



## Markers of inflammation in obese pregnant women: Adenosine deaminase and high sensitive C – reactive protein

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### ABSTRACT

**Introduction:** The worldwide increase in the prevalence of obesity over the years has emerged as a global health concern. The growing rate of obesity in women of child bearing age is particularly a matter of concern. Obesity is considered a risk factor that predisposes an individual to a proinflammatory state through the release of the inflammatory mediators. Recent studies have shown a positive correlation between the severity of inflammation and an increase in adenosine deaminase (ADA) and high sensitivity C- reactive protein (hs-CRP). Obese pregnancy women are at a higher risk for developing inflammation-mediated pregnancy complications like gestational diabetes, preeclampsia, and preterm delivery. Considering the fact that pregnancy, obesity and inflammation are closely linked, this study evaluated the inflammation associated with obesity during pregnancy by estimating changes in ADA and hs-CRP.

**Materials and methods:** The current study aimed to evaluate the levels of inflammation in obese pregnant women compared to non-obese pregnant women by correlating BMI with levels of ADA / hs-CRP. The study also aimed to examine the change in ADA and hs-CRP levels with gestational age (between the 1<sup>st</sup> and the 3<sup>rd</sup> trimester) in obese pregnant women as compared to non-obese pregnant women. We also examined whether changes in the levels of ADA correlate with changes in the levels of hs-CRP particularly in obese pregnant women.

Blood samples were collected from obese and non-obese pregnant women. ADA activity and hs-CRP levels were estimated by biochemical assays. BMI was evaluated in the 1<sup>st</sup> trimester and those women with BMI > 30 kg/m<sup>2</sup> were considered as obese. Thirty subjects were included in each of the two groups.

**Results:** ADA and hs-CRP levels were significantly higher in obese pregnant women in both the 1<sup>st</sup> and 3<sup>rd</sup> trimesters compared to non-obese participants (P value<0.05). Statistically significant higher values of ADA and hs-CRP were seen in obese participants in the 3<sup>rd</sup> trimester compared to the 1<sup>st</sup> trimester. A significant linear positive correlation was found between BMI and 3<sup>rd</sup> trimester ADA, and a linear positive correlation between BMI and hs-CRP both in the 1<sup>st</sup> and 3<sup>rd</sup> trimester. The relationship between  $\Delta$  ADA and  $\Delta$  hs-CRP was non-significant.

**Conclusions:** The observations of this study reveal increased inflammatory responses in obese pregnant women and suggests the importance of ADA and hs-CRP as early indicators of obesity-related complications prevailing thereafter, these markers can be useful for clinical diagnosis of impending maternal and neonatal complications.

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## Introduction

Obesity or overweight in simple terms is due to excessive fat accumulation that can cause various health complications. As per the WHO, a simple measure of obesity is the body mass index (BMI), calculated as a person's weight (in kilograms) divided by the square of his or her height (in meters). A person with a BMI of 30 kg/m<sup>2</sup> or more is generally considered obese. Obesity has been associated with significant adverse maternal and fetal outcomes, which provide a major challenge to obstetric practice. Studies have estimated that 25% of complications impacting the maternal body are attributable to overweight and obesity [1,2]. Increased maternal BMI is an independent risk factor for pre-eclampsia, type 2 diabetes, cardiovascular disease (CVD), preterm delivery, cesarean delivery, delivery of a macrosomic infant, and metabolic disorders in children later in life [1–4]. There exists a link between maternal and neonatal health [4–6], therefore children born to obese mothers are at risk of future obesity, heart disease, and diabetes, the cause of which is described as “fetal programming” or “developmental origin of adult disease”, that is associated with the abnormal intrauterine fetal development. The risk of intrauterine fetal complications is increased in obese women compared to normal-weight women [5]. These obesity-associated pregnancy and fetal complications may be associated with altered immune and inflammatory responses [7].

In recent years, increased interest has been developed in understanding the role of inflammation in type 2 diabetes, metabolic syndrome, depressed innate immunity, and its association with obesity [2, 8]. Although there exists a theoretical explanation of the possible pathophysiology that links BMI and adverse pregnancy outcomes, not many studies have examined the association of systemic inflammatory responses to obesity [9].

