EFFECT OF GLUCOCORTICOIDS EXPOSURE ON SERUM OSTEOCALCIN LEVELS

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ABSTRACT

Glucocorticoids (GCs) treatment has a profound effect on bone turnover and the mechanism is complex and not elucidated completely. One of the actions on bone explained by many studies is its effect on serum osteocalcin levels. To investigate changes in the osteocalcin levels among patients who are on GCs and to correlate these levels with various doses of different GCs, a prospective observational study on 88 subjects who were on GC therapy was undertaken. Mean age of subjects was 42.29 ±13.86 years, with a marginal female preponderance (58%). Median dose of glucocorticoids was 1mg/kg (0.89, 2.60). Median baseline serum osteocalcin concentration was 4.4ng/mL (2.2, 9.0), which reduced significantly after starting GC therapy to 2.2ng/mL (1.01, 4.74) (p value=0.009). The median dose of prednisolone was 0.9 (0.72, 0.90) and of methylprednisolone 2.6 (2.1, 3.4) mg/kg. The serum osteocalcin level was reduced significantly (p<0.01), irrespective of GCs. We found that osteocalcin levels were negatively correlated with the dose of prednisolone (r= -0.06, p=0.6) and methylprednisolone(r=-0.11 p=0.53). Further, a weak negative correlation was seen in patients taking less than 1mg/kg and more than 1 mg/kg (r=-0.21 p value=0.32 and r=-0.352 p value-0.005), showing greater reduction in patients on higher doses. Osteocalcin reduction is solely dependent on dose of glucocorticoids, not on type of glucocorticoids. This may have important clinical implications and may help to reduce bone related side effects.

Keywords: Glucorticoids, osteocalcin, prednisolone, methyl prednisolone

INTRODUCTION

Exogenous glucocorticoids (synthetic glucocorticoids), which are potent anti-inflammatory agents are normally used in the treatment of both acute and chronic illnessses. Glucocorticoid (GC) treatment has a significant impact on bone turnover and the mechanism is complex and not explained totally[1,2]. One of the actions on bone explained by many studies is its effect on serum osteocalcin levels. Osteocalcin, also known as bone gamma carboxy glutamic acid Gla protein (BGLAP) is a marker of bone formation. It is a vitamin K and vitamin D-dependent protein produced by osteoblasts. Many studies support the observation that reduced bone formation is predominantly responsible for the GC associated bone loss[3,4].

Some studies observed that patients receiving GCs therapy have reduced serum osteocalcin levels. These adverse effects of GCs are acute rather than chronic, and are related to the daily dose of GCs[5,6].

A prospective observational study was undertaken to know the effects of GCs on serum osteocalcin levels, in patients on glucocorticoid therapy at Kasturba Hospital, Manipal, which is a tertiary care centre in Karnataka, India. The aim of the study was to investigate changes in osteocalcin levels among patients who are on glucocorticoids and to correlate these levels with various doses of different glucocorticoids.

MATERIALS AND METHODS

A prospective observational time bound study on 88 non-diabetic subjects, aged between 18 to 70 years, taking glucocorticoids by oral or parenteral route of administration for different conditions, were included.

They were recruited from the outpatients or inpatients at Kasturba Hospital Manipal, Karnataka from December 2015 to December 2017. Ethical clearance was (IEC:

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Table I: Baseline demographics and clinical characteristics

Baseline demographics and clinical characteristics.		No of patients (N= 86)
Age(years) Mean±SD		42.29 ± 13.86
Gender	Male(%)	36(42)
	Female(%)	50(58)
Underlying Disease#	Connective tissue and Autoimmune disorder (%)	57(66.3)
	Lung Diseases (%)	29(33.7)
Type of glucocorticoid used	Prednisolone (%)	46(53.5)
	Methyl Prednisolone (%)	38(44.2)
	Hydrocortisone (%)	2(2.3)

#-Connective tissue and Autoimmune disorder : Systemic lupus erythematosus (SLE), Autoimmune hemolytic anemia(AIHA) Rheumatoid arthritis (RA), Immune thrombocytopenic purpura (ITP)

Lung Disease: Interstitial lung disease (ILD),Chronic obstructive pulmonary disease (COPD),Bronchial Asthma(BA). Sarcoidosis.

