Feasibility Of Immunological Markers And Osteocalcin As A Barometer Of Glucocorticoid Replacement

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Introduction

Adrenal Insufficiency (AI) is a condition characterised by an inability to produce sufficient amounts of cortisol to match the body's requirement. Whilst survivability has improved since the introduction of exogenous steroid replacement, this condition is still associated with increased mortality and morbidity[1]. It is possible that this is because replacement is challenging and replicating the diurnal cortisol rhythm with oral hydrocortisone tends towards over-replacement, as demonstrated in Figure 1. The absence of an objective marker makes gauging the adequacy of steroid replacement very difficult. It has been shown that excess glucocorticoids lead to increased bone loss as well as immune suppression.
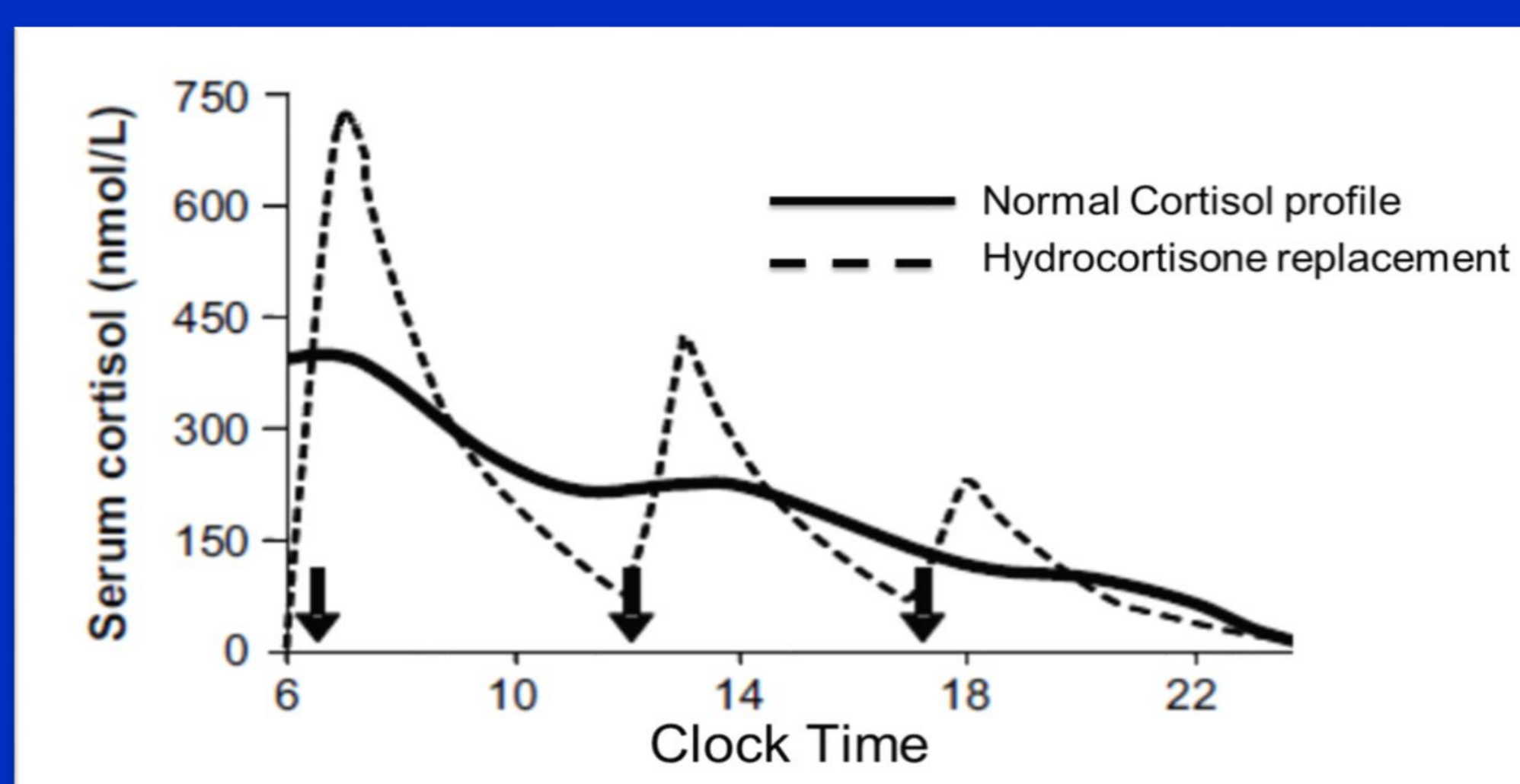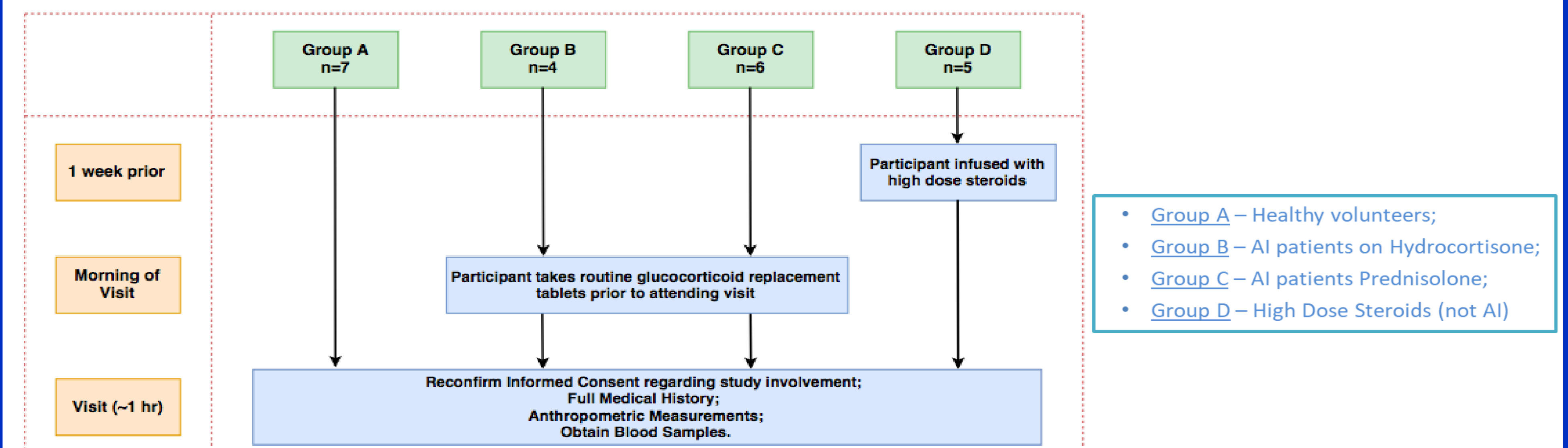

