THE RELATION BETWEEN MRI CHANGES, CALCITONIN GENE-RELATED PEPTIDE AND BLOOD LIPID IN MIGRAINE PATIENTS

Anmar Ahmed Ali [a], Nazaneen Akbar Omer [b], Zana A. Mohammed [c], and Sarwer Jamal Al-Bajalan [c]

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ABSTRACT

Background

Vascular-neuronal systems are highly affected by lipid levels, and migraine, associated with calcitonin gene-related peptide (CGRP) changes. Therefore, magnetic resonance imaging (MRI) has recently been employed to evaluate headaches in patients with migraines

Objectives

This study investigates the relationship between MRI changes and blood lipid and CGRP levels in migraineurs

Materials and Methods

This case-control study was carried out on 93 patients with migraines and 30 healthy individuals in Shar Teaching Hospital from Oct. 2018 to Oct. 2019. Detailed history and clinical examination were conducted for all of the participants. Data including weight, height, body mass index (BMI), fasting blood glucose (FBG), lipid profile, and CGRP levels were collected. In addition, MRI was performed for all of the participants.

Results

The mean age of the patients was 33 years; 86% of them were females. Brain MRI lesions were detected in 22 (23.7%) of the patients vs 2 (6.7%) of the controls with significant differences ($p=0.04$). The lesions were observed on fluid-attenuated inversion recovery (FLAIR) in 21(22.6%) or T2 in 6 (6.5%). No lesions were detected on T1 or diffusion-weighted images (DWI). There were no significant relationships between the MRI findings and the CGRP, lipid profile, FBG, or vitamin-D3 levels of the patients ($p>0.05$). Moreover, the patients and controls were comparable in their lipid profile, FBG, vitamin-D3, or CGRP levels ($p>0.05$).

Conclusion

Although MRI lesions were detected in a significant proportion of migraine patients, these findings were not significantly related to changes in CGRP and lipid profile levels.

Keywords: *Migraine, Lipid profile, Calcitonin gene-related peptide (CGRP).*

[a] Department of Neurology, Shar Teaching Hospital, Sulaimani, Iraq.
Correspondence: anmarahmed39@yahoo.com
[b] Department of Medical Biochemistry, College of Medicine, University of Sulaimani, Sulaimani, Iraq.
[c] Department of Medicine, College of Medicine, University of Sulaimani, Sulaimani, Iraq.

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INTRODUCTION

As a disabling headache disorder associated with no long-term morbidity or mortality, migraine has been reported to influence almost all age groups. Migraine can be accompanied by severe headaches, usually exacerbated by strong odors, loud sounds, or excessive visual lights. CGRP is a 37-amino acid peptide. The primary location of CGRP is C and A δ sensory fibres, which play a role in both sensory (nociceptive) and efferent (effectors) functions and present broad innervations all over the body with wide per vascular localization [1,2]. Discovering CGRP as one of the significant peptides gave way to finding a factor that plays an essential role in regulating the cerebral blood vessels[3]

As suggested by a large body of evidence, migraine is a chronic condition in many individuals and is associated with severe co-morbidities like cerebrovascular disease. There is a complex relationship between migraine and stroke. For example, during a migraine attack, ischemic stroke might happen, while migraine-like headaches can occur due to cerebral ischemia [4]. Moreover, ischemic stroke happening outside the setting of a migraine attack can be caused by migraine [5].MRI scans show clinically silent infarcts as hyperintense lesions, whose development can be induced by migraine. Stroke and migraine have been shown to share the exact pathogenesis; because both can occur with conditions like MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) and CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy)[6].

As a typical periodic initial headache disorder, migraine is closely connected to the vascular and neuronal systems. Migraine has also been associated with temporary neurological symptoms in some patients, known as migraine aura [7]. It is well documented that migraine aura is accompanied by an increase in the risk of developing ischemic strokes and other vascular diseases. In addition, some vascular biomarkers, which are risk factors for cardiovascular diseases, can develop due to migraines [8–11]. In the multitude of literature, migraines, particularly those with aura, have been associated with lipid levels [12,13].

