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Short communication

Calcitriol, but not FGF23, increases in CSF and serum of MS patients

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ABSTRACT

The anti-inflammatory role of the active metabolite of vitamin D, namely 1, 25-dihydroxyvitamin D_3 (calcitriol), has been reported in multiple sclerosis (MS). Moreover, recent studies have shown that fibroblast growth factor 23 (FGF23) is involved in the regulation of calcitriol biosynthesis. The probable changes of FGF23 and calcitriol concentrations in the CSF and serum of patients with MS were evaluated. Calcitriol concentration in the CSF and serum of MS patients was significantly higher than that in non-MS patients, while FGF23 concentration in MS patients was comparable to controls. We concluded that calcitriol concentration increases in the CSF and serum of MS patients independent of FGF23 status.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease which genetic and environmental factors are contributed to its etiology and pathogenesis (Bina et al., 2017; Steinman, 2014). To date, several anti-inflammatory drugs such as interferon beta-1a, natalizumab, and fingolimod (FTY720) have been developed against MS-induced inflammation (Cross and Naismith, 2014). 1, 25-dihydroxyvitamin D_3 (calcitriol) is an example of an endogenous anti-inflammatory factor with previous studies reporting its therapeutic potential in animal models of MS (Chang et al., 2010) and also MS patients (Wingerchuk et al., 2005). In addition, vitamin D_3 deficiency and its related genes are considered as important underlying factor in the etiology of MS (Pahlevan Kakhki et al., 2018; Smolders et al., 2008). Calcitriol is synthesized in renal cells and some other cells from its precursor, namely 25-hydroxyvitamin D_3, and acts as the bioactive metabolite of vitamin D_3 in the human body (Christakos et al., 2015). Recent studies have shown that, in addition to parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23) and Klotho protein are also involved in regulation of calcitriol biosynthesis (Khundmiri et al., 2016). In fact, in response to overproduction of calcitriol by the kidneys, FGF23 is secreted from osteocytes into the bloodstream and then by the help of Klotho protein, FGF23 binds to its receptor on renal cells and inhibits the biosynthesis of calcitriol (Kuro-o, 2008).

Recently, we found that Klotho concentration in the cerebrospinal fluid (CSF) of MS patients decreased in comparison to control individuals (Aleagha et al., 2015). Interestingly, there was a significant negative correlation between Klotho changes in CSF and the expanded disability status scale (EDSS) of MS patients (Aleagha, Siroos, 2015). Moreover, we reported that Klotho concentration in the serum of MS patients with prolonged disease duration tends to be higher than that in healthy individuals (Ahmadi et al., 2016). Accordingly, it seems that regulators of calcitriol biosynthesis may be involved in MS etiology and/or pathogenesis. However, there is no study regarding the FGF23 and calcitriol status in the CSF and serum of patients with MS. Thus, the aim of the present study was to measure the alterations of calcitriol and FGF23 in the CSF and serum of patients with relapsing-remitting MS (RRMS).

2. Materials and methods

A retrospective, case-control study was performed at Imam Khomeini Hospital, Tehran, Iran. This study was approved by the ethics committee of Tehran University of Medical Sciences (TUMS) according to the Declaration of Helsinki. Altogether, 14 patients (cases) who had been newly diagnosed with RRMS along with 14 controls with other non-inflammatory neurological disorders such as chronic headache, idiopathic intracranial hypertension (IIH) and cerebral venous sinus thrombosis (CVST) were recruited in this study. In order to diagnose RRMS patients, the revised version of the McDonald's criteria (Polman et al., 2011) was used by expert neurologists. MS patients were new case, so they had not received any specific treatments such as

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immunosuppressive drugs or vitamin D_3 supplementation. The demographic and clinical features of all individuals were recorded. We received informed consent from all participants to analyze their CSF and serum samples. The serum and CSF of all participants were obtained and sored at −80 °C for a period of < 3 months.

FGF23 was determined in the CSF and serum of all participants using commercial ELISA kit (Human FGF23 ELISA Kit, cat# LS-F4428; LifeSpan BioSciences, Inc.; USA) according to the protocol provided by the manufacturer. The CSF and serum concentrations of calcitriol were also assayed by the ELISA method (Human 1, 25-dihydroxyvitamin D_3 ELISA kit; cat# CSB-E05120h; CUSABIO™; USA). The results for FGF23 and calcitriol were presented as pg/ml and fmol/L, respectively.

