ORIGINAL ARTICLE

The Influence of Metformin on Serum Carbohydrate Antigen 19-9 (CA 19-9) Levels in Type 2 Diabetes Mellitus Patients

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Abstract

Objective: Diabetes mellitus has been claimed to be a risk factor for the development of pancreatic carcinoma. CA 19-9 has a great sensitivity in detection of pancreatic adenocarcinoma. Metformin exhibits a strong and consistent antiproliferative action on several cancer cell lines including pancreatic cancer. We aim to determine the influence of metformin on CA 19-9 levels in type 2 diabetes mellitus patients.

Methods: Total 193 patients with type 2 diabetes mellitus were registered for a single centre, cross-sectional study. On the basis of treatment modalities, patients were divided into metformin group (93 patients) and non-metformin group (100 patients). Detailed history, clinical examination, anthropometric measurements, serum CA 19-9 level, glucose and lipid metabolic profiles were determined. Results were presented as mean±SD. Association between CA 19-9 level and other variables were assessed with Pearson correlation and multiple stepwise regression analysis.

Results: Mean CA 19-9 level was 18.99±4.30 U/ml in the metformin group as compared to 30.49±5.61 U/ml in non-metformin group (p<0.001). Mean value of CA 19-9 was found highest among all i.e. 37.05±4.94 U/ml in patients taking insulin. Patients having lifestyle modification for the management of diabetes had their mean CA 19-9 level of 21.39±5.62 U/ml. CA 19-9 level is positively correlated with age, duration of diabetes, BMI, 2-hour Plasma Glucose level, HbA$_1$C, VLDL cholesterol, triglyceride, total cholesterol, LDL cholesterol (p<0.005) and negatively correlated with HDL cholesterol (p<0.001).

Conclusion: Metformin is associated with lower level of CA 19-9 in type 2 diabetes mellitus patients. It may have a protective role in preventing pancreatic damage and pancreatic cancer in diabetic individuals. CA 19-9 level could be an effective indicator of glycemic control, disease progression and lipid metabolism in patients with type 2 diabetes mellitus.

Introduction

Diabetes has been recognized as a key factor contributing to the development of solid organ malignancies including liver, pancreas, colorectal, breast, endometrial, uterine, and bladder.[1,2] The hepatic[3] and pancreatic carcinoma[4] shows the strongest association with type 2 diabetes mellitus. Insulin resistance, hyperinsulinemia, oxidative stress, and proinflammation have been suggested as the potential mechanisms[1]. Potential risk factors (modifiable and non-modifiable) common to both cancer and diabetes include aging, sex, obesity, physical activity, diet, alcohol, and smoking.

Carbohydrate antigen 19-9 (CA 19-9) was originally isolated from a human colorectal cancer cell line as a mucin like product by Koprowski and colleagues in 1979. The antigen is found in the normal epithelial cells of the gall bladder, biliary ducts, pancreas and stomach.[5] While elevations in serum CA 19-9 appear to be useful in the diagnosis of adenocarcinoma of the upper gastrointestinal tract and in the monitoring of colonic carcinoma, its greatest sensitivity is in the detection of pancreatic adenocarcinoma.[6] CA 19-9 is the only US Food and Drug Administration (FDA) approved biomarker for the diagnosis of pancreatic cancer. CA 19-9 level also increases in inflammatory conditions of the hepatobiliary system and in thyroid diseases as well as in pancreatic tissue damage that might be caused by diabetes.[7]

Metformin belongs to the biguanide class of oral hypoglycemic agents and is a widely used antidiabetic drug now prescribed to almost 120 million people in the world for the treatment of type II diabetes. Metformin exhibits a strong and consistent antiproliferative action on several cancer cell lines, including breast, colon, ovarian, pancreatic, lung and prostate cancer cells.[8]

Metformin exerts both indirect (insulin dependent) and direct (insulin independent) actions at the cellular level.[9,10] Its direct effect is mediated via AMPK activation and reduction of mTOR signaling pathway, which leads to inhibition of gluconeogenesis in the liver, protein synthesis and cell proliferation in cancer cells. The indirect effects of metformin are mediated through its blood glucose lowering ability and subsequent reduction of the circulating insulin level.