Figure 1: Diurnal Rhythm of Cortisol[2]

Aim

To investigate a selection of novel bone or immunomarkers which may act as indicators for steroid replacement in Adrenal Insufficiency (AI).

Materials & Methods

- Group A – Healthy volunteers;
- Group B – AI patients on Hydrocortisone;
- Group C – AI patients Prednisolone;
- Group D – High Dose Steroids (not AI)

This is a pilot cross-sectional study looking at 22 participants who were split into four groups based on the dose of exogenous steroid administered (high-dose steroids, replacement dose hydrocortisone, replacement dose prednisolone and healthy controls). Blood samples and anthropometric data were collected from participants. Carboxylated-Osteocalcin (Gla-OC) and bone-related immunological cytokines were investigated using a Takara Osteocalcin kit and Multiplex ELISA on Luminex platform respectively.

Figure 2: Effects of various dose regimes of exogenous steroids on carboxylated-osteocalcin (Gla-OC) levels
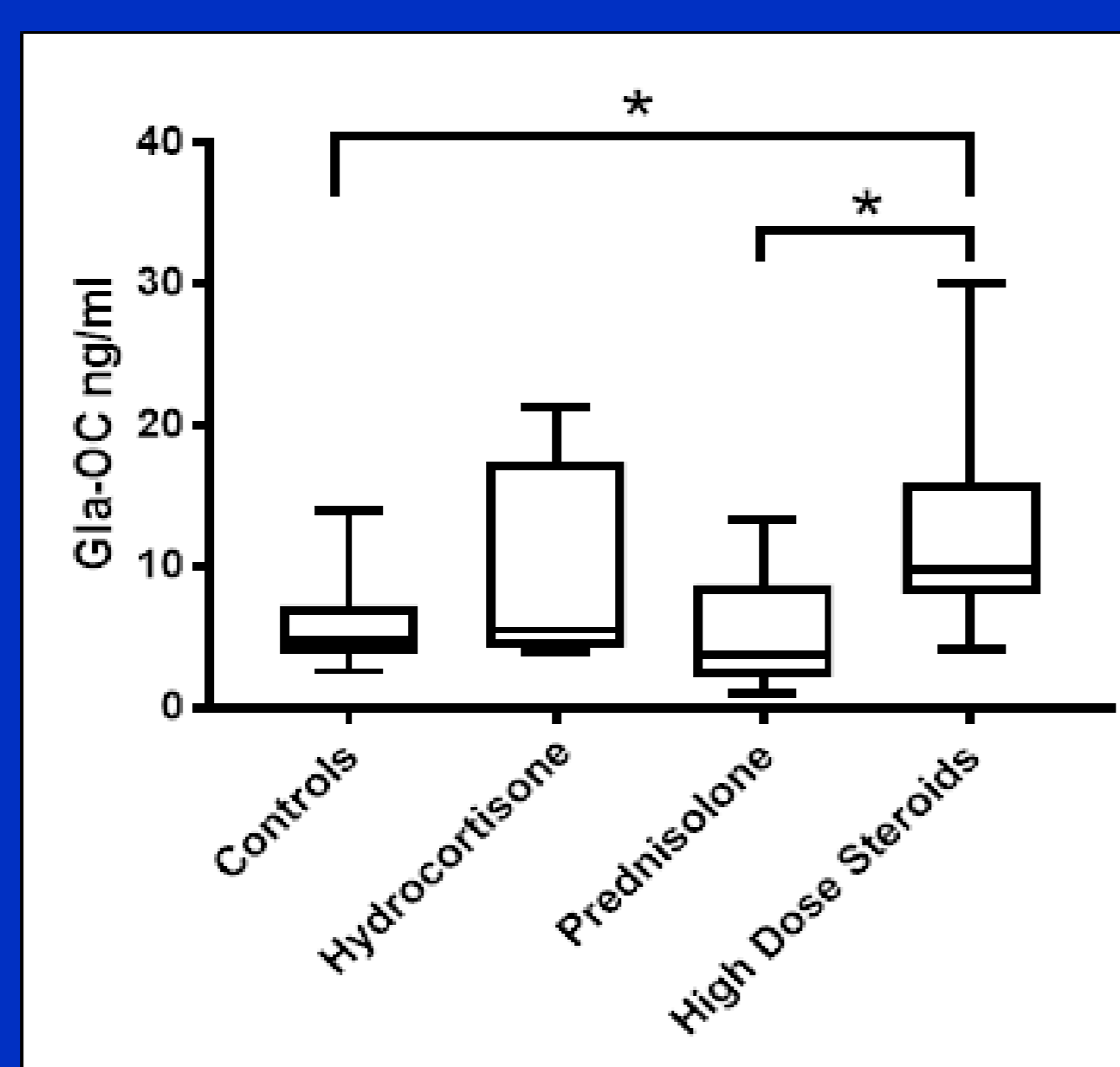
The high-dose steroid group had a significantly higher Gla-OC vs control (9.78ng/mL vs 4.70ng/mL, P=0.034) and vs the prednisolone group (9.78ng/mL vs 3.78ng/mL, P=0.032).