The association of migraine with stroke might be attributable to hypertension, adverse lipid profile, decreased sensitivity to insulin, and obesity. These are all known as characteristics of metabolic syndrome, which is a risk factor for cerebrovascular disease[14], Obesity, hypertension, abnormal concentrations of high-density lipoprotein cholesterol and triglycerides, and reduced insulin sensitivity in type 2 diabetes mellitus (T2DM) have been the significant characteristics of metabolic syndrome, which is a proven risk factor for cerebrovascular diseases. About the association of frank diabetes with migraines, studies have reported conflicting results, and in some cases, an inverse relationship between the two has been reported [15].

A large number of the patients suffering from recurrent or chronic headaches do not have any neurological abnormalities; however, computed tomography and magnetic resonance imaging (MRI) is conducted for a significant number of them to evaluate their headaches. However, MRI is costly and widely preferred to CT. In addition, MRI results might vary remarkably based on the acquisition sequences, paramagnetic contrast, and field strength (0.2 Tesla to 3 Tesla). However, MRI has been proved to be more sensitive, especially for vascular disease (venous and arterial infarctions), intracranial hyper/hypotension, pituitary lesions, cervicomedullary lesions, neoplasm, and posterior fossa lesions [16, 17].

The present study was carried out to investigate how MRI changes are associated with CGRP levels, lipid profile, BMI, vitamin D3, and FBG levels in migraine patients.

PATIENTS AND METHODS

Study design and setting

This case-control study was conducted at Shar Teaching Hospital in Sulaimani city, the Kurdistan Region of Iraq, from October 1, 2018, to October 1, 2019.

Study sample

Among the visitors of the outpatient department of Shar Teaching Hospital in Sulaimani city, 93 patients were recruited, consisting of 80 females and 13 males, with an age range of 18-65 years. The study sample was chosen based on the following inclusion criteria: diagnosis as classical or common migraine, age 18-65 years, both genders, and patients free from migraine attacks or abortive therapy at least 24 hours before sample collection. Patients with: any other primary or secondary headaches, diabetes mellitus, hypertension, ischemic heart and brain disease, pregnant women, and women on oral contraception pills (OCP) were excluded from the study. All of the patients had a

history of migraines of variable durations and received abortive treatment. The patients were grouped based on their age, sex, residence, blood pressure, mean arterial pressure (MAP), body mass index (BMI), and dyslipidemia. The study sample was compared to 30 healthy control subjects.

Data collection and procedures

All of the patients were subjected to a detailed history and clinical examination, and the diagnosis of migraine was confirmed according to the International Classification of Headache Disorders, 3rd edition ICHD-3 criteria[18], as migraine without aura (common migraine) and migraine with aura (classical migraine).

The intensity of pain was determined by the Visual Analogue Scale (VAS) [19] and disability rates were determined by the Migraine Disability Assessment Scale (MIDAS) [20]. The primary outcome measures were analyzed regarding the significance of brain MRI changes in migraine patients. Each patient completed a self-administered questionnaire and was interviewed by the researchers. The questionnaire included questions regarding duration of headache history, aura, frequency, severity, pain characteristics, location, attack duration, presence or absence of aggravating factors, accompanying symptoms, and autonomic features. A final diagnosis was made by applying the criteria in ICHD3 [19] after the questionnaire analysis, medical records, and neurological examination were compiled.

Five millilitres of venous blood were collected from the cubital vein of each study participant using disposable syringes, collected in straight gel tubes, and allowed 20 minutes at room temperature to clot. Serum was separated by centrifugation at 3000 rpm for 20 minutes and then stored at -70oC in the research laboratory in Shar Teaching Hospital until assayed. Biochemical tests, including total cholesterol (mg/dl), triglycerides (mg/dl), HDL (high-density lipoproteins, mg/dl), LDL (low-density lipoproteins, mg/dl), VLDL (very low-density lipoproteins, mg/dl), and glucose levels) mg/dl(was performed by spectrophotometer. CGRP (pg/dl) levels were assessed by the enzyme-linked immunosorbent assay (ELISA) technique.

Measurements of body weight and height were done for all individuals in light clothing wearing flat shoes or no shoes. BMI was calculated as weight/height ^{2} (kg/m^2), and all individuals in this study were classified as average weight or obese according to their BMI categories [21].