Various studies have shown the antineoplastic effect of metformin. However, very few data are available regarding the correlation between metformin and CA 19-9 levels. Therefore, we planned this study to determine the influence of metformin on CA 19-9 levels in type 2 diabetes mellitus patients.

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Aims and Objectives

We are aimed to determine serum carbohydrate antigen 19-9 (ca 19-9) levels in

1. Type 2 diabetes patients taking metformin.

2. Type 2 diabetes patients on life style modification only.

3. Type 2 diabetes patients with different treatment modality viz insulin, Sulphonylurea, Thiazolidinediones etc.

Material and Methods

Study design

Total 193 patients with type 2 diabetes mellitus were registered for a single centre, cross-sectional study. The study was conducted in department of medicine and diabetic care and research centre, S.P. medical college & associated group of P.B.M. hospitals, Bikaner during the period of October 2014 to September 2015. On the basis of treatment modalities, patients were divided into two groups; Patients taking metformin for more than 2 years were taken in the metformin group (93 patients) and patient taking treatment other than metformin were labeled as non-metformin group (100 patients).

Inclusion criteria:

• Known case of diabetes mellitus type 2

• Taking treatment for more than 2 years

Exclusion criteria:

1. Patients with type 1 diabetes mellitus

2. Patients with history of smoking and alcoholism

3. Patients with history of hypertension

4. Patients with history of liver and kidney disease

5. Patients with history of infectious disease and malignancy

6. Patients with history of acute and chronic pancreatitis

7. Patients with history of bronchial asthma and chronic allergic condition, Buerger's disease, systemic sclerosis, Raynaud's disease and other connective tissue disorders

8. Patients with history of hormonal replacement therapy

9. Patients with history of thyroid disorders

10. Patients with history of AIDS, hepatitis B and hepatitis C

Detailed history, clinical examination, anthropometric measurements, biochemical indices were assessed for all the selected patients.

Routine investigations performed were CBC, ESR, renal function test, blood sugar (fasting and postprandial), HbA_1C, liver function test, lipid profile, urine complete and microscopy and ECG.

Ca 19-9 Levels

Approximately 2ml of venous blood sample was withdrawn in a microtitre well after an overnight fasting. Samples were stored at 2° to 8°C. CA 19-9 level was measured from serum by commercially available CA 19-9 ELISA kit by complete automatic chemistry auto analyzer (ARK diagnostics private limited).

Statistical analysis

Statistical analysis was performed with SPSS software. Distributions of patients between case and control groups were tested for significance using $\chi 2$ test. Results are presented as mean±SD. Unpaired student's t-test was used to compare case and control group. To compare different treatment modality in type 2 diabetic patients, ANOVA test was used. Association between CA 19-9 level and other variables were assessed with Pearson correlation and multiple stepwise regression analysis. Multifactorial ANOVA (analysis of variance) test was used to study the independent effect of different variables on CA 19-9. p value less than 0.05 was considered significant.

Observations

The number of patients were more with CA 19-9 level in the range of 21-30 U/ml. There were 32 patients in metformin group and 43 patients in non-metformin group in the same range. Number of patients with level <10 U/ml of CA 19-9 level were 2 in metformin group and 1 in non-metformin group. The patients with CA 19-9 level in the range of 11-20 U/ml were 58 in patients taking metformin as compared to no patient in non-metformin group. 53 patients were reported with CA 19-9

level of 31-40 U/ml in non-metformin group as compared to only 1 patient in metformin group. Minimal cases were reported with the level of CA 19-9 level >40 U/ml i.e. 3 cases were found in non-metformin group and none was reported in metformin group. This difference was found to be highly significant (p <0.001) (Table 1, Figure 1).

In the present study, the mean value of CA 19-9 in the metformin group was found to be 18.99±4.30 U/ml that was less than that found in non-metformin group i.e. 30.49±5.61 U/ml. This difference was also found to be highly significant (p<0.001).

