were significantly lower compared to healthy controls (0.39 vs. 0.69 ng/mL, p=0.01).
Plasma INSL3 levels were lower in Klinefelter's Syndrome reflecting testicular failure. INSL3 levels were not lower in men with osteoporosis. The relationship between INSL3, its receptor and bone metabolism requires further study.

Key words

INSL3, RXFP2, Osteoporosis, hypogonadism, Klinefelter's Syndrome

Study

Insulin-like factor 3 (INSL3) is a member of the relaxin peptide family and produced in the leydig cells of the testes in the adult male. INSL3 is understood to be involved in the first trans-abdominal phase of testicular descent in the male foetus however little is known about the function of INSL3 in adult males (Ivell and Anand-Ivell, 2011). INSL3 binds to its receptor RXFP2 and the INSL3/RXFP2 signalling pathway in the human osteoblast has been determined (Ferlin *et al*. 2011).

INSL3 concentrations remain constant throughout life, only declining in the ageing male (Anand-Ivell *et al*. 2006). They are also low in hypogonadal Klinefelter Syndrome (KS) (47 XXY) patients (Bay *et al*. 2005). It was therefore suggested that low INSL3 levels could contribute to low bone mineral density in some men with osteo-

* Corresponding Author: Guys' Hospital, Diabetes & Endocrine Department, 3rd Floor Southwark Wing, Great Maze Pond, London SE1 9RT; E-mail: Barbara.McGowan@gstt.nhs.uk; Tel: 020 7188 1915. Fax: 020 7188 1926.

porosis or KS (Ivell and Anand-Ivell, 2011; Ferlin *et al*. 2008; Ferlin *et al.* (2010); Ferlin *et al.* (2011)).

The aim of this study was to determine whether INSL3 levels were reduced in male patients with osteoporosis compared to healthy subjects and KS patients.

The following groups of subjects: 14 healthy adult men, 4 KS (47 XXY) patients, and 21 males with osteoporosis were studied. Consent was obtained and plasma samples were collected. This study was approved by the Norfolk Research Ethics Committee.

INSL3 was measured using a commercial enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc. Burlingame, CA). Testosterone, LH, FSH and sex-hormone-binding globulin (SHBG) were measured using the Cobas e ® (Roche Diagnostics) by Electrochemiluminescence immunoassay.

Comparison for baseline characteristics including age, testosterone (total and free), LH, FSH, SHBG and INSL3 were tested for statistical significance using the Mann-Whitney U test for non-parametric data. Significance was defined as $P<0.05$.

Our results show that there was no significant difference ($p=0.2662$) in INSL3 levels between healthy controls and osteoporosis patients [Healthy controls n=14, 0.69 (0.175) ng/mL, osteoporosis patients n=21, 0.72 (0.3) ng/mL and KS patients n=4, 0.39 (0.11) ng/mL; INSL3 plasma levels are shown as median values with interquartile range in bracket]. Plasma INSL3 levels were significantly lower in KS patients compared to healthy controls ($p=0.0066$) and osteoporosis patients ($p=0.0054$).

In the osteoporosis group, 4 patients had primary osteoporosis and the remaining 17 had secondary osteoporosis. Eight healthy controls were subsequently age-matched to the primary osteoporosis group. INSL3 levels were significantly higher in patients with primary osteoporosis compared to the age-matched healthy controls (p

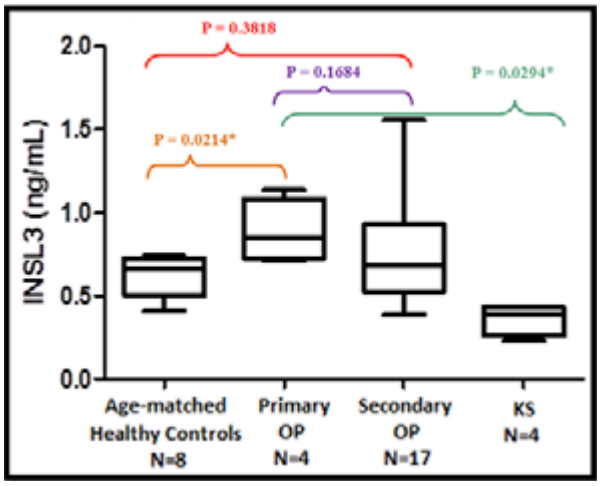

Figure 1. INSL3 concentrations (ng/mL) in age-matched healthy controls, primary and secondary osteoporosis patients and KS patients. The box plots display the median (middle line), the interquartile range (25th percentile and 75th percentile, represented by the box). The bottom whisker represents the 2.5th percentile and the top whisker represents the 97.5th percentile. * = $p<0.05$.

= 0.0214): [Healthy controls aged-matched to primary osteoporosis n=8, 0.665 (0.128) ng/mL, primary osteoporosis n=4, 0.845 (0.205) ng/mL, secondary osteoporosis n=17, 0.68 (0.33) ng/mL; INSL3 plasma levels are shown as median values with interquartile range in bracket]. Biochemical characteristics including testosterone (free and total), FSH, LH and SHBG were not significantly different between groups.

A significant difference in INSL3 was seen between primary osteoporosis (n=4) and KS patients (n=4) (p=0.0294). No significant difference in INSL3 was seen between the primary and secondary osteoporosis patients (p= 0.1684), or between healthy controls (n=8) and secondary osteoporosis patients (p=0.3818) (Figure 1).

Currently there are no published studies on circulating INSL3 in male patients with osteoporosis.

Our results show that plasma INSL3 levels are significantly higher between patients with primary osteoporosis compared to age-matched healthy subjects. Other biochemical characteristics were not statistically significant between the groups. This suggests that there may be a unique defect present in males with primary osteoporosis resulting in these high circulating INSL3 levels. Alternatively, the role of INSL3 in bone physiology may be more complex than first thought. This preliminary study was limited by the small number of participants.

In conclusion, our data does not support the hypothesis that low INSL3 levels are present in males with osteoporosis. Our data further confirms previous studies showing low INSL3 levels in KS patients. Further work is required in order to validate these findings.

Acknowledgements

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