During the later stages of gestation anti-inflammatory cells must prevail and towards the end of gestation, a proinflammatory state for initiating labor may be necessary [10,11]. Thus, in normal pregnancy, a properly balanced and regulated inflammatory response is vital for placentation, from implantation through the stages of pregnancy to labor at term, obesity-associated disruption of this balance may be associated with pregnancy complications [7,12].

Adenosine deaminase (ADA) is an enzyme, whose primary function in humans is the development and maintenance of the immune system, which is produced in all cells including adipocytes, lymphocytes and monocytes which are cells of the immune system, and this enzyme primarily deaminates adenosine to inosine.

ADA plays a significant role in the proliferation, differentiation, and maturation of lymphocytes and is considered as an important immunoenzyme marker for cell-mediated immunity [13]. An increase in the expression and activity of ADA is linked to the severity of inflammation and has a crucial role in neurological, immunological and cardiovascular systems, where it regulates the required concentrations of adenosine [13,14]. ADA is found to increase in several inflammatory conditions and is considered as a marker of inflammation [15,16]. ADA is expressed in adipose tissue, and increased release of ADA from adipocytes in individuals with higher BMI indicates obesity-associated inflammation [17,18].

C-reactive protein (CRP) is an acute phase protein of the pentraxin family, that is synthesized by liver macrophages and adipocytes in response to inflammatory stimuli like tumor necrosis factor, Interleukin 6 (IL-6), and IL-1 [19]. CRP is produced in response to inflammation of the blood vessel endothelium, and an increase in CRP is a direct measure of inflammation. High sensitive CRP (hs-CRP) is useful as an independent inflammatory marker for the prognosis of recurrent CVD, myocardial infarction, and stroke [20]. Obesity associated inflammation is marked by increased serum CRP levels [21].

Maternal systemic inflammation can lead to placental insufficiency, vascular dysfunction, preeclampsia, and preterm labor [22–24]. Adipose tissue is a prominent site that secretes proinflammatory IL6, a cytokine that stimulates the liver to release CRP, which marks systemic

inflammation [25].

Understanding of the association between obesity, inflammation, and pregnancy may help in mitigating inflammation-mediated maternal and neonatal complications. Very few studies have examined CRP and ADA levels in relation to prepregnancy weight. There is also insufficient data regarding changes in the levels of inflammatory markers (ADA and CRP) with gestational age (from 1<sup>st</sup> to 3<sup>rd</sup> trimester) in obese women. Considering the fact that pregnancy, obesity and inflammation are closely linked, this study was aimed to determine whether obesity during pregnancy is associated with increased ADA and hs-CRP.

To understand whether there exists any correlation between BMI and the levels of systemic inflammatory markers namely the ADA / hs-CRP, we estimated them in obese pregnant women in the 1<sup>st</sup> and in the 3<sup>rd</sup> trimester compared to non-obese women. We also examined whether the serum levels of ADA and hs-CRP increases with gestational age, and finally we examined whether changes in the levels of ADA correlate with changes in the levels of hs-CRP in obese pregnant women.

## Methods and procedures

### Study design

In this observational prospective study, a group of 30 obese and 30 non-obese pregnant women (control group) were included.

This study was conducted on pregnant women attending an antenatal clinic for a regular checkup at a health care center in Mangalore, Karnataka, India.

Ethical Committee of the same establishment approved the protocol of the study and all women signed written informed consent.

### Study participants, measurement of BMI ADA & hs-CRP

#### Inclusion criteria

Participants involved women within the age group of 21–30 years with ultrasonography corroborated gestational age without prenatal complications. Participants were defined as obese women when BMI in the first trimester of pregnancy was in the obese range. For every consenting woman with BMI in the obese range, a non-obese participant matched with the corresponding obese case in age (within two years) and parity (nulliparous versus multiparous) was selected as control.

#### Exclusion criteria

Smokers, women with any evidence of recent infections or morbidity like cardiac diseases, bronchial asthma, acute or chronic inflammatory diseases, autoimmune diseases, women who were on medications like steroids, antipsychotic drugs, women with menstrual disorders, polycystic ovary disease and all known parameters that may affect ADA and CRP levels were not included in the study.