Figure 3: Effects of various dose regimes of exogenous steroids on Interleukin-4 (IL-4) levels
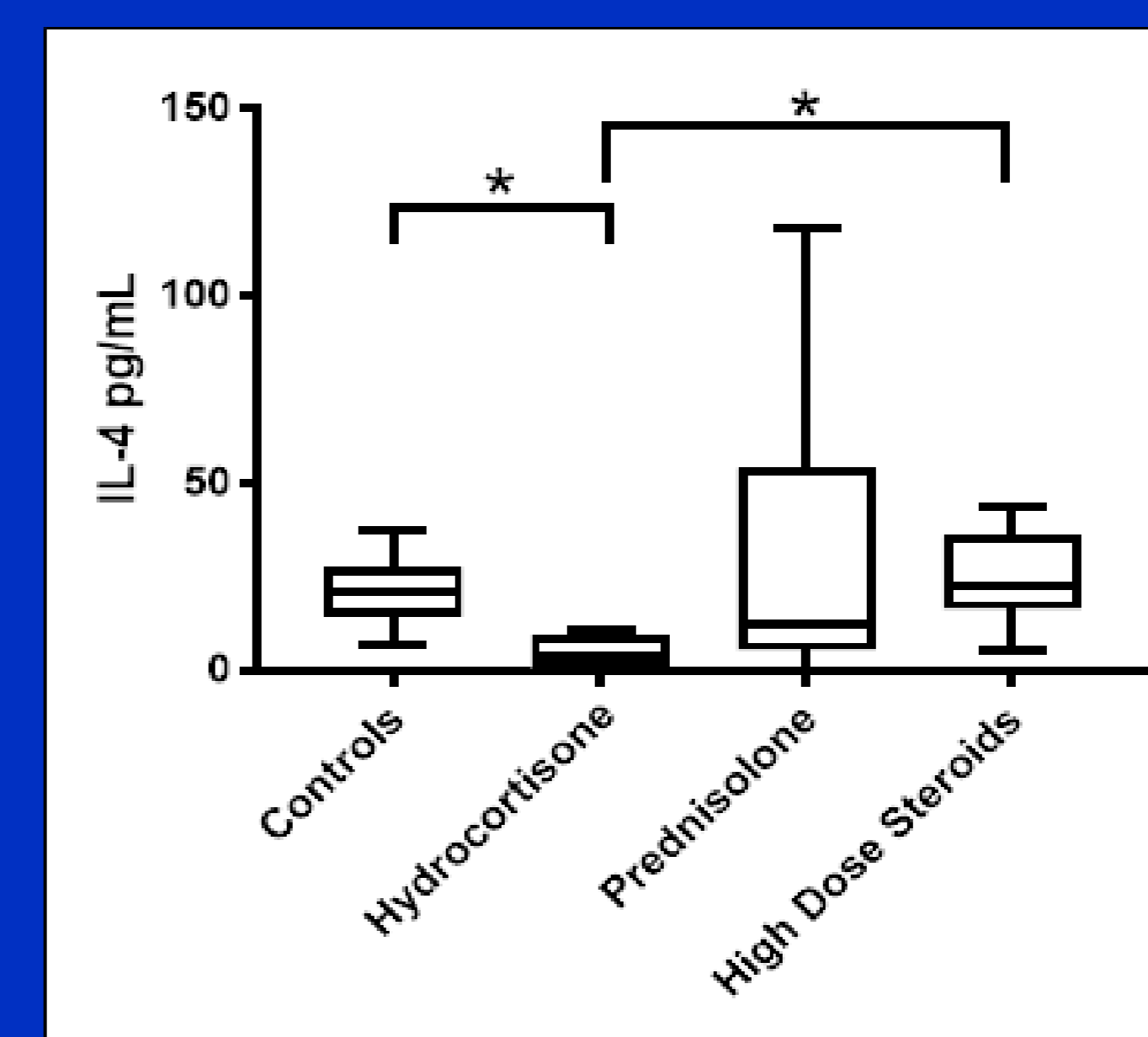
IL-4 was significantly higher between the high-dose steroid vs hydrocortisone group (22.6ng/mL vs 3.52ng/mL, P=0.033) and between the control vs hydrocortisone group (21.0ng/mL vs 3.52ng/mL, P=0.032).

Discussions

Gla-OC is exclusively produced by osteoblasts, hence acts as a marker of bone formation. Studies have also shown that dexamethasone directly suppresses the osteocalcin gene in osteoblasts[3]. Paradoxically, in our study, the high dose steroid had the greatest levels of Gla-OC.
- It is possible that the high-dose steroid group experiencing a compensatory rise in bone formation over the course of the week
    - Participants were screened on day 7 after their IV methylprednisolone
- Participants in control group had a significantly higher BMI
    - Polgreen et al[4] has demonstrated an inverse relationship between Gla-OC and BMI, perhaps this confounding factor is suppressing Gla-OC levels in the control group

IL-4 is secreted by activated T-cells and is primarily involved in regulating antibody production. However, it has an important role in inhibiting excess bone formation as well
- In-vitro study by Mangashetti et al[5] demonstrated a dose dependent relationship between IL-4 and inhibition of RANKL induced bone resorption by mature osteoclasts. RANK receptor-RANKL interaction results in the propagation of downstream signalling by increasing intracellular ionized calcium (Ca^{2+}). IL-4 inhibits this rise in intracellular Ca^{2+}, thus preventing the downstream signalling and osteoclast maturation.
- IL-4 levels support theory that high-dose steroid group is experiencing bone formation.

Conclusion

The discovery of an objective novel marker could pave the way for improved quality of life and reduced morbidity and mortality in patients with AI. This study has demonstrated that Gla-OC and IL-4 show significant detectable changes between healthy controls, steroid replacement regimens and anti-inflammatory steroid regimens. They display potential to be long-term markers of steroid replacement and a larger prospective study to evaluate these markers further, is warranted.

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