Evaluation of the efficacy of the combination of elements present in Zuckerage Retrospective analysis

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Introduction

Type 2 diabetes mellitus. In Mexico, there is a clear upward trend in type 2 diabetes mellitus (DM2) in the population, as evidenced by data obtained from the latest national health and nutrition surveys conducted by the National Institute of Public Health . Thus, the prevalence of a previous diagnosis of DM2 has been 7.0% in 2006, 9.2% in 2012 and 9.4% in 2016 (INSP, 2016).

In the last survey, although the majority of people with DM2 (87.8%)referred receiving medical treatment to control it, only a quarter is controlled (INSP, 2016; Gutiérrez, 2012). Furthermore, medical control of diabetes and its potential complications remains low. The determination of glycated hemoglobin (HbA1c) was reported as 15.2%, the search for lesions in the lower limbs was 20.9% and the measurement of microalbuminuria was 4.7%, during the year prior to the survey (INSP, 2016).

Therefore, it is not surprising that chronic complications have almost tripled compared to the previous survey. Thus, visual impairment was reported in 54.4%, the presence of ulcers in the lower limbs in 9.1% and amputations in 5.5% (INSP, 2016).

Complications represent clinically definable end points that occur as a result of a series of complex intracellular pathways that trigger serious adverse effects in multiple organs, and are related to the degree and duration of hyperglycemia (Ranjit, 2011).

Findings from the follow-ups of the Epidemiological Study of Diabetes Interventions and Complications derived from the Diabetes Control and Complications Trial (DCCT-EDIC) and the UK Prospective Diabetes Study (UKPDS, demonstrated the importance of glycemic control early in the course of the disease and its value in preventing late complications (Ranjit, 2011). *"Metabolic memory". The phenomenon of continued beneficial effects on diabetic complications after a period of improved glycemic control, even after a return to normal (often worse) metabolic control, was called "metabolic memory" by the DCCT-EDIC researchers. and “legacy effect” by UKPDS researchers (Ranjit, 2011).*

Metabolic memory begins with persistent hyperglycemia that triggers oxidative stress and non-enzymatic reactions of glucose, ascorbate, and other carbohydrates with proteins, lipids, and nucleic acids (glucose forms covalent adducts through glycation) and also by lipid peroxidation, leaving a heterogeneous group of chemical moieties called advanced glycation end products (AGEs) (Ranjit, 2011; Peppa, 2005). Although this process occurs during aging, it is markedly accelerated in DM2.

AGEs. AGEs directly induce the cross-linking of long-lasting proteins such as collagen and trigger vascular stiffness and thus alter vascular structure and function. AGEs also interact with certain receptors to induce intracellular signals that lead to increased oxidative stress and the production of key pro-sclerotic and

pro-inflammatory cytokines. The modification that AGEs produce in proteins and, especially, in mitochondrial ones, can be irreversible (lasting modification of substrates) and result in a decrease in mitochondrial function with excess in the formation of reactive oxygen species (Rhee, 2018 ; Peppa, 2005; Ranjit, 2011; Koska, 2018). Another couple of theories involve the anti-inflammatory effect of insulin and the epigenetic changes caused by oxidative stress (Ranjit, 2011).

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The AGE receptor system that includes specific and nonspecific receptors and soluble binding proteins plays an important role in their homeostasis. At least two types of cell receptors have been characterized: AGER and RAGE. AGER binds to AGEs and degrades them, protecting tissue from oxidative damage and minimizing inflammation. AGE-R1 is a 50 kD protein involved in its endocytosis and processing. AGE-R2 is an 80-90 kD protein involved in early signaling and AGE-R3 is a 30-35 kD protein that participates in cell clearance and activation. The other type of multiligand receptor, RAGE, is thought to promote and perpetuate cell activation and tissue damage through increased oxidative stress, class A (MSR-A) and B (MSR-B) scavenger receptors, and lysozyme. involved in cell uptake and degradation of AGEs (Peppa, 2003; Vlassara, 2014). AGEs interact with their plasma receptors (RAGE) and alter intracellular signaling, alter gene expression, release pro-inflammatory molecules and ROS in vascular myocytes and vascular endothelial cells, leading to micro- and macrovascular complications (Rhee, 2018; Peppa, 2003). Thus, the balance between these two receptors is critical in the maintenance of oxidative homeostasis or progression of diabetes (Vlassara, 2014).