207/2015) obtained from the Institutional Ethics Committee (IEC) of KMC & Hospital, Manipal.

Subjects who were pregnant, having major organ dysfunction or acutely ill, those receiving vitamin K and its inhibitors, vitamin D and calcium, glucocorticoids, anti-epileptic drugs, calcitonin, bisphosphonate, estrogen or known patients of osteoporosis were excluded from our study.

Eligible subjects based on inclusion and exclusion criteria, were recruited for the study. Written informed consent was obtained before doing any study related procedures. Detailed history including family history of diabetes and co-morbid conditions were recorded. Basic anthropometric measurements including height, weight, and waist circumference were recorded.

In every case after detailed examination, blood samples (3mL) were collected by venipuncture from peripheral veins under aseptic precautions after an overnight fasting for baseline measurement of osteocalcin. Immediately after fasting sample collection, blood samples were centrifuged. Samples for analysis are stored at -80^{0}C. The same procedure was repeated on the 3rd or 4th day after glucocorticoid administration. Serum osteocalcin was measured by Enzyme Linked Immunosorbant Assay method (ELISA) method. (DIA source hOST-EASIA Kit Belgium) normal value 5-25ng/mL).

STATISTICAL ANALYSES

The continuous variables which were normally distributed were expressed as Mean ±SD, and data not normally distributed were expressed as median and interquartile range. Categorical variables such as gender distribution and type of glucocorticoid, were expressed in percentage. Wilcoxon signed rank test was used to compare baseline and post treatment ostocalcin concentrations. Mann-Whitney test was used to compare median difference of serum osteocalcin between two glucocorticoids and between two dosage groups (less than and more than 1mg/kg). Spearman's rank correlation coefficient was applied to find out the relationship between dose of glucocorticoid therapy and serum osteocalcin level after glucocorticoid therapy. $P<0.05$ was considered to be statistically significant. Statistical analysis was done by using SPSS software version 15.0.

RESULTS

Data of two subjects were found to have uneven results, and were excluded from the analysis. The mean age of the subjects was 42.29 ±13.86 years, and many of them were females (58%) compared to males (42%), 66.3% had connective tissue and autoimmune disorders and 33.7% had lung diseases. 53.5% of patients were treated with prednisolone, 44.2% with methyl prednisolone and 2.3% with hydrocortisone. The demographic details and clinical characteristics measurements are shown in Table I.

We observed weak negative correlation between serum osteocalcin level with age of subjects after GC therapy (r=-0.162, P=0.1).

Table II: Median dose across prednisolone, methyl prednisolone and the respective serum osteocalcin values

	Dose(mg/kg)	Serum osteocalcin before glucocorticoid therapy (ng/mL)	Serum osteocalcin after glucocorticoid therapy (ng/mL)	P value
Prednisolone	0.9(0.72,0.90)[#]	7.01(3.2,10.5)[#]	3.7(1.6,6.0)[#]	<0.01*
Methyl prednisolone (converted to prednisolone)	2.6(2.1,3.4)[#]	3.1(1.4,6.1)[#]	1.44(0.75,3.05)[#]	<0.01*
#-Median and interquartile range *-Statistically significant				

Serum osteocalcin level and glucocorticoid treatment

The median baseline serum osteocalcin concentration in this study was 4.4ng/mL with interquartile range (2.2, 9.0). Three days after glucocorticoids treatment, the osteocalcin level reduced to 2.2ng/mL with interquartile range (1.01, 4.74) and it was statistically significant (p value=0.009). Fig. 1 represents a comparison of serum osteocalcin level before and after glucocorticoid treatment.

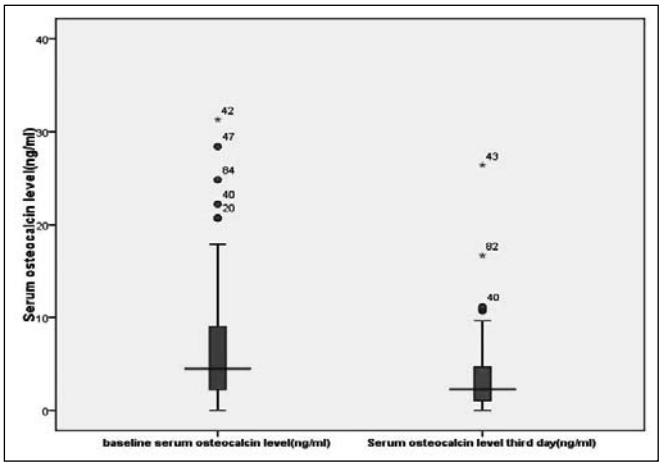

Fig. 1: Comparison of serum osteocalcin level before and after glucocorticoid treatment