MRI was performed at the radiology department of Shar Teaching Hospital using a 1.5 T Philips Intera Achieva scanner (Philips Medical Systems, Best, the Netherlands) using a 32- element phased-array receive head coil. As a result, T1, T2, FLAIR, and DWI sequences were obtained.

Data analysis

Data were collected and coded. The collected data were reviewed and analyzed using the Statistical Package for Social sciences (SPSS version 22). Descriptive statistics such as frequency and percentage were calculated. Measures of central tendency and dispersion around the mean were used to describe continuous variables. P-value was obtained for the continuous variable using T-independent test and for categorical variable chi-square and was considered significant if it was less than 0.05

Ethical considerations

Necessary approval for study conduction was obtained from the University of Sulaimani College of Medicine (no. 64; August 6, 2018). Moreover, written informed consent was signed by each of the participants.

RESULTS

Our sample included 93 migraine patients; for 60 of them, the level of CGRP was measured, while for 33 of them, the CGRP was not measured. The control group includes 30 health subjects. For all of them, the CGRP level was measured.

The mean age of the patients was 33.14 years with female gender predominance (86%), while the mean age of the controls was 35.5 years with male predominance (56.7%). Most of the study participants resided in large cities. Their occupations were housewives, employees, or manual workers. Most of them were married. All the demographic characteristics were comparable between the 2 study groups (p-value > 0.05) apart from the gender where males were more predominant in the control group (p-values < 0.05) see Table 1.

In terms of the anthropometric (age and BMI) and biochemical evaluation (Vit. D3, TC, TG, HDL, LDL, VLDL & FBG), the cases & the controls were comparable (p-value > 0.05). However, the blood pressure parameter was within the higher levels of the typical values in the control group (p-values < 0.05)

see Table 2., The mean CGRP serum level was higher in migraine patients than healthy subjects, although it did not reach a notifiable difference (p-value result was marginal 0.15). See Table 3 and Figure 1,Changes in T2, FLAIR or both MRI sequences s were observed in 22 out of 93 migraine patients (23.7%) with a significant difference from the control group (p-value 0.04) see Table 3 and Figure 1.

There were no detectable lesions on T1 or DWI sequences on MRI of the patients or controls. In 21 migraine patients, FLAIR lesions were detected; in>50% of them,> 4 lesions were counted. On the T2 sequence, six patients showed MRI lesions; only in 2 of them, the number exceeded four lesions. The lesions were not specified in the location in any of the patients. Moreover, 17 patients (18.3%) had lesions in their supra-tentorial region, with sub-cortical, per-ventricular, and juxta-cortical distribution in order of frequency. Subcortical WM defines as a WM 1 cm far from the inner of the GM , Table 4.

In our attempt to find other risk factors that may affect the accumulation of MRI lesions in migraine patients, we did a subgroup analysis of the data between patients with MRI lesions and those without MRI lesions. We found older migraineurs age, older age at onset of a migraine, higher BMI, higher systolic BP, more elevated diastolic BP, or higher MAP even in the absences of hypertension diagnosis to be associated with more MRI lesion loads. On the other hand, CGRP, vitamin D3 & other biochemical factors (TC, TG, HDL, LDL, VLDL, and FBG) fail to impact MRI lesion accumulations among migraine patients. Table 5.

Table 1. The demographic characteristics between migraine cases & the controls

		Controls: n (%) n= 30	Cases n (%) n=93	P*
Gender	Male	17(56.7)	13(14.0)	0.001
	Female	13(43.3)	80(86.0)	
Occupation	Employed	13(43.3)	27(29.0)	
	Worker	5(16.7)	14(15.1)	0.28
	Housewife	12(40.0)	52(55.9)	
Marital Status	Single	3(10.0)	23(24.7)	0.09
	Married	27(90.0)	70(75.3)	
Address	Inside city	27(90.0)	79(84.9)	0.76
	Outside city	3(10.0)	14(15.1)	

*Chi-square test

Table 2. Anthropometric & Biochemical characteristics between migraine cases & the controls.

