INTRODUCTION

Dipeptidyl peptidase 4 (DPP-4), an aminopeptidase which was isolated from rat liver in 1960, is excreted in the pancreas, brain, lungs, kidneys, intestines, adrenal glands, and lymph nodes . (1-2) Moreover, this enzyme causes glucogen-like peptide-1 (GLP-1) to break down in less than two minutes and gastric inhibitory polypeptide (GIP) in less than 5 to 7 minutes. (3-4) Dipeptidyl peptidase-4 inhibitors (DPP-4 inh) are oral antidiabetic (OAD) agents that increase circulating GLP-1 levels. Thus, they help to inhibit the rapid inactivation of GLP-1 and regulate blood sugar. In other words, they control blood glucose by stimulating insulin secretion in glucose-dependent pathways. (5) Although DPP-4 contains a wide range of substrates including chemokines, hormones, and neuropeptides, DPP-4 inh usually do not negatively affect type 2 diabetes (T2DM) patients. Several studies have evaluated the safety of DPP-4 inh in elderly patients as well as patients with renal insufficiency, liver disease, and/or heart failure. While most observational studies have demonstrated the safety of these inhibitors, nevertheless they have been associated with a slightly increased incidence of acute pancreatitis during placebo-controlled trials. In addition, the possible side effects of long-term DPP-4 inh use remain unclear. (6) Those side effects observed during the preclinical and clinical trials of sitagliptin and vildagliptin treatments are inconsistent with the increased risk of pancreatitis in T2DM patients, but the association between DPP-4 inh and pancreatic cancer, pancreatitis, and pancreatic enzyme elevation has been proven. (7) The Food and Drug Administration (FDA) has also reported that patients using sitagliptine and exanitide should be cautious in terms of developing pancreatic cancer. (8) On the other hand, various studies have demonstrated that the use of DPP-4 inh does not increase the risk of pancreatitis and/or pancreatic cancer. (9) Finally, although the number of studies involving pacreatic cancer patients is sufficient, those concerning patients with clinically non-pancreatic hyperamylasemia are rare. This study assessed changes in the amylase and lipase values of various patients who had been using DPP-4 inhibitors but did not exhibit acute pancreatitis symptoms.

MATERIALS AND METHODS

The participants of this study were diabetic patients who had been referred to the XXXXXXXXXXXXXXXXXX Prior to conducting the study, approval (approval no. 17/10/2016/268) was obtained from the XXXXXXXXXXXXX Committee. The study was prospective in nature, with patients being evaluated twice over a 3-month period (i.e. at 0 and 3 months). In addition, it was descriptive in nature and assessed the relationship among variables. All patients involved in this study met the following inclusion criteria developed by the American Diabetes Association (ADA):

1- Patients with T2DM
2- Patients who were not using anti-diabetic drugs other than metformin and previously had not used sitagliptin, saxagliptin, or vildagliptin
3- Patients with HbA1c level lower than 10
4- Patients without cholelithiasis
5- Patients without chronic alcohol consumption
6- Patients with triglyceride levels lower than 150 mg/dl
7- Patients in whom blood sugar regulation could not be achieved with metformin or any form of sitagliptin, saxagliptin, or vildagliptin treatment
8- Patients without pancreatitis or pancreatitis history
9- Patients with no history of renal or hepatic disease
10- Patients without acute malignnacy or malignancy history
11- Patients who were not pregnant

The 87 patients who met the above criteria were divided into 3 groups according to their use of saxagliptin, sitagliptin, and vildagliptin. All had been taking metformin at a dosage of 2 grams/day at the start of this study. Patients using saxagliptin had been taking 1 mg of 5 mg daily, patients using sitagliptin had been taking 100 mg daily, and patients using vildagliptin had been taking 50 mg daily (morning and evening). Prior to and following the three-month treatment, patients' fasting blood glucose, post-prandial blood glucose, hemoglobin A1c (HbA1c), serum creatinine, alanine transaminase (ALT), amylase, and lipase levels were recorded.

STATISTICAL ANALYSIS

A minimum of 80 patients were required for this study based on the results of power analysis conducted for sample selection. The normal distribution fitness of numerical data was

tested using a Shapiro-Wilk test, while a Kruskal-Wallis test was used to compare normal non-dispersive variables. The relationship among normally undistracted dependent variables was tested via a Wilcoxen Test, and the relationship among categorical variables was tested using a Chi-square test. The SPSS 22.0 software was employed for results analysis, and a *p*-value <0.05 was considered significant.

RESULTS

There was no statistically significant difference among the groups in terms of age or sex (p> 0.05). The demographic characteristics of each group of participants are shown ~~in the table 1~~

Table 1 below.