Data were analyzed by GraphPad Prism (version 6.01) and SPSS (version 16). In order to compare the mean scores between cases and controls, the unpaired *t*-test was selected. The chi-square test was applied in order to evaluate the sex distribution between MS and non-MS patients. The correlation between different molecular and clinical parameters was gauged by Pearson product-moment or Spearman's rank correlation test. The values are presented as mean ± SD. The statistical significance was considered < 0.05 (P < .05).

3. Results

As shown in Table 1, the case and control groups are well sex and age-matched. In addition, the mean of EDSS is shown in Table 1. The results showed that the calcitriol concentration in the CSF of MS patients and controls was about 1928.5 ± 512.7 fmol/L and 1071.1 ± 227.6 fmol/L, respectively (P < .0001; Fig. 1). The calcitriol concentration in the serum of MS patients was also higher than non-MS patients' (4819.7 ± 460.0 fmol/L Vs. 4441.0 ± 277.6 fmol/L, respectively; P = .0139; Fig. 1). As indicated in Fig. 1, FGF23 concentration in the CSF and serum of MS patients was comparable to that of the controls (P > .05).

The CSF: Serum ratio for calcitriol and FGF23 concentrations was also calculated. The results showed that CSF: Serum ratio for calcitriol in MS patients was approximately 1.6-fold higher than controls' (P < .0001), while regarding CSF: Serum ratio for FGF23, there was no significant difference (P = .5925) between MS and non-MS patients (Fig. 2).

As shown in Fig. 3, the correlation studies in MS patients revealed that there was a significant negative (inverse) correlation between calcitriol concentration in CSF and EDSS (r = −0.3502; P = .0323). In addition, we found significant negative correlations between FGF23 concentration in CSF and EDSS (r = −0.566; P = .035), and calcitriol concentration in serum and age (r = −0.7867; P = .0008).

4. Discussion

It has been shown that the storage form of vitamin D, namely 25-hydroxyvitamin D_3, is lower than normal in the serum of patients with MS (Martinelli et al., 2014). However, there is little information regarding the status of the active metabolite of vitamin D (1, 25-dihydroxyvitamin D_3; calcitriol) in the CSF and serum of MS patients. As an attempt to address this lacuna, in the present study, we investigated the calcitriol changes in CSF and serum of patients with RRMS. In addition, FGF23 concentration, as the upstream regulator of calcitriol biosynthesis, was also targeted to be measured.

Interestingly, in spite of its very low concentration, we were able to detect the calcitriol in patient's CSF, and concentrations in the CSF were almost in the same range as those in serum. In fact, in the present study, we utilized a very high sensitive and specific ELISA kit with ability to measure the calcitriol concentration as low as 250 femtomole per liter (fmol/L). In addition, Balabanova et al. (1984) measured the concentration of calcitriol in the serum and CSF of patients with suspected or proved prolapse of a disc using the high performance liquid chromatography (HPLC) method. In concomitant with our result, they found that calcitriol concentration in the CSF and serum were within the same range. However, there are some more new data that contradict our result. For example, Holmøy and Moen (2010) stated that they were not able to detect calcitriol in the CSF of human. It can be deduced that the measurement of vitamin D metabolites is still a matter of controversy.

Surprisingly, we found that calcitriol concentration in the CSF and serum of RRMS patients was higher than non-MS patients' (Fig. 1). In addition, the CSF: Serum ratio was also higher in RRMS patients when compared to control subjects (Fig. 2). One possible explanation for these results is that calcitriol concentration might first increase in the serum of MS patients, followed by the recruitment of calcitriol into the CSF circuit. In this line, our results showed that there is a significant inverse correlation between CSF calcitriol concentration and the disease severity (EDSS) in MS patients (Fig. 3). Consequently, it is possible that calcitriol, as an anti-inflammatory factor, increased in the serum and CSF of MS patient in order to alleviate MS-induced inflammation and disability (Chang et al., 2010; Wingerchuk et al., 2005).