Mean value of CA 19-9 in patients taking metformin was 18.99±4.30 U/ml, while who only had life style modification had a value of 21.39±5.62 U/ml. Patients taking sulfonylurea had a mean CA 19-9 value of 29.59±3.40 U/ml and in patients taking insulin it was found to be highest of all i.e. 37.05±4.94 U/ml. In patients taking both thiazolidinedione and sulfonylurea, mean CA 19-9 level was found to be 28.96±4.31 U/ml, whereas in patients having a combination of sulfonylurea and alpha-glucosidase inhibitor it was 32.04±4.50 U/ml, in patients taking insulin+sulfonylurea it was 32.25±2.0 U/ml while in patients treated with insulin + DDP-4 inhibitor mean value of CA 19-9 level was 36.40±4.39 U/ml. The highest levels were reported in patients taking insulin only whereas lower levels of CA 19-9 were found in patients who took metformin and this difference was found to be highly significant statistically (p<0.001) (Table 2).

Correlation of CA 19-9 level with different variables shows that age, duration of diabetes, BMI, 2-hour Plasma Glucose level, HbA_1C, VLDL cholesterol, triglyceride, total cholesterol (p<0.001) and LDL cholesterol (p<0.005) was positively correlated while HDL cholesterol was negatively correlated with CA 19-9 level (p<0.001) (Table 3, Figure 2).

When we applied multiple stepwise regression analysis for different variables on CA 19-9 level, age had a significant correlation and when we added the duration of diabetes, BMI, HbA_1C, HDL cholesterol, LDL, cholesterol and triglyceride, significance level had a higher range (p <0.001) (Table 4, Figure 3).

Table 1: Distribution of cases according to CA 19-9 level (U/ml) in both groups

CA 19-9 (U/ml)	Groups		Total
	Metformin	Non-Metformin	
	No. (%)	No. (%)	No. (%)
<10	2 (2.15)	1 (1)	3 (1.55)
11-20	58 (62.37)	0	58 (30.05)
21-30	32 (34.40)	43 (43)	75 (38.86)
31-40	1 (1.07)	53 (53)	54 (27.97)
>40	0	3 (3)	3 (1.55)
Total	93	100	193
c^2	112.9		
p	<0.001		

Table 2: Mean CA 19-9 level (U/ml) in different treatment modalities

Individual Group	No. of cases (%)	Mean ± SD
Metformin	93 (48.2)	18.99 ± 4.30
Lifestyle modification	9 (4.7)	21.39 ± 5.62
Sulfonylurea	36 (18.7)	29.59 ± 3.40
Insulin	13 (6.7)	37.05 ± 4.94
Thiazolidinedione + Sulfonylurea	18 (9.3)	28.96 ± 4.31
Sulfonylurea+Alpha-glucosidase inhibitor	10 (5.2)	32.04 ± 4.50
Insulin + Sulfonylurea	10 (5.2)	32.25 ± 2.03
Insulin + DDP-4 inhibitor	4 (2.1)	36.40 ± 4.39
Total	193	
f	64.68	
p	<0.001	

Discussion

CA 19-9 is typically used as a screening tool to diagnose pancreatic cancer and as a marker of pancreatic damage that might be caused by diabetes. Limited numbers of studies showed that diabetic patients have increased CA 19-9 levels as compared with control group. Antineoplastic effect of metformin on pancreatic cancer has been demonstrated in various preclinical studies. In the present study, we observed the influence of metformin on serum carbohydrate antigen 19-9 (CA 19-9) levels in type 2 diabetes mellitus patients.

In our study, both the groups were comparable in terms of age, duration of diabetes, BMI, 2 h plasma glucose, HbA_1C, HDL cholesterol, VLDL cholesterol, LDL cholesterol, triglyceride, total cholesterol.