At the beginning of the study, no significant difference in baseline fasting glucose level and postprandial blood glucose, HbA1C, serum creatinine, ALT, amylase, and lipase was observed (p> 0.05) (Table 2). A statistically significant decrease in all groups (p> 0.05) was found in fasting blood glucose, postprandial blood glucose, and HbA1C values after 3-month treatment. Moreover, amylase and lipase values increased in all groups, but there was no significant difference in these values when comparing the groups. (p> 0.05) (Table 3). By the end of the study, there was a statistically significant decrease in fasting blood glucose and HbA1C in all groups (p <0.05). However, in comparing the groups, the decline rates did not significantly differ. There was no statistically significant increase in lipase or amylase levels within the saxagliptin and vildagliptin groups (p> 0.05) after 3 month treatment . However, there was a statistically significant increase in amylase and lipase levels within the sitagliptin group (p <0.05). There was no statistically significant increase in ALT levels within the sitagliptin and vildagliptin groups (p> 0.05), yet there was a statistically significant increase in ALT level within the saxagliptin group (p <0.05) (Table 4).

CONCLUSION

Sitagliptin, which has been used since 2006, is the longest in-use DPP-4 inh; thus, more information is available regarding its use compared to other DPP-4 inh. According to FDA's reported side effect database, patients using sitagliptin or exenatide are more prone to developing pancreatic cancer than those using other drugs. (10) Moreover, it has been claimed

that chronic subclinical pancreatitis due to GLP-1 therapy may cause an increase in the incidence of pancreatic cancer. (11)

The above assertions have been supported by studies demonstrating that long-term exendin-4-mediated GLP-1R activation proliferates the development of pancreatic duct glands (PDG) and the formation of dysplastic lesions (low grade intraepithelial neoplasia-mPANIN) in rats as well as chronic pancreatitis in the KrasG12D mouse model. (12) These studies have observed GLP-1-induced changes in pancreatic duct glands as well as genetic predisposition to cellular dysplasia. Therefore, it has been assumed that the absence of both a clear pancreatitis pattern and tumors in lean and non-diabetic animals receiving extendin-4 therapy owes itself to two facts. First, these animals are not genetically pre-dispaosed to dysplasia, and secondly, the methodological analysis used to detect changes in the pancrease is not suitable for these animals. (13,14) For this reason, it has been suggested that in the case of chronic pancreatitis, pancreatic duct glands can easily transform into PanIN-like lesions. (15,16) Although exendin-4 administration leads to the development of mPanIN, it has not been scientifically proven that drug treatment in subjects does not lead to pancreatic cancer in genetically modified mice since this treatment has not been long enough in terms of duration. (17) At the same time, Butler, et al. have reported that patients with incretin therapy experience exocrine pancreatic dysplasia and endocrine pancreatic cell proliferation. (18) On the other hand, the limited number of patients and treatment involved in Butler et al.'s study as well as a lack of statistical data might have caused this link between therapy and cancer (19.20)

In previous studies, cases of pancreatitis associated with the use of DPP-4 inh have been presented as case reports. (21) However, retrospective studies and meta-analyses have reported that these drugs are not statistically significant in terms of causing pancreatitis compared to other anti-diabetic drugs. (22) In the current study, although there was an amylase and lipase increase in patients, no significant difference was seen in any patient possessing the characteristics of acute pancreatitis. In their study of acute pancreatitis among T2DM patients in Taiwan, Hsin-Chuo, et al. determined the use of DPP-4 inh to be a risk factor for the presence of cholelithiasis and uncontrolled diabetes mellitus. (23) On the other hand, in another retrospective cohort study conducted by Daisuke Yabe, et al. in Japan among 93,280 anti-diabetic patients who had screened for acute pancreatitis and used 27,962 DPP-4 inh, no significant difference was observed in terms of acute pancreatitis development. (24)

Studies have also emerged regarding the use of DPP-4 inh for elevating amylase and lipase from pancreatic exocrine enzymes. In a 3-month propsective study observing the amylase and lipase levels of 36 patients, 24 of whom were using DPP-4 inh and 12 of whom were taking

other anti-diabetic drugs, Hirotake, et al. observed the elevation of amylase and lipase levels among 11 out of 24 patients receiving DPP-4 inh , which was regarded as statistically significant (25). In the present study, a baseline increase in amylase and lipase was observed in all groups. However, there was no statistically significant increase in the saxagliptin and vildagliptin groups ($p> 0.05$), wherease a statistically significant increase was observed in the sitagliptin group.

A greater amount of studies concerning sitagliptin in DPP-4-associated pancreatitis cases presented as case reports may be related to the longer treatment duration of sitagliptin in comparison to other DPP-4 inh. Still, some theoretical information (especially that derived from animal experiments) is available which speculates the cause of the increase in pancreatic enzymes with the use of DPP-4. Studies examining the relationship between sitagliptin and oxidative stress as well as inflammation have indicated that sitagliptin increases the levels of tumor necrosis factor-alpha (TNF-α) in serum inflammatory stores. Moreover, it has been found to decrease oxidative stress in serum by decreasing serum IL-1β levels and reducing serum total malondialdehyde levels but not total antioxidant levels. Another study examining the effects of sitagliptin on oxidative stress has reported that the total oxidant levels in diabetic rat pancreatic tissues did not change but increased oxidative stress due to decreased total antioxidant levels. This increased oxidative stress, which is likely to occur in the pancreas tissue, causes some inflammation in the exocrine pancreas. However, it is unclear whether this level can cause pancreatitis. Despite the inflammation and dysplastic changes in animal experiments examining the exocrine pancreas, the use of DPP-4 inh has been shown to increase function and mass in pancreatic beta cells. (26,27)

Based on the above findings, one could assume that the use of DPP-4 inh may increase amylase and lipase levels in patients who do not exhibit symptoms of acute pancreatitis. Thus, DPP-4 inh should be used with caution in patients at risk for pancreatitis and pancreatic cancer. Moreover, patients using DPP-4 inh, especially sitagliptin, should be evaluated carefully for pancreatitis risk factors.