Serum osteocalcin level and glucocorticoids types

In our study, most of the patients were treated with prednisolone and methyl prednisolone; so we compared the serum osteocalcin level in prednisolone and methylprednisolone.

Irrespective of glucocorticoids, serum osteocalcin level was reduced significantly after starting therapy.

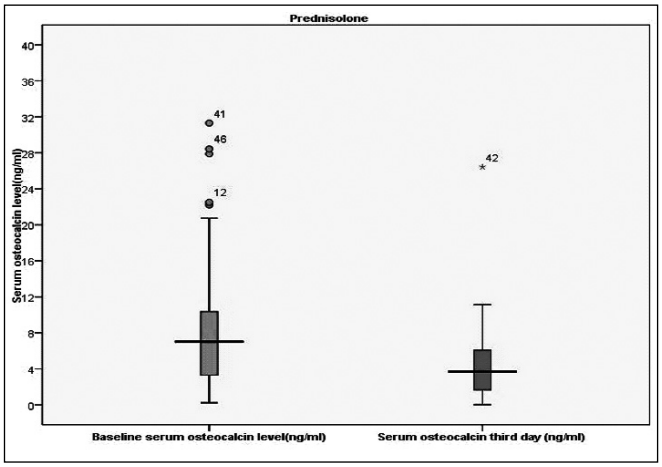

Fig. 2: Comparison of serum osteocalcin before and after prednisolone treatment

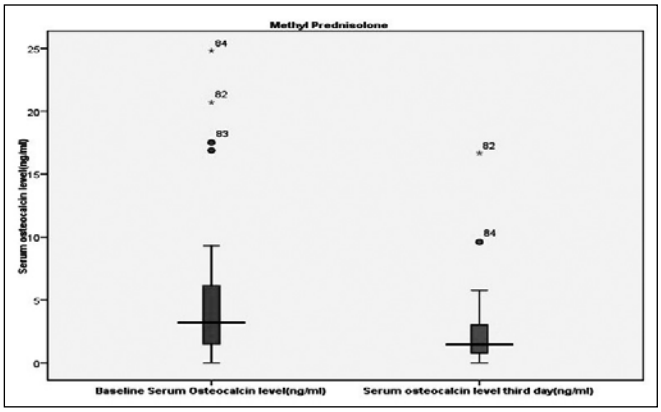

Fig. 3: Comparison of serum osteocalcin before and after methylprednisolone treatment

Figs. 2 and 3 represent the median osteocalcin difference before and after prednisolone and methylprednisolone treatment. There was a weak negative correlation with prednisolone (r= -0.06, p=0.6) and methylprednisolone (r=-0.11 p=0.53).

Serum osteocalcin level and dose of glucocorticoids

Based on their potency, the dose of the glucocorticoids was converted in to equivalent prednisolone dose. The median dose of glucocorticoids prescribed to patients was 1mg/kg with interquartile range (0.89, 2.60). The median dose across prednisolone, methyl prednisolone and the respective serum osteocalcin values are shown in Table II.

There was a weak negative correlation between serum osteocalcin level (ng/mL) after glucocorticoid therapy and dose of glucocorticoid therapy (r= -0.328.p=0.003). Scatter plot depicting the relationship between serum osteocalcin level (ng/mL) after glucocorticoid therapy and dose of glucocorticoid therapy is shown in Fig. 4.