AGEs are powerful toxic molecules that promote cell death and contribute to organ damage. AGEs can induce the development or progression of not only diabetic complications, but also the pathophysiology of many other diseases,

including cardiovascular, neurodegenerative diseases such as Alzheimer's, Parkinson's, and brain damage from alcoholism (Byun, 2017).

The serum levels of AGEs are an independent risk factor for endothelial dysfunction in DM2. They are also an independent risk factor for cardiovascular mortality in women with diabetes (Rhee, 2018).

The circulating levels of AGEs reflect the balance between endogenous formation, ingestion, catabolism, tissue degradation and renal elimination. At the tissue level, macrophages and other cells engulf and degrade AGEs through receptor or non-receptor pathways, resulting in the formation of low molecular weight AGE peptides. These peptides undergo a variable degree of reabsorption and further catabolism in the proximal nephron, with the remainder excreted in the urine (Peppa, 2005).

One of the most studied AGEs is the glucoxidation epitope, N-carboxymethyl-lysine (CML). CML is formed when glucose and its oxidation products, glyoxal and methylglyoxal, react with the ε -amino group of lysine. CML is a product that accumulates in tissues with age and its rate of accumulation accelerates in diabetes. CML is formed from the oxidation of carbohydrates and lipids, and exists in free and peptide-bound forms, making it a general oxidative stress biomarker (Shaw, 2002; Ames, 2008). Furthermore, CML levels are a biomarker of glycoxidation in DM2 and are related to the development of microvascular complications (Wautier, 2003).

AGE modulators. Drugs that can modulate AGEs include: pravastatin, atorvastatin, telmisartan, ramipril, rosiglitazone, linagliptin, etc. These drugs act mainly through the inhibition of damage caused by AGEs (Rhee, 2018). Hence, additional interventions are required for the prevention of disease complications with innovative mechanisms of action, products that act before the formation of AGEs considering that their effect is prolonged.

One of the elements prevents the activation of three important pathways of hyperglycemic damage (the hexosamine pathway, the intracellular AGE formation pathway, and the diacylglycerol protein kinase C pathway) by increasing the activity of transketolase, the regulatory enzyme for the non-oxidative branch of the phosphatepentose pathway. In animal studies, high doses of another element and a corresponding therapy increase transketolase expression in the renal glomeruli and inhibit the development of microalbuminuria and diabetes-induced hyperfiltration. One element improves peroneal nerve conduction speed in people living with diabetes and one study showed relief from painful neuropathy. Also,

another element prevents micro- and macrovascular endothelial dysfunction and oxidative stress after a meal high in AGEs (Goh, 2008).

It prevents the activation of three important pathways of hyperglycemic damage (the hexosamine pathway, the intracellular AGE formation pathway, and the diacylglycerolproteinase C pathway). Another element that prevents the degradation of intermediate proteins from Amadori to AGE of protein products. It has also been seen to reduce hyperlipidemia and prevent the formation of AGEs. That element antagonizes the angiotensin II-induced elevation of serum and renal AGEs, prevents renal hypertrophy, and decreases salt retention in animal models. It also prevents retinal vascular lesions induced by diabetes.

Two of the elements have been shown in vitro to inhibit the formation of AGEs and therefore have therapeutic potential in the prevention of diabetic vascular complications Thus, one of them acts as a post-Amadori inhibitor, reducing the final levels of AGEs formed . On the other hand, one element, can inhibit glycation reactions and the formation of AGEs. Their mechanisms of action include: (i) inhibition of the formation of AGEs by blocking oxidative degradation of the Amadori intermediate of the Maillard reaction; (ii) clearance of toxic carbonyl products from glucose degradation and lipids; and, (iii) ROS entrapment.

The glycation process can also be inhibited, in its three stages, by a better and more powerful agent than aminoguanidine, the third element, a bioavailable dietary antioxidant flavonol as demonstrated by other authors. In an in vitro study, this third element and others were combined in different proportions and the products generated from this reaction were analyzed. Low amounts of the third element monoglyoxal adducts and diglyoxal adducts were found with long incubation periods. In a bovine serum albumin incubation system with methylglyoxal or glyoxal, the third element bound them directly and thus, significantly inhibited the formation of AGEs

In another more recent study, quercetin more efficiently inhibited the formation of AGEs mediated by glucose or ribose than nine other polyphenols (Bhuiyan, 2017).