In addition, we demonstrated that there was a significant negative correlation between serum calcitriol and age of MS patients (Fig. 3). This result corroborates the finding of Smolders et al. (2008), who found a similar correlation. Moreover, Smolders et al. (2008) conducted a cross-sectional study among patients with different types of MS disease. They discovered that calcitriol concentration is higher in the serum of RRMS patients than that of patients with other types of MS disease. However, Smolders et al. (2008) did not compare the calcitriol concentration between MS patients and non-MS controls.

In contrast to our results, Moen et al. (2012) found that there is no significant difference in the serum calcitriol level between MS patients and healthy individuals as controls. In the Moen et al. study the mean duration from first symptom of demyelinating disease to study examination was 1.6 ± 1.3 years (Moen et al., 2012) and patients received medication, while in our study, we recruited new cases which were evaluated immediately after MS diagnosis. In addition, they measured the calcitriol concentration using radioimmunoassay (Moen et al., 2012), while we used ELISA method. Consequently, it is plausible to expect different results from these two studies because of different stages of MS disease which have been evaluated and also different methods used for calcitriol determination.

Another interesting finding of our study was that FGF23 concentration in the CSF and serum of MS patients was unchanged when compared to control subjects (Fig. 1). However, our results showed that there is a significant negative correlation between FGF23 changes in CSF and EDSS of patients with RRMS (Fig. 3). Consequently, it seems that, in spite of its probable role in the pathogenesis of MS disease, FGF23 plays no important role in the calcitriol metabolism of MS patients. In addition, FGF23 is not the sole regulator of calcitriol

Table 1
Demographic and clinical features of controls and patients with MS.

	Control individuals (n = 14)	MS patients (n = 14)	Statistical test (*P* value)
Gender (Female/Male ratio)	10/4	10/4	Chi-square test (P = 1.0)
Age (mean years ± SD)	35.5 ± 8.64	32.28 ± 7.79	Unpaired *t*-test (P = .311)
EDSS* (mean, range of scores)	–	1.53 (1–5)	–

*EDSS stands for Expanded Disability Status Scale.