The mean value of CA 19-9 for the metformin group was much lower i.e. 18.99±4.30 U/ml as compared to 30.49±5.61 U/ml in non-metformin group. This difference was highly significant (p<0.001). In non-metformin group, the patients were subjected to
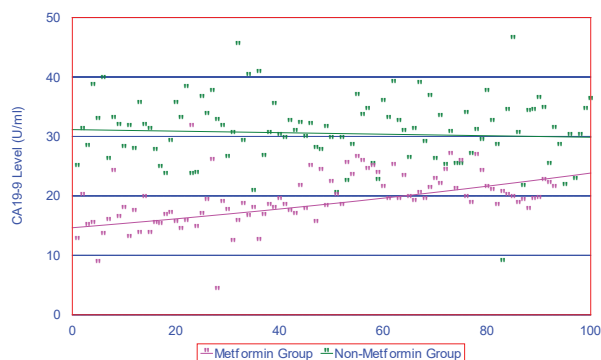
Fig. 1: Geo mapping of case clusters in neighbouring districts

different modes of treatment. Mean CA 19-9 level was found highest among all i.e. 37.05±4.94 U/ml in patients taking insulin. Patients who took sulfonylureas had mean CA 19-9 level of 29.59±3.40 U/ml while patients having lifestyle modification for the management of diabetes had their mean CA 19-9 of 21.39±5.62 U/ml. Patients taking combination therapy for treatment also had their mean CA 19-9 level more than metformin group patients. The difference in the mean value of CA 19-9 in different treatment modalities was found to be statistically highly significant (p<0.001).

In a study, Zhang et al[11] (2015) showed that incidence of elevated CA 19-9 level in diabetes mellitus patients was more in non-metformin group than in short-term and long-term metformin group patients. After a 1-year follow-up, the decrease in CA 19-9 level was highest in long-term metformin group than short-term metformin and non-metformin group patients.

In diabetic patients, hyperglycemia with endogenous hyperinsulinemia is associated with increased cancer incidence and progression by over activation of insulin receptor and IGF-1 receptor (INSR/ IGF-1R) signaling pathways.[12] Hyperinsulinemia has been indicated as a possible factor for pancreatic cancer. CA19-9 is a tumor marker mainly used for the diagnosis of pancreatic cancer[6]. Metformin by its direct action on cellular level induces p53-dependent autophagy, inhibits mTOR and protein synthesis, induces cell cycle arrest through a decrease in cyclin D1 protein level and reduces cellular metabolism.[9,13-15] The indirect effects of metformin are mediated through its blood glucose lowering ability, improving insulin resistance and subsequent reduction of the circulating insulin level. Metformin

exhibits a strong antiproliferative action on several cancer cell lines including pancreatic cancer.[16] This possibly explains our finding in the present study that lower level of CA 19-9 was found in metformin group while patients taking insulin have a higher CA 19-9 level.

In our study, CA 19-9 level is positively correlated with age, duration of diabetes, BMI, 2-hour Plasma Glucose level, HbA_1C, VLDL cholesterol, triglyceride, total cholesterol, LDL cholesterol (p<0.005) and negatively correlated with HDL cholesterol (p<0.001).

In the year 1986, Nakamura N et al[17] found that CA 19-9 level was positively correlated with fasting plasma glucose level and HbA_1C. They concluded that even though diabetes mellitus is not a malignant disease, serum CA19-9 levels were increased in diabetic patients.

Benhamou et al[18] in 1991 found a significant correlation between CA 19-9, fasting blood glucose, serum creatinine, bicarbonate level and HbA_1C. They concluded that CA 19-9 in diabetic patients is positively correlated with blood glucose concentration and raised acute metabolic situations.

In the year 2011, Gul K et al[19] found that median CA 19-9 level in diabetes was significantly higher with diabetes patients than non-diabetic controls. CA 19-9 level was positively correlated with age, duration of diabetes, HbA_1C and number of complications.

In a study, Huang Y et al[20] (2012) observed that CA 19-9 levels were positively and significantly associated with fasting plasma glucose, 2 hour post-load plasma glucose, and HbA_1C.

In a study by Haoyong Yu et al[21] (2012) noted that mean CA 19-9 level in type 1 and type 2 diabetes mellitus patients was higher than non-diabetic

Table 3: Correlation of different parameters with CA 19-9 level (U/ml)

Parameters	Regression (r)	Significant (p)
CA 19-9	1.000	-
Age (years)	0.246	0.001
Duration of diabetes (years)	0.332	<0.001
BMI (kg/m^2)	0.431	<0.001
2 h Plasma Glucose	0.348	<0.001
HbA$_1$C	0.308	<0.001
HDL cholesterol (mg/dl)	-0.297	<0.001
VLDL cholesterol (mg/dl)	0.540	<0.001
LDL cholesterol (mg/dl)	0.202	<0.001
Triglyceride (mg/dl)	0.540	<0.001
Total cholesterol (mg/dl)	0.309	<0.001

individuals. They showed that CA 19-9 was positively correlated with fasting plasma glucose, 2-hour plasma glucose, HbA$_1$C, total cholesterol and negatively correlated with LDL cholesterol.