Finally, the fourth element is a main polyphenolic component derived from Another element. that has been shown to have a wide variety of biological activities and pharmaceutical properties (antioxidants, anti-inflammatory and immunoregulatory) that provide protection and promotion of human health. Among the properties that have been studied are its antineoplastic, antidiabetic, cardio- and hepatoprotective activities. Likewise, it can protect endothelial cells

from damage induced by carbonyl stress by trapping dicarbonyl components such as methylglyoxal

Additionally, the fourth element shows antidiabetic activity characterized by the control of hyperglycemia by downregulating the activity of alpha-glucosidase and alpha-amylase. It also improves the response of tissues to insulin (Xu, 2019).

Thus, the combination of the four elements (Zuckerage®), according to the principles of U.S. Application No. 16/108,239, inhibits the formation of AGEs and therefore has therapeutic potential. The objective of the present study was to evaluate the safety and efficacy of the nutraceutical Zuckerage in preventing the complications of DM2 associated with endothelial damage (neuropathy, nephropathy and retinopathy).

Material and methods

Design. Retrospective, open, interventional, not blinded (retrospective analysis).

Patients Subjects with DM2.

Period. 24 weeks.

Goals:

Primary. To determine the effect of the ingestion of Zuckerage® at therapeutic doses on the serum levels of CML.

Secondary. Quantify the effect of Zuckerage® on monthly serum glucose and quarterly HbA1c.

Variables Demographic (sex, age), clinical (time of evolution of DM2, anthropometry, vital signs and symptoms of diabetic complications) and paraclinical (CML, serum glucose, cholesterol, triglycerides, HbA1c).

Intervention. The intervention consisted of ingesting two Zuckerage® capsules daily throughout the study. People with DM2 maintained their conventional antidiabetic management, including the diet and physical activity that they were previously doing.

Method. CML quantification was performed with the competitive ELISA kit (Oxiselect N- (carboxymethyl) Lysine (CML) competitive ELISA Kit, Cell Biolabs, Inc.). The system determines the carboxymethylation adducts of lysine in proteins. A logarithmic equation was used to calculate the concentration in ng / mL of CML, for a better fit to the standard curve.

Statistic analysis. Descriptive statistics (frequencies, proportions, arithmetic means, standard deviations, intervals) and inferential statistics (Student's t test for paired samples). A p value <0.05 was considered significant.

Results

Forty-three subjects entered the study, most of whom were male, 42 (95.4%). The mean age was 50.6 ± 6.5 years (34-61 years).

Regarding anthropometric variables and vital signs, no statistically significant differences were found between baseline and final values.

Primary objective. The mean baseline CML values were 823.3 ± 947.4 ng / mL (146.2 - 1,012.8 ng / mL) and the final values were 607.2 ± 187.7 ng / mL (80.5 - 4,124.2 ng / mL), finding a percentage decrease in the 26.2% (p = 0.138) (Fig. 1).

Figure 2 shows a greater dispersion of the baseline CML values, while the final values are more homogeneous. At a general level, the results suggest a positive effect of adding the supplement to the usual treatment, with a clear tendency to stabilize the CML values at low levels. It is necessary to mention that subjects are observed in whom the CML does not decrease. In 19 patients a considerable decrease in the concentration of CML is observed.

Secondary objective. With respect to the baseline and final values of the paraclinical tests analyzed, decreases were found in their figures, highlighting a significant decrease in serum glucose (p <0.0001) (Fig. 3) and HbA1c, although the latter did not reach statistical significance .

Findings. Within the additional laboratory tests that were determined, decreases were found in cholesterol (p = 0.0028) (Fig. 4) and triglycerides (p = 0.6708) values, with no change in the nitrogen values.

Regarding the symptoms of diabetic complications, at the beginning of the study there was one patient with paresthesia / dysesthesia, another with erectile dysfunction, another with insomnia and another with gastrointestinal dysfunction. At the end of the study, no patient reported symptoms of diabetic complications.

Conclusions

In the studied population of people with T2D, a clear tendency to decrease AGEs values (p = 0.138) was found, reaching the primary objective of the study. Regarding the secondary objectives, a statistically significant decrease in the decrease in serum glucose was determined (<0.0001) and the HbA1c values remained stable, although it is necessary to mention that the study period was short, 24 weeks.