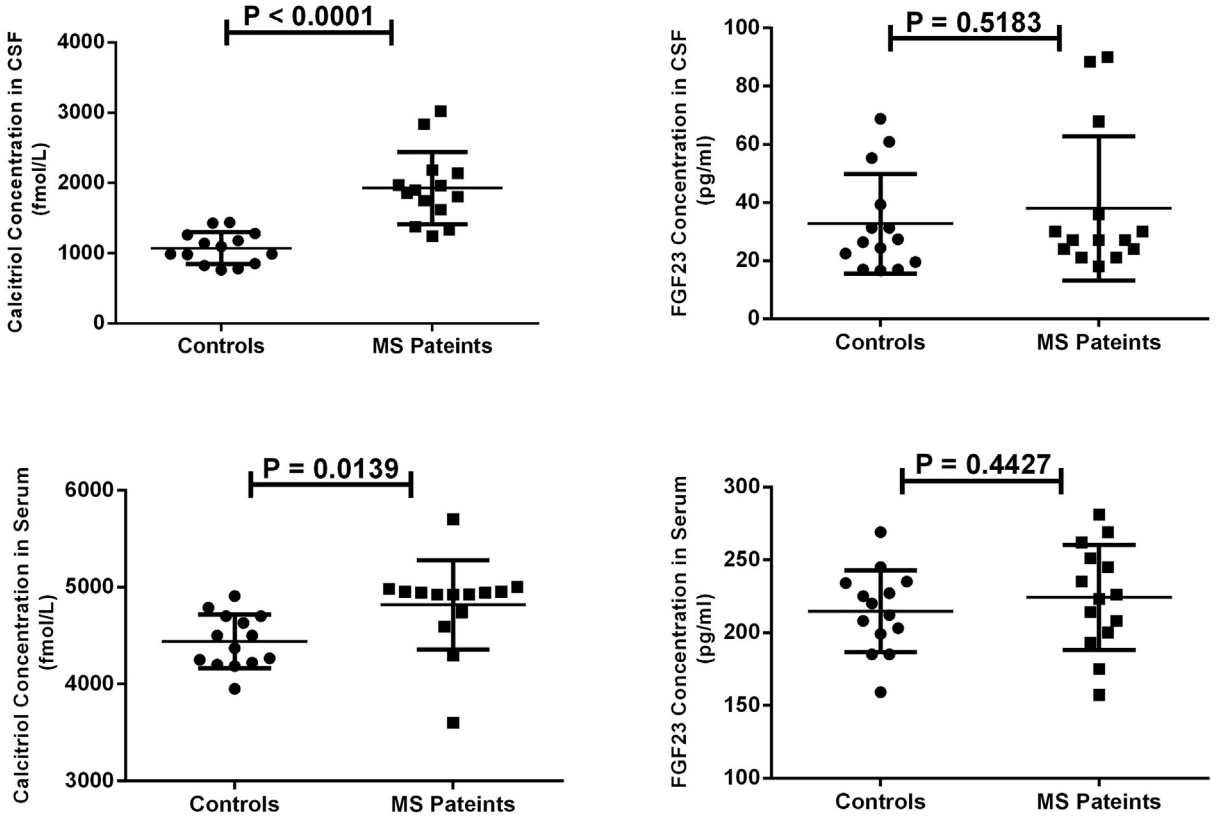

Fig. 1. The mean concentrations of calcitriol and FGF23 in MS patients and controls.

metabolism. For example, PTH and CYP27B1 enzyme are also key regulators of calcitriol metabolism (Chanakul et al., 2013; Khundmiri et al., 2016). To our knowledge, this is the first study evaluating the FGF23 concentration in the CSF of patients with MS. On the other hand, to date, there has been just one published study, by Ellidag et al. (2016), measuring the FGF23 concentration in the serum of patients with MS. In contrast to our results, Ellidag et al. (2016) reported that FGF23 increases in the serum of RRMS patients. This contradictory result may be due to the effects of drug therapy. There are some studies indicating that FGF23 expression might be affected by inflammation and medications (Ito et al., 2015; Moe et al., 2015). All RRMS patients in our study were new case and so had not received any type of medication, while almost all RRMS patients in Ellidag et al. (2016) study were receiving interferon or glatiramer acetate during the time of data collection.

Further studies are required to elucidate the exact roles of the FGF23 and calcitriol in the pathogenesis of MS disease. It is important to note that the present study was conducted among MS patients which had been newly diagnosed and so they had not received medication or supplementation. However, this leads to a limitation of the sample size of case and control groups. It should also be noted that using the low sample size, the correlations with clinical features, especially EDSS, will not be accurate enough. Future studies should be performed in large sample sizes in order to confirm the importance of calcitriol metabolism in the pathogenesis of MS disease.

5. Conclusion

In this study, we found that calcitriol concentration increases in the CSF and serum of RRMS patients independent of FGF23 alterations. We suggest that calcitriol may increase in the CSF and serum of MS patients in order to attenuate MS-induced inflammation and disability.

Conflict of interest

The authors declare that there is no conflict of interest.

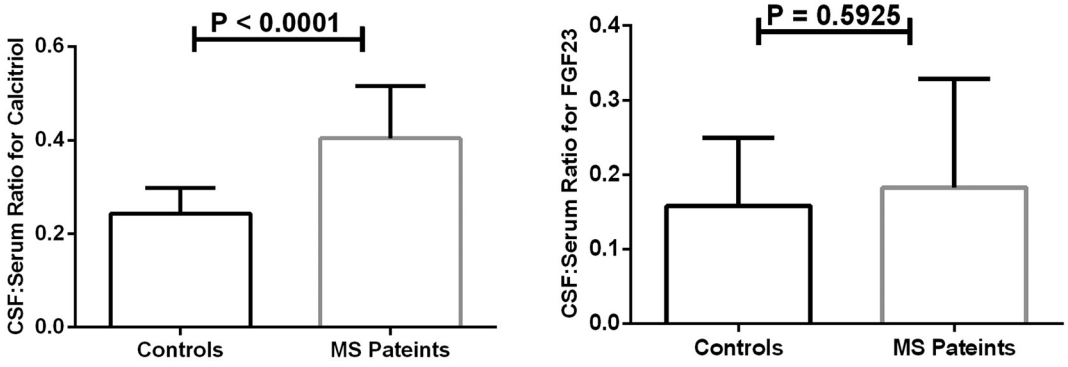

Fig. 2. CSF: Serum ratios for calcitriol and FGF23 in MS patients and controls.