Esteghamati A *et al*[22] in 2014 showed that CA 19-9 level was significantly higher in diabetic patients. Fasting plasma glucose, postprandial plasma glucose, HbA$_1$C insulin resistance and β-cell function were directly correlated with CA 19-9 levels.

In another study, Yinfang Tu *et al*[23] (2014) showed significant positive correlation of CA 19-9 with fasting plasma glucose, 2-hour post-challenge plasma glucose levels, glycated hemoglobin levels, glycated albumin levels, total cholesterol, insulin resistance and β-cell function.

Limitations of study

There were few limitations to our study. This was a single centre study. It was cross-sectional in design and therefore, a cause and effect relationship determination was not possible. The sample size (n=193) in this study was relatively small.

Conclusions

- Metformin is associated with lower
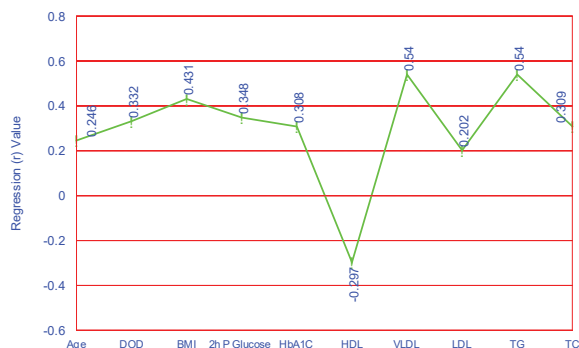
Table 4: Multiple stepwise regression analysis showing variables independently associated with CA 19-9 level (U/ml)

Parameters	
Age	0.246
Age+ Duration of diabetes	0.333
Age+ Duration of diabetes +BMI	0.442
Age+ Duration of diabetes BMI+ HbA$_1$C	0.442
Age+Duration of diabetes +BMI+ HbA$_1$C +HDL	0.482
Age+ Duration of diabetes +BMI+ HbA$_1$C +HDL+ LDL	0.484
Age+ Duration of diabetes +BMI+ HbA$_1$C +HDL+LDL+ Triglyceride	0.574

level of CA 19-9 in type 2 diabetes mellitus patients. It may have a protective role in preventing pancreatic damage and pancreatic cancer in diabetic individuals.

- CA 19-9 level was higher in patients taking insulin or insulin secretagogues. Insulin, because of its known mitogenic effects, may increase the incidence of pancreatic cancer in diabetes mellitus patients.

- CA 19-9 level has a positive correlation with duration of diabetes, 2-hour plasma glucose, HbA$_1$C, body mass index and lipid profile. Hence, it could be an effective indicator of glycemic control, disease progression and lipid metabolism in patients with type 2 diabetes mellitus.

References

1. Giovannucci E, Harlan DM, Archer MC et al. Diabetes and cancer: a consensus report. *CA Cancer Journal for Clinicians* 2010; 60:207–21.

2. Vigneri P, Frasca F, Sciacca L, et al. Diabetes and cancer. *Endocr Relat Cancer* 2009; 16:1103–23.

3. Jee SH, Ohrr H, Sull JW, et al. Fasting serum glucose level and cancer risk in Korean men and women. *JAMA* 2005; 293:194-202.

4. Inoue M, Iwasaki M, Otani T, et al. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006; 166:1871-7.

5. Eskelinen M, Haglund U. Developments in serologic detection of human pancreatic adenocarcinoma. *Scand J Gastroenterol* 1999; 34:833–844.

