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Adipokines are associated with pediatric multiple sclerosis risk and course

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ABSTRACT

Background: Obesity during adolescence confers an increased risk of multiple sclerosis (MS) in both adults and children. However, obesity-mediated inflammatory mechanisms require elucidation. In models of MS, leptin and fatty acid binding protein-4 (FABP-4) have been identified as proinflammatory adipokines, while adiponectin has anti-inflammatory effects.

Methods: Morning serum samples from 32 pediatric MS (POMS) patients (22 females;10 males) and 67 pediatric healthy controls (PHC) (47 females; 20 males) followed at Massachusetts General Hospital were studied. Levels of leptin, FABP-4 and adiponectin were compared between POMS and PHC groups, adjusting for sex, age and vitamin D3 levels. Associations between each marker and the time to next relapse was assessed using a Cox proportional hazards model. The association between each marker and EDSS was assessed using linear regression.

Results: Pediatric MS patients had significantly higher levels of leptin and FABP4 and significantly lower adiponectin than healthy controls. Higher levels of adiponectin were associated with a lower hazard of relapse. Similar differences were observed between POMS and PHC males for both leptin and adiponectin, and within females for FABP4. In females with MS, there was a trend for a positive association between higher leptin levels and higher disability scores. In males with MS, paradoxically, higher leptin levels were associated with longer time to next relapse. All these results remained significant after adjusting for Vitamin D.

Conclusions: FABP4 and leptin levels are higher, while adiponectin levels are lower in pediatric MS compared to controls in sex-specific patterns. These adipokines could serve as biomarkers and therapeutic targets of disease risk and course in early forms of MS.

1. Introduction

Obesity during the adolescent period is associated with an increased risk of multiple sclerosis (MS) in both adults (Munger et al., 2009) and children (Langer-Gould et al., 2013). A pivotal study from the Nurses Health cohort demonstrated that obesity at the age of 20 was associated with MS risk (Munger et al., 2009). In a large cohort of children with MS in the U.S, we found that approximately 2/3 of children with MS were overweight or had obesity and increased body mass index (BMI), both a risk factor for MS and associated with younger age at onset of disease (Chitnis et al., 2016).

The mechanisms of obesity-induced autoimmunity in MS are relatively unexplored. White adipose tissue acts as an energy storage and endocrine secretory organ, which secretes both pro-inflammatory and anti-inflammatory cytokines called *adipokines* (Ouchi et al., 2012; Kershaw and Flier, 2004). Adipokines further balance between energy supply and demand of whole body. Obesity now is believed to cause a chronic inflammatory disorder connected to metabolic dysfunction and autoimmunity (Hotamisligil, 2006; Ouchi et al., 2011). Leptin a well-studied adipokine, is secreted from white adipose tissue as well as lymphocytes and plays a crucial role in energy homeostasis, neuroendocrine system and immune-modulation (Procaccini et al., 2012; Matarese et al., 2005). The potential role of leptin in MS development and course is supported by studies demonstrating that leptin is required for experimental autoimmune encephalomyelitis (EAE) induction and progression (Matarese et al., 2001), and human studies showing increased levels of leptin in adult relapsing remitting MS patients (Messina et al., 2013). Leptin is also found to decrease after two months

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Table 1
Demographics—PMS vs. PHC groups.

Demographics	PMS (n = 32)	PHC (n = 67)	P-value
Sex	21 Female 11 Male	47 Female 20 Male	0.82
Age (years)	15.4 ± 2.8	14.4 ± 3.2	0.098
BMI (kg/m^2)	27.3 ± 7.3	22.8 ± 5.3	0.0033
Vitamin D3 (ng/ml)	20.0 ± 10.8	17.7 ± 7.6	0.28
EDSS	1.5 ± 0.8, Median 1.5	NA	

PHC: Pediatric healthy controls; PMS: Pediatric multiple sclerosis, BMI: Body mass index, NA: Not applicable.