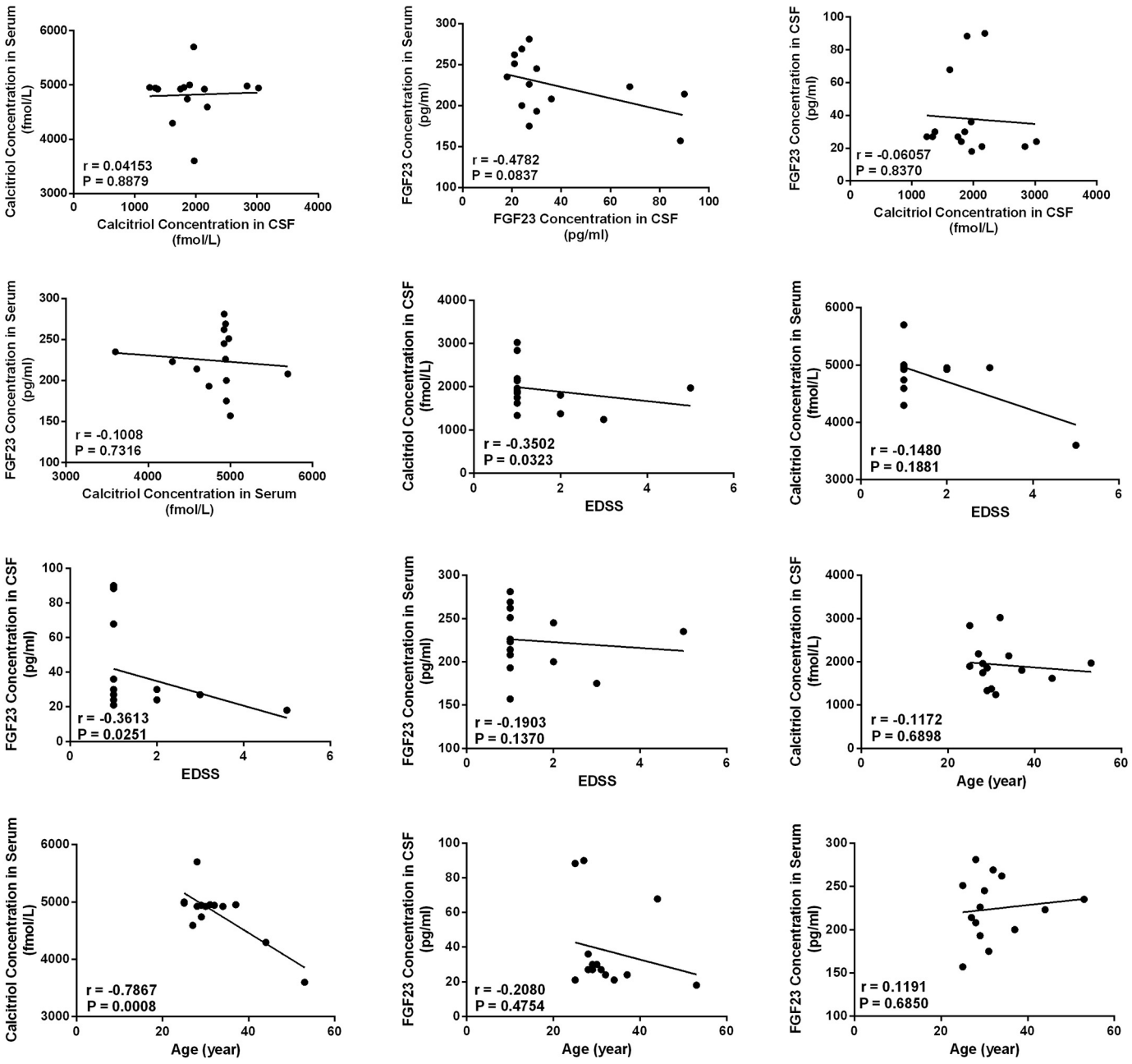

Fig. 3. Scatter plots and correlations between different molecular and clinical parameters in MS patients.

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