	Controls n (±SD)	Cases: mean (±SD)	P*
Age	35.50 ± 10.08	33.14 ± 9.25	0.24
BMI	26.17 ± 3.82	26.83 ± 4.37	0.46
Systolic BP	120.20 ± 7.69	115.32 ± 16.82	0.03
Diastolic BP	78.33 ± 6.06	72.76 ± 10.40	0.001
MAP	92.27 ± 6.16	86.94 ± 12.09	0.001
Vit D3(ng/ml)	18.66 ± 9.42	16.86 ± 11.86	0.45
TC(mg/dl)	166.93 ± 41.53	178.08 ± 85.56	0.49
TG(mg/dl)	120.60 ± 80.27	128.75 ± 66.27	0.58
HDL(mg/dl)	46.07 ± 11.16	49.16 ± 13.86	0.27
LDL(mg/dl)	92.90 ± 21.08	97.80 ± 33.01	0.35
VLDL(mg/dl)	24.12 ± 16.05	25.48 ± 13.52	0.65
FBG(mg/dl)	99.80 ± 14.44	97.26 ± 17.62	0.48

*Independent t-test, MAP: mean arterial pressure

Table 3. Comparing the controls and patients in terms of CGRP level & MRI findings

	Control	Case	P-value
CGRP level (pg/ml) (Mean±SD)	n: 30 209.23±103.85	n: 60 244.97±111.87	0.15*
+ve Brain MRI Finding	n: 30 2(6.7)	n: 93 22(23.7)	0.04**

***Independent t-test,** Chi-square test**

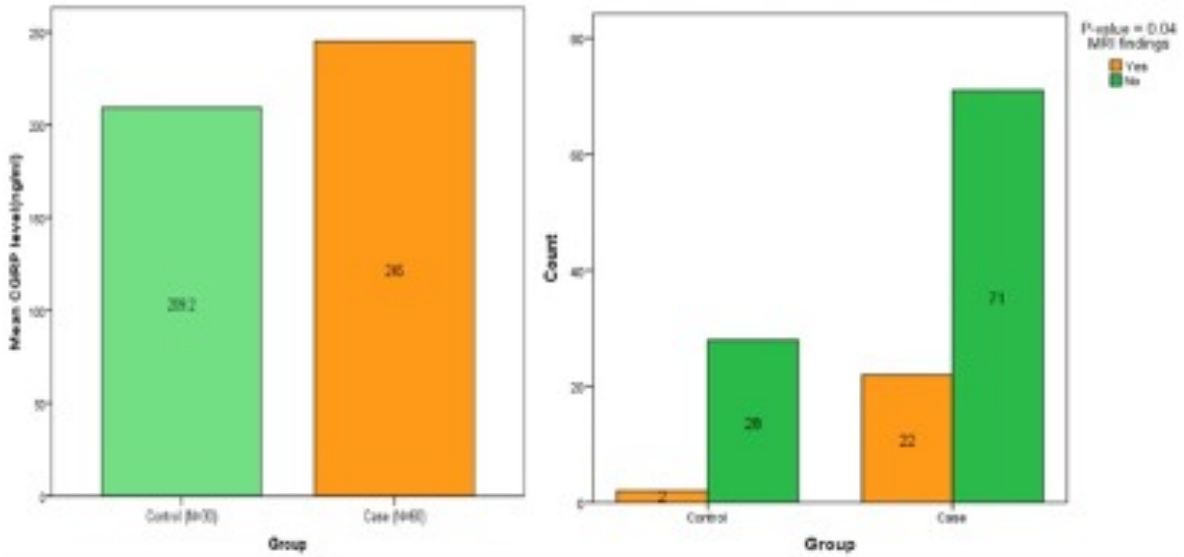

Figure 1. A significant number of migraine patients had MRI lesions in comparison to the controls & the level of CGRP was higher in migraine patients, although the statistical significance was marginal.

Table 4. Number and location of MRI lesions among migraine patients (n: 93).

No. of lesions	Frequency (%)	Location of lesions	Frequency (%)
No. on T1	0 (0%)	Supratentorial	17 (18.3)
No. on T2	6 (6.5%)	Infratentorial	1 (1.1)
≤ 3	4 (4.3)	Subcortical	9 (9.7)
> 4	2 (2.2)	Periventricular	6 (6.5)
No. on FLAIR	21 (22.6)	Juxtacortical	5 (5.4)
≤ 3	10 (10.8)		
> 4	11 (11.8)		
No. on DWI	0 (0%)		
Total patients with MRI lesions	22 (23.7)		

Table 5. Comparison of the study parameters between the patients with MRI findings vs the patients with no MRI finding