Mean ± SD reported unless otherwise indicated.

of treatment with interferon beta (Batocchi et al., 2003; Kraszula et al., 2012). Among other pro-inflammatory adipokines we found Fatty Acid Binding Protein 4 (FABP4) to be associated with MS severity (Bove et al., 2018). FABP4 later found to be among biomarkers in RNA expression signature in patients with relapsing remitting MS (Sheng et al., 2015). If the effect of FABP4 is mediated through degrading PPAR-gamma causing alteration of adipogenesis and immunity is a possibility (Garin-Shkolnik et al., 2014; Drew et al., 2008). Conversely, adiponectin is an anti-inflammatory adipokine, and adiponectin deficient mice experienced a more severe course of EAE as well as psoriasis (Piccio et al., 2013; Shibata et al., 2015). Adiponectin is reported to be decreased in serum of adult MS patients (Musabak et al., 2011), but was found to be elevated in cerebrospinal fluid of MS patients in a Finnish twin cohort study (Hietaharju et al., 2010). Adolescents with MS represent the earliest stages of MS and the disease presents during a key peri-pubertal risk period (Chitnis, 2013). The goal of this study is to dissect the association of obesity-related adipokines in adolescents with MS and age and sex-matched controls. In addition we evaluate the associations of specific adipokines with disease course in these early MS patients who experience a particularly inflammatory course of disease (Gorman et al., 2009).

2. Methods

2.1. Subject selection

32 pediatric MS patients (POMS) (21 females and 11 males) and 67 pediatric healthy control (PHC) subjects (47 females, 20 males) between 6–19 years old were enrolled from an ongoing biomarker study at the Partners Pediatric MS Center at Massachusetts General Hospital. Participants' parent(s) or guardian(s) signed a written consent after risk and benefits of study were explained in full length.

Pediatric MS subject selection: Pediatric MS subjects were selected using the following inclusion/exclusion criteria: (1) age at sample < 19 years; (2) serum sample drawn before 11 am; (3) absence of steroids or disease modifying therapy for at least one month before their blood draw date.

Pediatric healthy controls samples were used based on the following inclusion/exclusion criteria: (1) age at sample < 19 years; (2) serum sample drawn before 11 am; (3) no history autoimmune disorders (other than asthma or eczema); (4) no history of treatment with immunosuppressive therapy or other severe health conditions.

2.1.1. Data collection

BMI was recorded as kg/m^2 on all patients and controls at the time of blood draw. Pediatric MS patients were diagnosed based on International Pediatric MS Study Group criteria published in 2007, with onset before 18 years of age (Krupp et al., 2013).

Relapses and Expanded Disability Status Scale (EDSS) were recorded at regular visits every 6 months. Disease severity was assessed using EDSS by Kurtzke using a standardized data collection form (Kurtzke, 1983). Clinical relapse was defined as state of experiencing acute to sub-acute development of new or increasing neurological

Table 2
Comparison of mean adipokine levels in PMS and PHC using linear regression to adjust for sex and age in all models.

	All	Females	Males	Interaction
Leptin-unadjusted	19.56; 95% CI: 2.82, 36.3; $\boldsymbol{p = 0.023}$	20.45; 95% CI: −0.62, 41.52; $p = 0.057$	20.51; 95% CI: 3.42, 37.59; $\boldsymbol{p = 0.02}$	0.997
Leptin-age adjusted	16.75; 95% CI: 2.26, 31.25; $\boldsymbol{p = 0.024}$	12.9; 95% CI: −7.07, 32.88; $p = 0.202$	20.57; 95% CI: 3.43, 37.7; $\boldsymbol{p = 0.02}$	0.621
Leptin-age and vitamin D adjusted	14.99; 95% CI: 0.05, 29.93; $\boldsymbol{p = 0.049}$	10.42; 95% CI: −10.3, 31.13; $p = 0.318$	20.05; 95% CI: 2.05, 38.05; $\boldsymbol{p = 0.03}$	0.547
FABP4-unadjusted	5.75; 95% CI: 1.79, 9.71; $\boldsymbol{p = 0.005}$	5.91; 95% CI: 1.44, 10.39; $\boldsymbol{p = 0.01}$	5.73; 95% CI: −2.62, 14.07; $p = 0.171$	0.966
FABP4-age adjusted	6.39; 95% CI: 2.4, 10.38; $\boldsymbol{p = 0.002}$	6.53; 95% CI: 1.94, 11.11; $\boldsymbol{p = 0.006}$	6; 95% CI: −2.4, 14.4; $p = 0.154$	0.903
FABP4-age and vitamin D adjusted	5.95; 95% CI: 1.81, 10.1; $\boldsymbol{p = 0.005}$	5.63; 95% CI: 0.89, 10.37; $\boldsymbol{p = 0.021}$	6.07; 95% CI: −2.79, 14.92; $p = 0.171$	0.921
Adiponectin-unadjusted	−1.49; 95% CI: −2.58, −0.41; $\boldsymbol{p = 0.008}$	−1.08; 95% CI: −2.37, 0.2; $p = 0.097$	−2.45; 95% CI: −4.46, −0.44; $\boldsymbol{p = 0.019}$	0.236
Adiponectin-age adjusted	−1.08; 95% CI: −2.05, −0.12; $\boldsymbol{p = 0.029}$	−0.44; 95% CI: −1.58, 0.7; $p = 0.446$	−2.29; 95% CI: −4.16, −0.42; $\boldsymbol{p = 0.018}$	0.073
Adiponectin-age and vitamin D adjusted	−1.05; 95% CI: −2.04, −0.07; $\boldsymbol{p = 0.037}$	−0.35; 95% CI: −1.52, 0.82; $p = 0.549$	−2.27; 95% CI: −4.18, −0.37; $\boldsymbol{p = 0.021}$	0.068