As our primary aim was to study the inflammatory markers (ADA and hs-CRP) in obese pregnant women, the weight and height of the consenting women were recorded in the first trimester (before 8 weeks of gestation) from which the BMI was calculated according to the formula: weight (kg)/height<sup>2</sup> (m). BMI classification was based on the World Health Organization classification (underweight  $\leq 18.5$  kg/m<sup>2</sup>, normal 18.5–24.99 kg/m<sup>2</sup>, preobese 25–29.99 kg/m<sup>2</sup>, obese  $\geq 30$  kg/m<sup>2</sup>), thus women with BMI  $\geq 30$  kg/(m<sup>2</sup>) in the first trimester were considered as obese. Prepregnancy weight of every participant was obtained by self-reporting questionnaire to confirm BMI calculated in the 1<sup>st</sup> trimester. As gestational weight gain is insignificant in the 1<sup>st</sup> trimester of pregnancy, BMI measured before the 12th week of gestation is largely used to ascertain prepregnancy weight [26].

Venous blood samples were collected twice from all participants, the first sample was drawn during the 1<sup>st</sup> trimester (before 8 weeks) and the second sample was drawn during the 3<sup>rd</sup> trimester (between 24 and 28 weeks).

Levels of ADA and CRP were measured in sera separated from venous blood collected from all participants.

Additionally, systolic and diastolic blood pressure, fasting blood glucose, lipid profile, glucose challenge test with 50 gm glucose were also measured in all participants during their 1<sup>st</sup> trimester.

Glucose challenge test was performed for screening gestational diabetes. The glucose challenge test was performed by estimating blood glucose after 1 h of consuming 50gms glucose dissolved in one glass of water.

The level of ADA activity in the serum was estimated based on the principle of deaminating adenosine to inosine, further converting the products to hypoxanthine by purine nucleoside phosphorylase. Hypoxanthine is converted to uric acid and hydrogen peroxide by xanthine oxidase. Hydrogen peroxide is then quantified by a Trinder reaction in Selectra Pro S biochemistry analyzer. Commercially available kit from Bio-systems was used for this purpose. ADA activity expressed as U/L represents moles of substrate converted by ADA at standard pH and temperature. hs-CRP (high sensitive CRP) was measured instead of CRP as CRP readings lacks sensitivity. Serum hs-CRP was measured by immunoturbidometric method using Agappe diagnostics nephelometer with a sensitivity of 0.04 mg/L.

### Statistical analysis

The study data was analyzed with Statistical Package for Social Sciences (SPSS) for windows version 23 (IBM Corp, Armonk, N.Y, USA). Quantitative data is presented as mean±SD. Differences in serum ADA, hs-CRP and other variables were compared between non-obese and obese women by Student's t-test, assuming equal variance between the groups. Serum ADA, hs-CRP were compared between the 1<sup>st</sup> and the 3<sup>rd</sup> trimester using paired Student's t-test. The relationship between BMI and inflammatory markers were determined using Pearson's correlation coefficient and multiple linear regression analysis. The relationships between  $\Delta$  ADA and  $\Delta$  hs-CRP ( $\Delta$ =Change in the value of ADA and hs-CRP between 1<sup>st</sup> and 3<sup>rd</sup> trimester) was also determined using Pearson's correlation coefficient. Multiple linear regression was used to understand the percentage of predictors specifying variances. P Value  $\leq$  0.05 with confidence intervals at 95% was considered as statistically significant in all analysis.

### Results and observations

Demographic description and clinical characteristics between obese and non-obese pregnant women are shown in Table 1. As prepregnancy obesity is one of the risk factors for gestational diabetes which in turn

**Table 1**  
Demographic data and biochemical parameters in the study participants.

Variable	Non-Obese (n = 30) Mean (SD)	Obese (n = 30) Mean (SD)
Age (years)	26.65 (3.9)	26.32 (4.02)
BMI- kg/m <sup>2</sup> ( 1 <sup>st</sup> trimester/< 8 weeks gestation)	22.61 (1.66)	32.69 (1.49) *
Systolic Blood Pressure(mm/Hg)	112.32 (13.57)	110.74 (11.74)
Diastolic Blood Pressure	72.97 (6.02)	75.29 (6.70)
Glucose ( mg/dl)	100.4 (11.5)	105.71 (10.80)
Total Cholesterol ( mg/dl)	142.81 (14.52)	164.55 (15.95)
Triglycerides (mg/dl)	100.65 (10.24)	149.51(11.86)
HDL (mg/dl)	45 (13.7)	41(14.5)
LDL(mg/dl)	71.5(15.1)	85.0 (23.6)
glucose challenge test values (50 gm glucose)	121.19 (8.50)	123.19 (10.69)