The results are promising, and require a larger study including a larger sample size and a longer study period of treatment with Zuckerage®.

The symptoms of diabetic complications present in four patients disappeared at the end of the study. It is necessary to mention that this was not the objective of the study, however, considering that the final therapeutic effect of Zuckerage® is the reduction of the symptoms of the complications of DM2, it is an important finding.

These results require additional studies to confirm the positive results of the combination of thiamine, pyridoxine, quercetin and curcumin on the serum levels of AGEs for the reduction of the frequency and severity of diabetic complications with a larger sample size and a longer follow-up. strict.

Conclusions

In the studied population of people with T2D, a clear tendency to decrease AGEs values ($p = 0.138$) was found, reaching the primary objective of the study. Regarding the secondary objectives, a statistically significant decrease in the decrease in glucose was determined.

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Table 1. Baseline and final values of the analysis population.

Variable	Basal	Final	p
Weight, Kg	87.9 ± 12.9	89.8 ± 12.1	0.5417
Body mass index, Kg / m2	31.0 ± 4.3	31.4 ± 3.8	0.7146
Heart rate, beats / min.	80.5 ± 9.5	78.5 ± 5.4	0.4073
Respiratory rate ,resp./min.	19.3 ± 2.9	20.4 ± 13.8	0.7042
Systolic blood pressure mm Hg	124.9 ± 12.3	121.4 ± 11.5	0.2246
Diastolic blood pressure mm Hg	79.0 ± 5.5	78.5 ± 6.0	0.6919

Table 2. Baseline and final paraclinical values of the analysis population.

Variable	Basal	Final	p
Glucose,	201.9 ± 43.9	148.6 ± 44.7	<0.0001
HbA1c,%	10.5 ± 1.3	10.2 ± 1.3	0.6176
Cholesterol, mg / dL	191.7 ± 32.5	169.8 ± 31.8	0.0028
Triglycerides, mg/dL	206.5 ± 123.6	195.2 ± 114.3	0.6708
BUN, mg / dL	14.6 ± 3.7	15.5 ± 3.6	0.1950
Creatinine, mg / dL	0.8 ± 0.2	0.9 ± 0.2	0.1414

Fig. 1. Decreasing trend of mean carboxymethyl-lysine (CML) levels in people with type 2 diabetes mellitus from week 1 to week 24.