Legend: Estimated difference in mean adipokine level comparing PMS and PHC. Positive values indicate higher mean in PMS.

Table 3
Estimated hazard ratio for time to next relapse for each standard deviation increase in each adipokine.

	All	Females	Males	Interaction
Leptin-unadjusted	0.68; 95% CI: 0.4, 1.16; $p = 0.16$	1.12; 95% CI: 0.59, 2.14; $p = 0.728$	0.26; 95% CI: 0.06, 1.06; $p = 0.061$	**0.026**
Leptin-age adjusted	0.83; 95% CI: 0.44, 1.55; $p = 0.56$	1.2; 95% CI: 0.58, 2.48; $p = 0.625$	0.27; 95% CI: 0.06, 1.16; $p = 0.08$	**0.043**
Leptin-age and vitamin D adjusted	0.71; 95% CI: 0.38, 1.32; $p = 0.277$	1.04; 95% CI: 0.48, 2.24; $p = 0.926$	0.22; 95% CI: 0.05, 0.96; $\boldsymbol{p = 0.044}$	**0.03**
FABP4-unadjusted	0.81; 95% CI: 0.51, 1.3; $p = 0.38$	0.83; 95% CI: 0.39, 1.76; $p = 0.631$	0.83; 95% CI: 0.47, 1.47; $p = 0.524$	0.915
FABP4-age adjusted	0.87; 95% CI: 0.57, 1.33; $p = 0.516$	0.8; 95% CI: 0.34, 1.87; $p = 0.602$	0.75; 95% CI: 0.41, 1.38; $p = 0.357$	0.929
FABP4-age and vitamin D adjusted	0.91; 95% CI: 0.61, 1.35; $p = 0.628$	0.95; 95% CI: 0.39, 2.32; $p = 0.918$	0.75; 95% CI: 0.42, 1.35; $p = 0.338$	0.668
Adiponectin-unadjusted	1.29; 95% CI: 0.85, 1.95; $p = 0.226$	1.14; 95% CI: 0.68, 1.91; $p = 0.63$	3.35; 95% CI: 1.02, 11.07; $\boldsymbol{p = 0.047}$	**0.017**
Adiponectin-age adjusted	1.2; 95% CI: 0.7, 2.06; $p = 0.501$	1.17; 95% CI: 0.65, 2.12; $p = 0.598$	2.82; 95% CI: 0.54, 14.56; $p = 0.217$	**0.17**
Adiponectin-age and vitamin D adjusted	1.11; 95% CI: 0.6, 2.07; $p = 0.735$	1.22; 95% CI: 0.63, 2.37; $p = 0.564$	2.43; 95% CI: 0.24, 24.15; $p = 0.449$	0.36

Legend: Cox proportional hazards model to adjust for age, sex and vitamin D3. Hazard ratios, 95% confidence intervals and *p*-values are reported. Each hazard ratio represents the change in the hazard of a relapse for a one standard deviation increase in the adipokine.

symptoms, not associated with underlying fever or infection (Lublin et al., 2014).