6. Safi F, Beger HG, Bittner R, et al. CA 19-9 and pancreatic adenocarcinoma. *Cancer* 1986; 57:779–83.

7. Locker GY, Hamilton S, Harris J, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol* 2006; 24:5313-27.

8. Foretz M, Hébrard S, Leclerc J, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest* 2010; 120:2355–69.

9. Dowling RJ, Zakikhani M, Fantus IG, et al. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. *Cancer Res* 2007; 67:10804.

10. Cusi K, Consoli A, DeFronzo RA. Metabolic effects of metformin on glucose and lactate metabolism in non insulin-dependent diabetes mellitus. *J Clin Endocrinol Metabol* 1996; 81:4059–67.

11. Zhang D, Hou W, Liu F, et al. Metformin reduces serum CA199 levels in type 2 diabetes Chinese patients with time-effect and gender difference. *Diabetes Technol Ther* 2015; 17:72-9.

12. Ciaraldi TP, Sasaoka T. Review on the in vitro interaction of insulin glargine with the insulin/insulin-like growth factor system: potential implications for metabolic and mitogenic activities. *Horm Metab Res* 2011; 43:1-10.

13. Long YC, Zierath JR. AMP-activated protein kinase signaling in metabolic regulation. *J Clin Invest* 2006; 116:1776–83.

14. Zhuang Y, Miskimins WK. Cell cycle arrest in Metformin treated breast cancer cells involves activation of AMPK, downregulation of cyclin D1, and requires p27Kip1 or p21Cip1. *J Mol Signal* 2008; 3:18.

15. Wang LW, Li ZS, Zou DW, et al. Metformin induces apoptosis of pancreatic cancer cells. *World J Gastroenterol* 2008; 14:7192–8.

16. Schneider MB, Matsuzaki H, Haorah J, et al. Prevention of pancreatic cancer induction in hamsters by metformin. *Gastroenterology* 2001; 120:1263–70.

17. Nakamura N, Aoji O, Yoshikawa T, et al. Elevated serum CA19-9 levels in poorly controlled diabetic patients. *Jpn J Med* 1986; 25:278-80.

18. Benhamou PY, Vuillez JP, Halimi S, et al. Influence of metabolic disturbances of diabetes mellitus on serum CA 19-9 tumor marker. *Diabete Metab* 1991; 17: 39-43.

19. Gul K, Nas S, Ozdemir D, et al. CA 19-9 level in type 2 diabetes mellitus and its relation to the metabolic control and microvascular complications. *Am J Med Sci* 2011; 341:28–32.

20. Huang Y, Xu Y, Bi Y, et al. Relationship between CA 19-9 levels and glucose regulation in a middle-aged and elderly Chinese population. *J Diabetes* 2012; 4:147–152.

21. Yu H, Li R, Zhang L, et al. Serum CA19-9 Level Associated with Metabolic Control and Pancreatic Beta Cell Function in Diabetic Patients. *Exp Diabetes Res* 2012; 2012:745189.

22. Esteghamati A, Hafezi-Nejad N, Zandieh A, et al. CA 19-9 is associated with poor glycemic control in diabetic patients: role of insulin resistance. *Clin Lab* 2014; 60:441-7.

23. Tu Y, Yu H, Zhang P, et al. Decreased serum CA19-9 is associated with improvement of insulin resistance and metabolic control in patients with obesity and type 2 diabetes after Roux-en-Y gastric bypass. *J Diabetes Investig* 2014; 5:694–700.

Fig. 2: Correlation of different parameters with CA 19-9
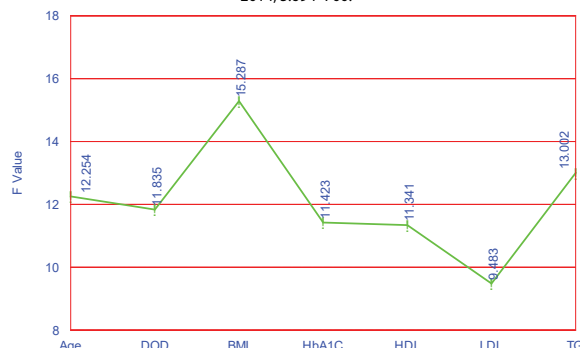
Fig. 3: ANOVA test of different parameters in relation to CA 19-9