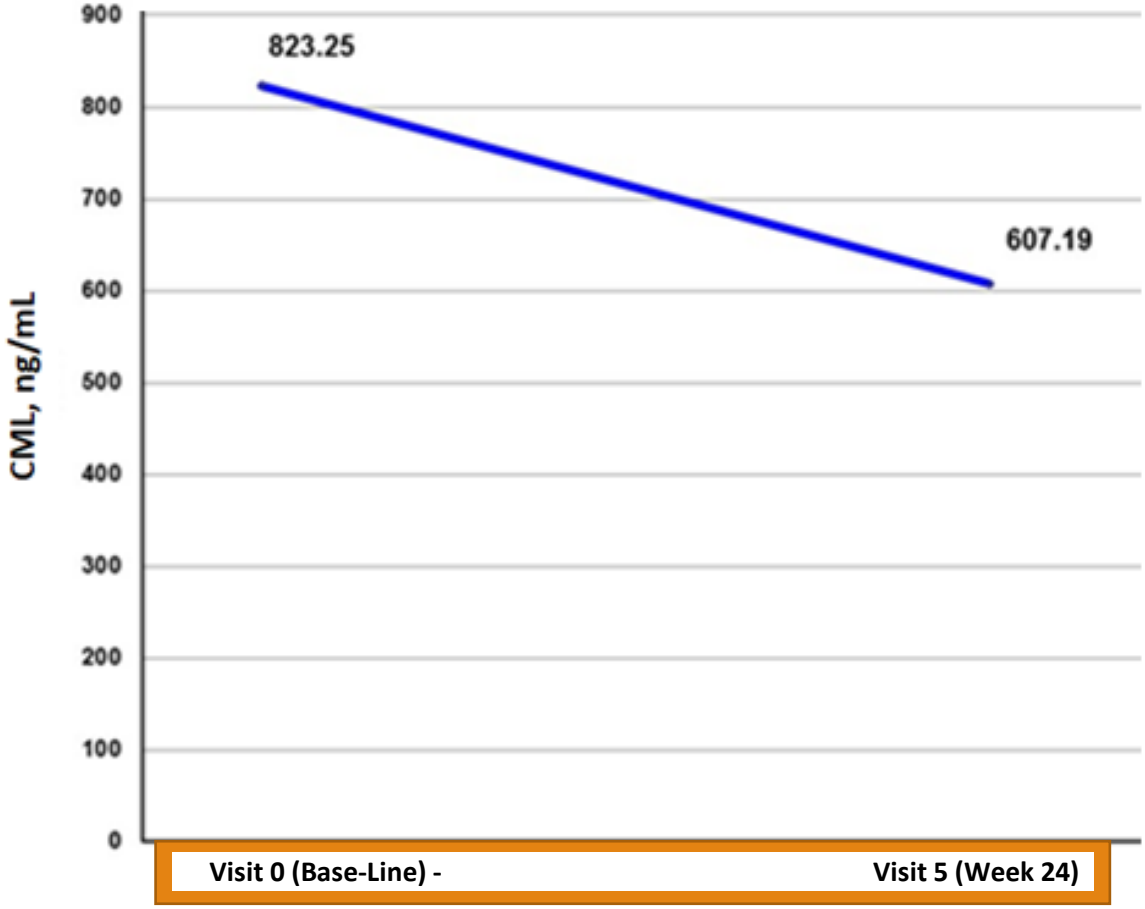

Fig. 2. Change in carboxymethyl-lysine (CML) levels in people with type 2 diabetes mellitus from week 1 to week 24 (DM2).