2.2. Biochemical assessments

Serum was isolated from morning blood samples drawn from POMS and PHC subjects, while seated position. Serum levels of leptin, adiponectin, and FABP4 were measured. FABP4 was measured by ELISA (Biovendor, Asheville, NC, USA), Limit of detection: 0.066 ng/ml, Intra assay CV%: 5.4–6.2% and Inter assay CV%: 5.6–6.6%.

Leptin was measured by ELISA (Biovendor, Asheville, NC, USA), Limit of detection: 0.2 ng/ml, Intra assay CV%: 4.2–7.6% and Inter assay CV%: 4.4–6.7.

Adiponectin was measured by ELISA (ALPCO Diagnostics, Salem, NH), Dynamic range: 0.10–24 ug/mL, Intra assay CV%: 5.0–5.4% and Inter assay CV%: ~6%.

25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were measured by Liquid Chromatography-°©-Tandem Mass Spectrometry (BRAC Lab, Boston, MA), Dynamic range: 1–100 ng/mL, Intra assay CV%: < 5% and Inter assay CV%: < 8%.

2.3. Statistical methods

Clinical characteristics in POMS and PHC groups were compared using a chi-squared test for comparison of gender and a two sample *t*-test for comparison of other features. Levels of leptin, FABP-4, and adiponectin were compared between the POMS and PHC groups using linear regression to adjust for sex and age in all models. In addition, the difference between the groups was estimated stratifying by gender and the interaction between sex and each adipokine was used to assess if the difference between POMS and PHC was different in males compared to females. Because Vitamin D levels have been associated with MS disease course and is sequestered in adipose tissue, subsequent models also controlled for vitamin D3 level. In addition to group comparisons, the association between each marker and the time to next relapse was assessed using a Cox proportional hazards model to adjust for age, sex and vitamin D3. The association between each marker and EDSS was assessed using linear regression adjusting for the same features. The analyses were run in all subjects as well as stratified by gender. Further, the interaction between gender and each adipokine was assessed to see if the relationship between adipokines and the outcomes differed in males compared to females. A p value of < 0.05 was used to indicate significance. Data is presented as mean ± SD, unless otherwise indicated.

2.4. Data sharing

We adhere to the Neurology data-sharing policy. De-identified limited datasets from this study can be made available to qualified investigators with appropriate Ethics/IRB approval. Data is stored for up to five years post-publication.

3. Results

3.1. Clinical features

POMS and PHC subjects had a similar distribution of sex and age. Of the participants with reported race and ethnicity, 71% of the pediatric MS patients were white, compared to 85% of the controls. 7% of the pediatric MS patients reported Hispanic/latino ethnicity versus 88% of the controls. Vitamin D3 levels were similar in both groups. However, as in our previous studies, mean BMI was significantly higher in the POMS as compared to PHC groups ($P = 0.0008$) (Chitnis et al., 2016). POMS and PHC clinical characteristics are listed in Table 1.

3.2. Adipokine levels in MS compared to controls

We compared adipokine levels in POMS and PHC participants (Table 2). All adipokines were significantly different between groups in the unadjusted as well as adjusted analyses. POMS subjects had significantly higher leptin (unadjusted: 19.56; 95% CI: 2.82, 36.3; $p = \mathbf{0.023}$) and FABP4 (unadjusted: 5.75; 95% CI: 1.79, 9.71; $p = \mathbf{0.005}$) levels and significantly lower adiponectin levels (unadjusted: −1.49; 95% CI: −2.58, −0.41; $\boldsymbol{p = 0.008}$) as compared to PHC in underadjusted analyses. Analysis adjusting for age and Vitamin D yielded similar and statistically significant results. After stratifying the groups by sex, similar differences were observed between POMS and PHC within males for leptin and adiponectin and within females for FABP4 in both adjusted and unadjusted analyses (Table 2).

3.3. Effect on disease course

3.3.1. Relapse risk - time to next relapse

The association between leptin, FABP4 and adiponectin levels and time to the next relapse was estimated and the data are summarized in Table 3. Although no significant association was observed between any of the adipokines and the time to next relapse in the group as a whole, a statistically significant interaction was observed between sex and both leptin and adiponectin. In males, a higher leptin level was associated with a lower hazard of relapse in adjusted analysis. This was not observed in females. For adiponectin, in males (but not females), higher levels were associated with a lower hazard of relapse on unadjusted analysis.