\* =P value obtained from Students T test showing significant difference in BMI between obese and nonobese women.

can influence the levels of inflammatory markers, a glucose challenge test was performed with 50 g glucose on all participants. Glucose challenge test values did not were not significantly different between obese and non-obese participants, thus eliminating any association of gestational diabetes with ADA and hs-CRP levels. Mean and SD value of glucose challenge test are shown in Table 1. Mean and SD of BMI of participants designated as obese was 32.69  $\pm$  1.49 and of non-obese participants was 22.61  $\pm$  1.66.

ADA and hs-CRP levels were significantly higher in obese pregnant women during both 1<sup>st</sup> and 3<sup>rd</sup> trimesters compared to non-obese participants (Table 2 and Table 3).

We observed statistically significant higher values of ADA and hs-CRP in obese participants in the 3<sup>rd</sup> trimester compared to the 1<sup>st</sup> trimester (P value =0.037 and 0.001), suggesting a significant increase in the levels of these markers with gestational age particularly in obese participants.

Regarding the correlation of BMI with ADA/hs-CRP, a significant linear positive correlation (shown in Table 4) was found between BMI and 3<sup>rd</sup> trimester ADA (r = 0.44, P = 0.004), a linear positive correlation between BMI and hs-CRP both in the 1<sup>st</sup> (r = 0.036, P = 0.003) and the 3<sup>rd</sup> trimester (r = 0.534, P = 0.0001), indicating higher levels of the inflammatory markers in pregnant women with higher BMI.

Multiple linear regression showed statistically significant influence of 1<sup>st</sup> trimester /pregnancy BMI (P value =0.01, r = 0.31), 1<sup>st</sup> trimester hs-CRP (P value = 0.03, r = 0.25) and 1<sup>st</sup> trimester ADA (P value =0.01, r = 0.3) on the levels of hs-CRP, suggesting that these factors determine increased levels of hs-CRP in the 3<sup>rd</sup> trimester. However, only 1<sup>st</sup> trimester /pregnancy BMI (P value=0.01, r = 0.35) was seen to significantly influence the increased levels of 3<sup>rd</sup> trimester ADA.

As both ADA and hs-CRP increased significantly in obese pregnant women, we sought to understand the correlation (Pearson's) between the two markers from the deduced  $\Delta$  values. The correlation between  $\Delta$  ADA and  $\Delta$  hs-CRP was non significant with very small negative relationship (P = 0.370), indicating that these two markers rise independent of each other.  $\Delta$  values reflect ADA and hs-CRP levels between the 1<sup>st</sup> and the 3<sup>rd</sup> trimester which was determined by subtracting the 1<sup>st</sup> trimester value from the 3<sup>rd</sup> trimester value for each participant.

Multiple linear regression appropriated that BMI and 1<sup>st</sup> trimester hs-CRP are statistically significant predictors for  $\Delta$  hs-CRP. [Delta hs-CRP = -0.673491 + 0.143302 (BMI) - 0.596047 (1<sup>st</sup> trimester hs-CRP), R<sup>2</sup> = 0.27] indicating that predictors specify 27% of the variance.

### Discussion

Obesity during pregnancy poses a risk to both maternal and fetal health, the exact mechanism of which is still unclear [5]. Prepregnancy weight is an important factor that determines gestational weight gain and higher prepregnancy BMI is associated with adverse maternal and neonatal outcome [27]. The present study highlights increased inflammatory responses in obese pregnant women. Statistically significant higher levels of serum ADA and serum hs-CRP in obese pregnant women (in whom 1<sup>st</sup> trimester /pregnancy BMI was in obese range),

**Table 2**  
Comparison of ADA values in Non-Obese & Obese Women.

		Non-Obese Women (n = 30)	Obese Women (n = 30)	P Value #
ADA U/L	1 <sup>st</sup> trimester	25.06 (4.02)	28.61 (4.7)	<b>0.001 #</b>
Mean (SD)	3 <sup>rd</sup> trimester	26.54 (5.15)	31 (4.03)	<b>0.0003 #</b>
P Value \$		0.02	<b>0.03\$</b>	