We looked at the osteocalcin levels in patients taking two different dose groups, i.e <1mg/kg and > than 1mg/

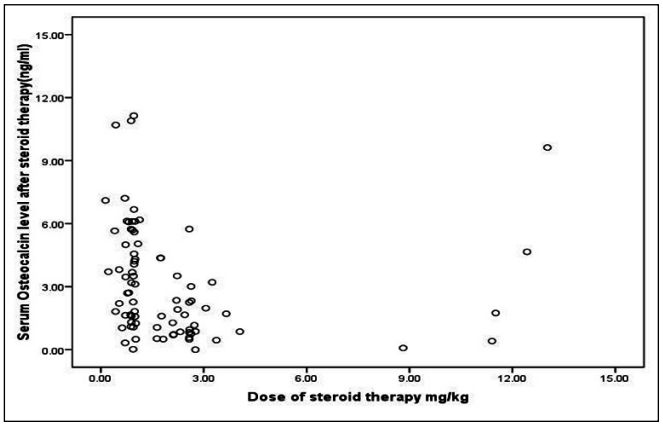

Fig. 4: Scatter plot showing negative correlation between Serum osteocalcin level (ng/mL) after glucocorticoid therapy and dose of glucocorticoid therapy

kg. It showed mild negative correlation in both the groups but significant in higher dosage group(less than 1mg/kg was r=-0.21 and p value=0.32 and more than 1mg/kg is r=-0.352 p value-0.005).

DISCUSSION

Glucocorticoid therapy and its bone related side effects have been known from years. Many studies have shown the relationship between bone markers such as osteopontin osteocalcin, alkaline phosphatase (ALP) and glucocorticoids[4-6].

Among participants in our study, majority of them were females. This was because autoimmune disorders are seen most commonly in females and glucocorticoids are the primary drug of choice in most of the autoimmune disorders[7].

The negative correlation between serum osteocalcin and age indicates that as age increases, the serum osteocalcin level decreases. Bao et al observed similar results in Chinese populations and Ernesto et al in type 1diabetes mellitus patients [8,9]. This was different from the study by Kindbolm et al where they observed a positive correlation with age in Swedish populations[10]. There has been no major studies from India in this regard.

In our study, we demonstrated the significant reduction of serum osteocalcin in patients who were on glucocorticoid therapy. This is similar to the study by Fleishaker et al in American population and Kaichi Kaneko et al in Japanese population[11,12]. Furthermore, this adverse effect was similar in both groups of patients, prednisolone and methyl prednisolone, which is depicted in Table II. The mechanism behind this may be that glucocorticoids inhibit osteoblast differentiation and function, induce mature osteoblast and osteocytes apoptosis, and activate osteoclasts. These multiple effects may result in rapid intense suppression of osteocalcin level[3,13,14].

In this study, we observed a significant weak negative correlation between doses of glucocorticoids with serum osteocalcin level. Further, we observed same trend in two different glucocorticoids, prednisolone and methylprednisolone though statistically not significant. It is well known that osteocalcin levels are lower in patients with stress. Napal et al in their study on Spanish patients documented this[15]. In our study, the baseline serum osteocalcin level of methyl prednisolone group is significantly low compared to prednisolone group. This may be due to methyl prednisolone subjects have more serious illness, causing higher level of stress than prednisolone group.

Later, we divided patients and studied two different dosage groups, those receiving less than and more than 1mg/kg. We observed negative correlation between osteocalcin level and dosage in both the groups. However, it was found that osteocalcin levels were significantly lower in the more than 1 mg /kg groups. Our observation from this study may indicate the reduction of serum osteocalcin is solely depending on dosage, not on what type of glucocorticoids are used. Therefore, by reducing glucorticoid dosage, concentration of osteocalcin may be improved, which is similar to the observation from TOMMOROW study (Total Management of Risk Factors in Rheumatoid Arthritis Patients to Lower Morbidity and Mortality,) in rheumatoid arthritis patients[15].

These findings have potentially important clinical implications because for many diseases, glucocorticoids

are still the first line drugs. According to literature search and our knowledge, this may be the first study to compare the reduction in serum osteocalcin level in two different glucocorticoids in Indian population.

This study has some limitations. We compared only two types of steroids as these were commonly used in our setup. Several other types of steroids have not been analysed in our study. We also did osteocalcin levels on the third day. Hence, we could not comment on the change in osteocalcin levels after first dose steroids. In conclusion, the reduction in osteocalcin level following glucocorticoid therapy is solely dependent on the dose of glucocorticoids and not on the type of GCs. Hence, by using minimum dose of GCs the reduction in osteocalcin level may be kept at minimum and the adverse effects are minimised.

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