3.3.2. EDSS

The association between leptin, FABP4 and adiponectin and EDSS was estimated (Table 4). No statistically significant associations were observed in all subjects, and no statistically significant interaction between sex and adipokines was observed. There was a trend for higher adjusted leptin scores to be associated with higher EDSS in females.

Table 4
Adipokine association with EDSS:.

	All	Females	Males	Interaction
Leptin-unadjusted	0.27; 95% CI: −0.04, 0.57; $p = 0.082$	0.26; 95% CI: −0.21, 0.72; $p = 0.266$	0.13; 95% CI: −0.37, 0.62; $p = 0.579$	0.73
Leptin-age adjusted	0.24; 95% CI: −0.15, 0.63; $p = 0.211$	0.46; 95% CI: −0.06, 0.98; $p = 0.077$	−0.03; 95% CI: −0.54, 0.47; $p = 0.885$	0.227
Leptin-age and vitamin D adjusted	0.22; 95% CI: −0.17, 0.61; $p = 0.252$	0.37; 95% CI: −0.01, 0.76; $p = 0.057$	−0.01; 95% CI: −0.26, 0.23; $p = 0.888$	0.189
FABP4-unadjusted	0; 95% CI: −0.3, 0.3; $p = 0.989$	−0.09; 95% CI: −0.61, 0.42; $p = 0.71$	0.04; 95% CI: −0.25, 0.34; $p = 0.752$	0.649
FABP4-age adjusted	−0.02; 95% CI: −0.33, 0.28; $p = 0.876$	−0.01; 95% CI: −0.59, 0.56; $p = 0.96$	0.04; 95% CI: −0.25, 0.33; $p = 0.756$	0.865
FABP4-age and vitamin D adjusted	0.01; 95% CI: −0.3, 0.31; $p = 0.961$	0.17; 95% CI: −0.29, 0.63; $p = 0.445$	0.02; 95% CI: −0.13, 0.17; $p = 0.736$	0.545
Adiponectin-unadjusted	0.05; 95% CI: −0.25, 0.35; $p = 0.724$	0.18; 95% CI: −0.2, 0.56; $p = 0.329$	−0.39; 95% CI: −0.77, −0.01; $\mathbf{p = 0.045}$	0.106
Adiponectin-age adjusted	0.09; 95% CI: −0.24, 0.42; $p = 0.578$	0.15; 95% CI: −0.27, 0.56; $p = 0.474$	−0.37; 95% CI: −0.93, 0.19; $p = 0.168$	0.277
Adiponectin-age and vitamin D adjusted	0.07; 95% CI: −0.26, 0.4; $p = 0.67$	0.21; 95% CI: −0.11, 0.53; $p = 0.193$	0.23; 95% CI: −0.19, 0.65; $p = 0.238$	0.959

Legend: Adjusted change in mean EDSS score, 95% confidence intervals and *p*-values are reported. Each adjusted change was estimated using linear regression and the reported coefficient is the estimated change in the mean EDSS score for a one standard deviation increase in the adipokine.

4. Discussion

In this study, we evaluated adipokine levels in pediatric MS patients compared to age and sex-matched controls. Since adipokine levels differ by sex and we have previously found sex-specific associations of FABP4 associated with disease course in adult MS (Bove et al., 2018), we stratified our results by sex. Here, we demonstrate higher FABP4 levels in POMS compared to healthy controls, driven mainly by females suggesting a role of this adipokine in MS risk. In contrast, adiponectin, an anti-inflammatory adipokine was significantly lower in males with MS. Higher leptin levels were associated with a shorter time to next relapse in males, and increased disability scores in females with MS.

Obesity has been demonstrated to be a clear risk factor for MS, with the likely critical risk period being adolescence to early adulthood. Our results inform potential early mechanisms of obesity-mediated inflammation in adolescence. Moreover, the identified adipokines could be utilized in monitoring for MS disease risk and are potential therapeutic targets for early forms of MS. In our study, BMI is higher in the pediatric MS patients than controls, which matches results from other risk and epidemiological studies (Munger et al., 2009; Langer-Gould et al., 2013; Chitnis et al., 2016), supporting the fact that higher BMI is associated with MS risk. We did not control for BMI in this study, because our goal is to identify the hormonal associations of increased BMI in pediatric MS, which may have biological significance.