Statistically significant P values are displayed in bold characters  
# P Value for comparison between Non-Obese & Obese Women.  
\$ P value between 1<sup>st</sup> & 3<sup>rd</sup> trimester

**Table 3**  
Comparison of hs-CRP values in Non-Obese & Obese Women.

		Non-Obese Women (n = 30)	Obese Women (n = 30)	P Value #
hs-CRP mg/L Mean (SD))	1 <sup>st</sup> trimester	3.17 (1.31)	4.04 (1.2)	<b>0.01</b> #
	3 <sup>rd</sup> trimester	3.79 (1.24)	5.7 (1.78))	<b>0.0001</b> #
P Value \$		0.06	<b>0.001</b> \$	

Statistically significant P values are displayed in bold characters

# P value for comparison between Non-Obese& Obese Women.

\$ P value between 1<sup>st</sup> & 3<sup>rd</sup> trimester

**Table 4**  
Pearson's Correlation analysis between BMI & ADA,BMI & hs-CRP.

	1 <sup>st</sup> trimester (n = 60)		3 <sup>rd</sup> trimester (n = 60)	
	r value	P value	r value	P value
BMI Vs ADA	0.534	0.058	0.44	<b>0.0003</b> *
BMI Vs hs-CRP	0.3696	<b>0.004</b> *	0.534	<b>0.00001</b> *

\*The result is significant at  $p < 0.05$ .and shows significant positive correlation.  
Statistically significant P values are displayed in bold characters

compared to non-obese in both the 1<sup>st</sup> and the 3<sup>rd</sup> trimesters, statistically significant rise in the levels of serum ADA and serum hs-CRP with gestational age (from 1<sup>st</sup> to 3<sup>rd</sup> trimester) in obese pregnant women when compared to non-obese counterparts, significant higher levels of serum ADA and hs-CRP corresponding to higher BMI, are important indicators of inflammation noted in this study.

Increased levels serum ADA and hs-CRP confirms the pregnancy-associated increased inflammatory /immune responses, particularly in the early and late pregnancy periods [10,11]. Statistically significant higher levels of serum ADA and serum hs-CRP in obese pregnant women during the 3<sup>rd</sup> trimester compared to the 1<sup>st</sup> trimester is an important finding of this study indicating progressing inflammatory events along with progression in gestational age, particularly in women with pre-pregnancy obesity. Increased inflammatory responses in obese pregnant women underlie the pathophysiology of pregnancy-associated preeclampsia, preterm delivery, and other complications. Elevated ADA and CRP are known to hallmark underlying immunopathology in several diseases [22, 23, 28–30].

Higher levels of serum ADA and CRP in obese pregnant women confirm the strong link between various inflammatory-mediated events and depressed innate immunity in these individuals [8].

Rise in serum ADA during pregnancy, particularly in obese pregnant women, may be because of the increase in anti-inflammatory metabolite adenosine in response to inflammation produced by expanding adipose tissue. The enzyme ADA acts on adenosine and converts it into inosine, thereby causing increasing activity of serum ADA. Adenosine can influence immune-altered cytokine release, thereby increasing the release of CRP. Even though adipose tissue is primarily considered as a storehouse of excess energy, it is also a metabolically highly active endocrine organ that secretes numerous inflammatory markers [25,30]. Serum ADA in pregnancy may be also contributed by the monocyte- macrophage system, lymphocytes, and hematopoietic cells, such as T helper cells, besides adipocytes. T helper cells are important regulators of immune resort during pregnancy; they produce various cytokines that release ADA and CRP [31–35].

A close correlation between the severity of inflammation and a localized surge in activity and expression of ADA has been previously reported, for which obesity-associated increase in macrophage accumulation within adipose tissue is identified as the cause [36].

Additionally, elevated serum ADA in obese pregnant women reflects altered cellular immunity, as ADA is crucial for lymphoid cell differentiation. Obesity during pregnancy can affect cellular and humoral immunity along with augmented inflammatory responses[28]. Previous

studies have reported increased serum ADA activity in pregnancy-associated preeclampsia [29,30], and the risk of preeclampsia increases in overweight pregnant women due to higher levels of inflammation [37].