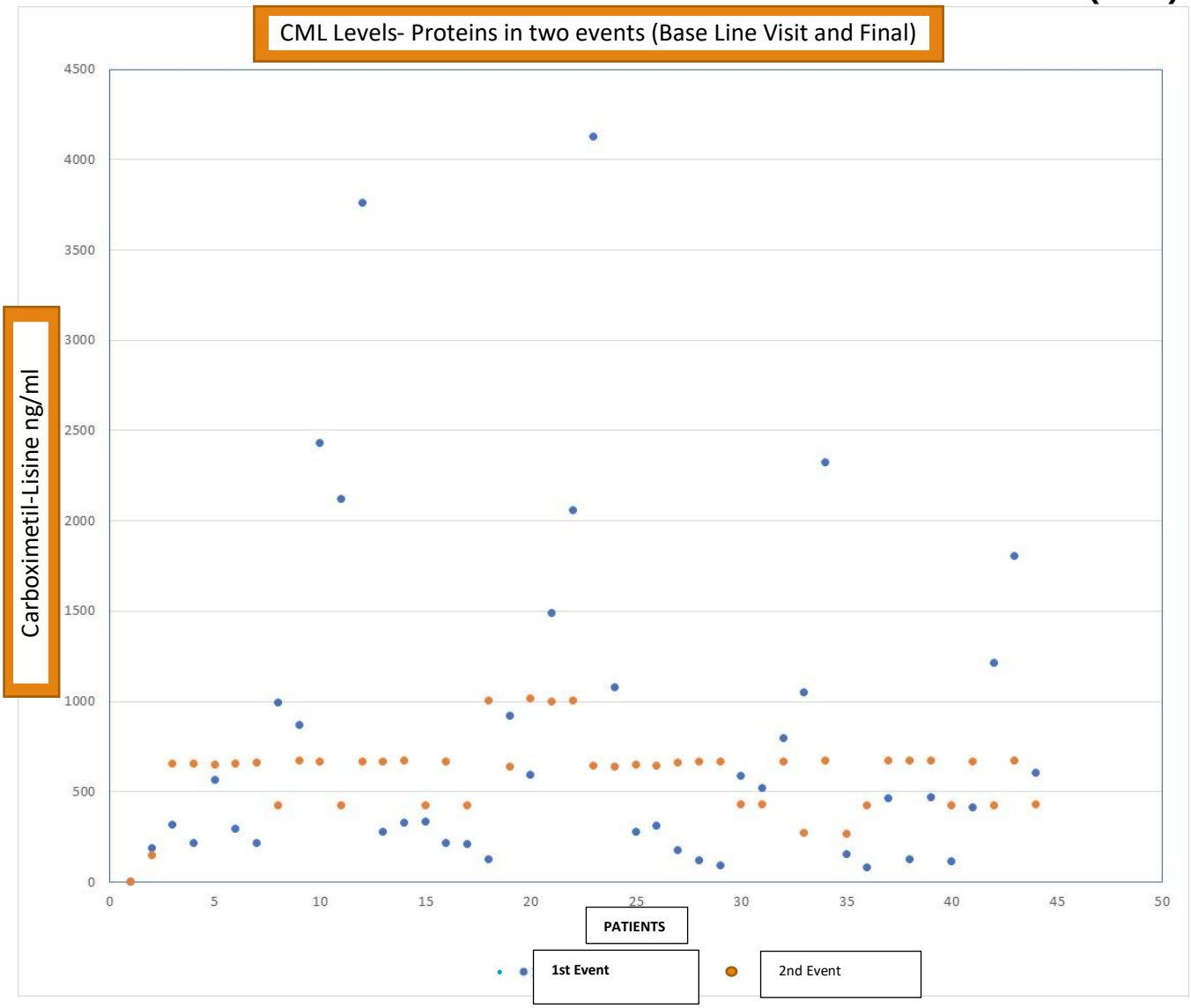

Fig. 3. Decreasing trend of mean serum glucose levels in people with type 2 diabetes mellitus from week 1 to week 24.

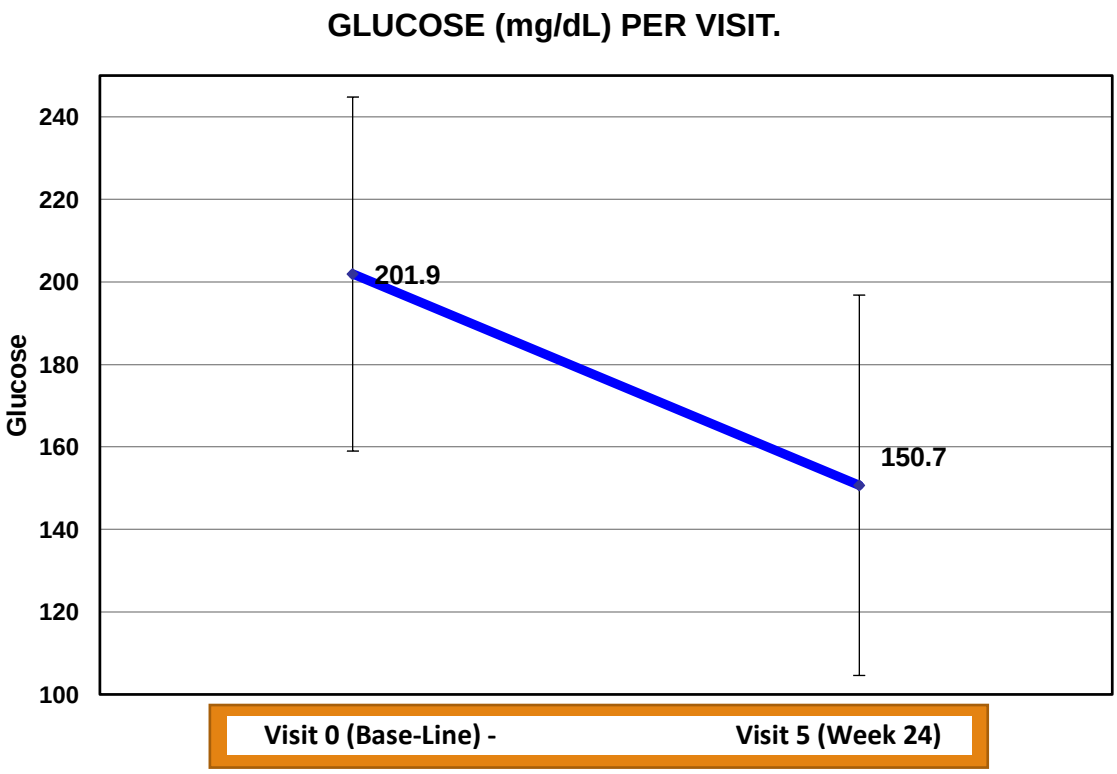

Fig. 4. Decreasing trend of mean serum cholesterol levels in people with type 2 diabetes mellitus from week 1 to week 24.