We found higher FABP4 levels in females with MS compared to controls. FABP4 is found in adipose tissue as well as macrophages. Higher FABP4 levels are associated with risk of the metabolic syndrome (Xu et al., 2007). FABP4 is induced through toll like receptor 4 (TLR-4) signaling and modulates inflammatory responses in macrophages through a positive feedback loop involving c-Jun NH2-terminal kinases and activator protein-1 (Hui et al., 2010). We recently found that FABP4 was associated with increased MS disability scores in adults with MS (Bove et al., 2018). This along with our current findings, suggests a pivotal role for this adipokine in MS.

Adiponectin deficient mice developed worse EAE with greater CNS inflammation, demyelination and axonal injury (Piccio et al., 2013) which corroborates our findings of lower levels of adiponectin in children with MS. A study in adult MS patients similarly found lower adiponectin levels compared to controls (Musabak et al., 2011). There appears to be a sex-dependency in our findings, with MS males having significantly lower levels than controls, with no difference found in females. Adiponectin has been associated with lower frequencies of regulatory T cells in adult MS patients (Kraszula et al., 2012) and may play a pivotal role in suppressing the immune response, particularly in males. Interestingly, Vitamin D has been shown to induce regulatory T cells in female mice, but not males (Correale et al., 2010), suggesting that different hormones influence regulatory T cells in males vs. females.

We found that leptin levels were higher in POMS compared to controls. Studies in EAE have found that leptin increases susceptibility and severity of disease33 and that chronic caloric restriction, associated with decreased leptin levels, attenuates EAE.34 Small studies in MS patients have found conflicting results with respect to leptin levels during relapse and remission states.12, 35 Our previous studies in adult MS did not find an effect of leptin on disability outcomes14, which matched findings from a recent Norway study36. However, pediatric MS represents an early and highly inflammatory form of MS, with an increased relapse rate, which may account for differences from adult studies.23, 37 Interestingly, in this study we found that in the males with MS, increased levels of leptin were associated with a longer time to subsequent relapse, controlling for appropriate covariates. This contrasted with the results in females, suggesting a sex-specific difference in leptin effects by gender. Testosterone administration lowers leptin levels in men (Luukkaa et al., 1998), and we have previously found lowered testosterone in men to be associated with a worsened MS disease course (Bove et al., 2014). Our present analysis did not assess

for testosterone levels and this along with other sex hormones is an area for future study. An immunological study found an inverse correlation between leptin concentration and the mean fluorescence intensity of the transcription factor Foxp3 in natural regulatory T cells in patients with RRMS (Kraszula et al., 2012).

Limitations of this study include the limited number of subjects, however pediatric MS is rare, and it is challenging to find untreated subjects. We were able to match for sex and age, however our POMS and PHC differed by Hispanic/latino ethnicity which could impact results. In addition, while nutrition has direct effects on both body mass index and adipokine levels, we did not have any control or survey on participants calorie intake and nutrition pattern in this study. Measuring body fat percentage and finding its association with adipokines is optimal which can be considered in future studies.

In summary, we identified several adipokines that are differentially present in pediatric MS patients compared to controls. These adipokines including leptin, FABP4 and adiponectin may exert key effects on the initiating inflammatory response and may serve as biomarkers and potentially therapeutic targets to curb the onset of MS. Childhood obesity is a worldwide epidemic and in genetically susceptible adolescents can result in the initiation of MS. Strategies to optimize body mass index, and the use of specific adipokines identified in this work as markers of adiposity-associated inflammation, could reduce the onset and disease course of MS.

5. Author contributions & disclosures

Name	Role	Contribution
Kiandokht Keyhanian, MD	Author	Study concept, study conduct, writing first draft
Shrishti Saxena, MSc	Author	Study conduct, revision and review of manuscript
Grace Gombolay, MD	Author	Study conduct, revision and review of manuscript
Brian C. Healy, Ph.D	Author	Statistical analysis, revision and review of manuscript
Madhusmita Misra MD	Author	Collection of data and samples, revision and review of manuscript
Tanuja Chitnis, MD	Author	Study concept, collection of data and samples, supervision of study, manuscript draft editing and review.

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Declaration of Competing Interest

None declared.

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