Study parameters	Patients With MRI findings mean (±SD)	Patients with No MRI Findings mean (±SD)	P*
Age	38.41 ± 9.80	31.51 ± 8.50	0.00
Age at Onset	29.00 ± 9.94	23.59 ± 9.14	0.02
BMI	28.96 ± 4.84	26.17 ± 4.03	0.01
Systolic BP	125.23 ± 20.38	112.25 ± 14.39	0.01
Diastolic BP	76.18 ± 11.56	71.70 ± 9.87	0.08
MAP	92.52 ± 13.74	85.21 ± 11.07	0.01
CGRP level(pg/ml)	257.86 ± 133.58	241.04 ± 105.77	0.63
Vit D3(ng/dl)	17.35 ± 13.28	16.70 ± 11.49	0.82
TC(mg/dl)	183.77 ± 34.73	176.31 ± 96.15	0.72
TG(mg/dl)	150.68 ± 74.09	121.96 ± 62.67	0.08
HDL(mg/dl)	50.27 ± 13.79	48.82 ± 13.97	0.67
LDL(mg/dl)	105.77 ± 30.33	95.32 ± 33.62	0.20
VLDL(mg/dl)	30.14 ± 14.82	24.04 ± 12.86	0.06
FBG(mg/dl)	101.68 ± 18.84	95.89 ± 17.14	0.18

***Independent t-test**

DISCUSSION

In the detection of brain lesions, FLAIR can be employed in brain imaging to suppress cerebrospinal fluid (CSF) impacts on the image to bring out the periventricular hyperintense lesions like multiple sclerosis (MS) or migraine plaques [22, 23]. The results of the present study demonstrated that 22.6% of patients had FLAIR lesions. FLAIR MRI, which is a heavily T2-weighted technique, dampens the ventricular CSF signal; therefore, the highest signals on the sequence belong to specific brain parenchyma abnormalities like MS lesions, while the CSF appears black [23]. Many studies showed the strong association of migraine with MRI lesion for unclear pathogenesis [24,25], that in some individuals are challenging to differentiate from MS plagues based on sole conventional imaging [26]. The conventional T1-weighted and T2-weighted sequences are utilized to identify and characterize disease pathology. In the present study, there was no T1 lesion in the participants, 4.3% of patients had three or fewer T2 lesions, and 2.2% of patients had four or more T2 lesions. The result of the present study showed that none of the subjects had diffusion-weighted imaging or space-occupying lesion. Moreover, the location of the lesions was not specific in pattern in any of them.

As a differential diagnosis of migraine-related white matter changes in young childbearing female age, MS may come to the mind. In this study, we did not find the specific pattern of MS distribution like periventricular, juxtacortical, infratentorial, or optic nerve lesions. The lesions were smaller, more discrete than the lesion usually seen in MS, and subcortical was the central location. The migraine-related white matter lesion in this study was more predominant in those migraineurs with older age, older at time of first migraine attack, and those who had high BMI or high MAP, systolic or diastolic blood pressure even if not reach the level required for the diagnosis of hypertension. In the present study, the process of identification of lesion locations demonstrated that in 18.3% of the patient's supratentorial and just in 1.1% of the patient's infratentorial lesions were identified. This observation is in favour of the vascular origin of migraine-related lesions rather than demyelination [27].

CGRP is one of the strongest microvascular vasodilators discovered (10-fold more significant than the prostaglandins and 100–1,000 times greater than other vasodilators) [28]. Moreover, equivalents to the well-known blood oxygen level-dependent (BOLD) MRI response can be artificially induced by CGRP variants. BOLD MRI response forms the basis of most functional brain imaging experiments[29]. However, here we did not find a significant relationship between CGRP level and MRI findings among our cases.

In this work, the status of vitamin D3 had no significant relationship with MRI findings of brain images.

Similarly, Mowry EM et al. assessed the relationship between vitamin D status and developing contrast-enhancing lesions or new T2 lesions on brain MRI. They indicated that new T2 lesions are more likely to develop in active smokers. In addition, their results revealed no significant association between vitamin D and the development of T2 lesions, as shown by MRI findings [30]. According to the study results conducted by Nowaczewska et al. (2020), vitamin D3 might have beneficial effects on some patients with headaches and migraines. It helps decrease the frequency of headaches, particularly in those with vitamin D deficiency [31],However, the low vitamin D3 in MS had been well established over many years[32], this relation with migraine may not be the same as we mentioned above. Furthermore, the migraine-related lesions were vascular rather than demyelinating in nature.