Estimation of serum ADA is largely being used to monitor diseases involving proinflammatory events and altered immunity [38]. Further studies are warranted to understand the mechanisms that cause the increase in serum ADA levels, the regulatory mechanisms involving increased ADA, and altered cell-mediated immunity in pregnancy. Such studies may reveal information regarding obesity-linked pregnancy complications more clearly.

Increase in serum CRP with gestational age in healthy pregnant women was reported by Mei. Z et. al. [39], in our study, we did not find any statistically significant higher levels of hs-CRP in non-obese women with gestational age, however, we found significant higher levels of hs-CRP with gestational age in obese participants. Increase in the levels of CRP in obese pregnant women as gestation progresses indicates higher levels of ongoing pro-inflammatory responses [40]. Another study conducted to understand the effect of glucose challenge on BMI reported that CRP, along with other markers of inflammation like leptin (adipokine) was strongly correlated with BMI at 24<sup>th</sup> – 29<sup>th</sup> week gestation [41].

Mild elevation in serum CRP during pregnancy may be due to maternal response, but higher levels are associated with adverse pregnancy outcomes such as preeclampsia with fluid retention, proteinuria, intrauterine growth restriction, endothelial dysfunction, preterm delivery and other neonatal complications [1, 22, 23, 42]. Higher levels of both serum ADA and hs-CRP in obese pregnant women and the increase in their values as pregnancy advances, can be attributed to adipocytes that secrete the proinflammatory cytokines like the IL6 and adenosine which in turn causes CRP release, and increase in ADA activity [25,43]. Thus women with higher levels of ADA and CRP must be carefully monitored for complications prevailing thereafter.

Statistically significant positive correlation between BMI and hs-CRP observed in this study complements the findings of the previous study that report higher CRP levels in pregnant women with increased prepregnancy/1<sup>st</sup> trimester BMI [44].

Obesity is associated with an ongoing state of low-grade inflammation and higher values of hs-CRP in pregnant women with higher BMI pinpoints that, in pregnant women with higher prepregnancy adiposity there is a chronic ongoing state of inflammation that can adversely influence pregnancy and neonatal outcomes [21, 40, 44].

The present study also indicates that the increase in the levels of ADA and increase in the levels of hs-CRP along with gestational age are independent of each other and both markers can independently display inflammatory status.

The results of this study convey that estimation of ADA and hs-CRP in pregnant women and estimating the increase in the levels of these markers with gestational age will be helpful indicators of altered inflammatory responses in pregnant women with increased BMI, reflecting the risk of adverse outcomes like preeclampsia, preterm delivery, sub-optimal placental development, and risk of neonatal complications. Maternal obesity augments already active metabolic changes in adipose tissue and along with obesity-related elevation of inflammatory responses, may all together play an important role in the pathophysiology of adverse pregnancy outcomes.

## Conclusions and recommendations

The results of the present study convey that obesity in pregnancy is associated with increased levels of ADA and hs-CRP, confirming the association of inflammation in obese pregnant women.

Adverse pregnancy outcomes that are most often associated with increased prepregnancy/ 1<sup>st</sup> trimester obesity may be originating from increased systemic inflammatory mediators.

Furthermore, our results indicate that the inflammatory state in

obese pregnant women increases as pregnancy advances. Further studies to identify the reference levels of serum ADA and hs-CRP in obese and non-obese pregnant women will help to utilize these easy-to-perform and economical markers to gauge inflammation during pregnancy and can serve as useful markers for the diagnosis of impending inflammation-associated pregnancy complications. Studies are warranted to elucidate the mechanisms regulating changes in the levels of inflammatory markers in obese pregnant women. To the best of our knowledge, this is the first study to confirm the inflammatory status by estimating changes in the levels of ADA and hs-CRP in obese pregnant women during the 1<sup>st</sup> and 3<sup>rd</sup> trimester. Furthermore, understanding the inflammatory status in the early trimesters of pregnancy will help to identify women at increased risk of adverse outcomes so that appropriate management can be planned.

Limitations of this study are the relatively small sample size. Further studies with larger sample size and following pregnancy to term to check for adverse maternal and neonatal outcomes in women with higher levels of inflammatory markers are required.

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### Conflicts of interest

Authors state no conflict of interest.

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