The present study results indicated that there were no significant associations between MRI findings and total cholesterol levels, HDL, and LDL. Unlike this finding, it has been reported that lower total brain grey matter volume correlates with high LDL and total cholesterol. In addition, MRI indexes of decreased neuronal integrity in posterior brain regions have been reported to be associated with higher levels of total cholesterol and non-HDL cholesterol and lower levels of high-density lipoprotein (HDL) cholesterol [33],However, in the present study, there was no association between MRI findings and triglycerides level. In this regard, the results of the study conducted by Choi et al. (2016) revealed that there is a relationship between β-amyloid (Aβ) positron emission tomography (PET) and higher triglycerides [34]. Moreover, brain amyloid deposition measured with PET is reported to be associated with an increased number of midlife vascular risk factors and midlife dyslipidemia [35, 36].

The results of the present study revealed no significant association between MRI findings and VLDL. In this regard, there is minimal information on how brain disease progression is affected by the roles of HDL and VLDL levels and serum triglycerides and cholesterol levels. Brain MRI images in clinically isolated syndrome patients after a first clinical demyelinating event have shown an association between increased total cholesterol and rises in the number of contrast-enhancing lesions [37]. Moreover, it has been indicated that the values of TC and TG undergo significant changes during migraine attacks [38]. However, Kurth et al. (2008) did not find any significant association between the frequency of migraine attacks and TC, LDL-C, and HDL-C levels [39].

Moreover, Gruber et al. (2010) reported raised oxidized LDL levels are associated with an about 8-fold increase in the risk for migraine (12). The discrepancies between this study and previous studies and other previous investigations can be attributed to various factors, ranging from different MRI devices and features to different types of migraine and associated underlying diseases or complications. Therefore, it is necessary to conduct more clinic- and region-based studies in the future to find out the actual usefulness of MRI images in diagnosing migraines.

In a study carried out by Mortimer et al. (2010), there was an association between higher FBG and dementia apart from vascular risk factors and MRI indicators of vascular disease and remained a significant risk factor when analyses were restricted to subjects with normal FBG. The results of that cross-sectional study revealed that dementia might develop as a result of an average high level of FBG [40]. However, the results of the present study showed no significant association between MRI findings and FBG, bearing in mind those patients with DM have been excluded in this study.

According to the results, no significant difference was observed between the patients and controls in terms of vitamin D3, lipid profile, and FBG (p-value>0.05). The CGRP level was higher in migraine patient rather than the control subjects although did not reach a statistically significant level. In this regard, plasma CGRP levels have been reported to rise during migraine attacks [41–43]. It has also been shown that CGRP has a role in migraines [44]. Our result may give an impression that CGRP assay may not be helpful in the diagnosis of migraine during attack-free intervals, as an attack of migraine during 24 hours of blood sampling was one of our exclusion criteria. Moreover, it has been indicated that the values of TC and TG undergo significant changes during migraine attacks [38]. However, Kurth et al. (2008) did not find any significant association between frequency of migraine attacks and TC, LDL-C, and HDL-C level

The present study's study sample consisted of more females than males. In line with this, other studies have reported a higher prevalence of migraine at about three times among women compared to men. For example, migraine prevalence among American women and men has been reported to be 18 and 6 per cent, respectively. Furthermore, migraine prevalence has been reported to be 43% among women in reproductive years. Of those

with migraines, 50% experienced over one attack per month, and 25% had four or more severe attacks each month [45,46]. According to the studies carried out by the Global Burden of Diseases, Injuries, and Risk Factors (GBD) in 2016, one of the significant debilitating causes, especially in young adult and middle-aged women, is migraine [47]. In line with this, the present study results showed that over three-fourths of migraine patients were less than 40 years.

In conclusion; MRI lesions were detected in a significant proportion of migraine patients versus control subjects. However, the MRI findings were not significantly related to changes in CGRP, lipid profile (TG, LDL, VLDL, and HDL), vitamin D3, and FBG serum levels.

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