Acknowledgement: There is no funding in this study. There is no conflict of interest between the authors.

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Table 1. Demographic Characteristics of Groups

	Saxagliptin n=27	Sitagliptin n=30	Vildagliptin n=30	P
Gender (Female/Male)	21/6	7/23	12/18	0,241
Age (year)	54.26±7.906	56.33±6.572	54.00±10.316	0,577

Table 2. Comparison of Laboratory Parameters of Groups at the Beginning Treatment

		N	Mean	Std. Deviation	p
Fasting Glucose (mg/dl)	SAXAGLIPTIN	27	204,41	69,841	0,704
	SITAGLIPTIN	30	190,67	66,093	
	VILDAGLIPTIN	30	215,73	91,480	
	Total	87	203,57	76,679	
Postprandial Glucose (mg/dl)	SAXAGLIPTIN	27	344,30	112,314	0,621
	SITAGLIPTIN	30	275,97	114,564	
	VILDAGLIPTIN	30	327,90	126,681	
	Total	87	315,08	120,458	
HbA1C (%)	SAXAGLIPTIN	27	9,41	2,005	0,700
	SITAGLIPTIN	30	8,97	1,650	
	VILDAGLIPTIN	30	9,27	1,874	
	Total	87	9,21	1,831	
Creatinin (mg/dl)	SAXAGLIPTIN	27	,93	,267	0,885
	SITAGLIPTIN	30	,90	,305	
	VILDAGLIPTIN	30	,93	,254	
	Total	87	,92	,274	
ALT (U/L)	SAXAGLIPTIN	27	23,07	10,954	0,905
	SITAGLIPTIN	30	24,57	10,448	
	VILDAGLIPTIN	30	22,63	9,368	
	Total	87	23,44	10,168	
Amylase (U/L)	SAXAGLIPTIN	27	60,74	21,506	0,792
	SITAGLIPTIN	30	56,77	22,660	

	VILDAGLIPTIN	30	56,03	21,714	
	Total	87	57,75	21,821	
Lipase (U/L)	SAXAGLIPTIN	27	19,65	12,776	0,394
	SITAGLIPTIN	30	25,32	17,035	
	VILDAGLIPTIN	30	21,82	14,274	
	Total	87	22,35	15,297	

Table 3. Laboratory Parameters of Groups by the End of Treatment

		N	Mean	Std. Deviation	p
Fasting Glucose (mg/dl)	SAXAGLIPTIN	27	155,48	49,431	0,171
	SITAGLIPTIN	30	162,67	52,948	
	VILDAGLIPTIN	30	182,37	61,222	
	Total	87	167,23	55,484	
Postprandial Glucose (mg/dl)	SAXAGLIPTIN	27	277,67	99,458	0,402
	SITAGLIPTIN	30	251,73	108,690	
	VILDAGLIPTIN	30	271,97	109,553	
	Total	87	266,76	105,579	
HbA1C (%)	SAXAGLIPTIN	27	8,11	1,450	0,154
	SITAGLIPTIN	30	7,80	1,375	
	VILDAGLIPTIN	30	8,57	1,569	
	Total	87	8,16	1,485	
Creatinin (mg/dl)	SAXAGLIPTIN	27	,96	,192	0,800
	SITAGLIPTIN	30	,93	,254	
	VILDAGLIPTIN	30	,97	,183	
	Total	87	,95	,211	
ALT (U/L)	SAXAGLIPTIN	27	26,00	10,269	0,937
	SITAGLIPTIN	30	25,13	11,697	
	VILDAGLIPTIN	30	25,47	13,364	
	Total	87	25,52	11,763	
Amylase (U/L)	SAXAGLIPTIN	27	63,33	22,675	0,646

	SITAGLIPTIN	30	67,13	20,416	
	VILDAGLIPTIN	30	62,00	27,188	
	Total	87	64,18	23,456	
Lipase (U/L)	SAXAGLIPTIN	27	35,41	18,158	0,249
	SITAGLIPTIN	30	42,23	20,992	
	VILDAGLIPTIN	30	34,10	18,713	
	Total	87	37,31	19,482	

Table 4. Comparison of Labaratory Parameters at the Beginning and End of Treatment

		Beginning	**3rd Month**	
		Mean ∓Std	**Mean ∓Std**	**p**
Fasting glucose	SAXAGLIPTIN	204,4∓69,9	155,5∓49,4	0,001
	SITAGLIPTIN	190,7∓66,1	162,7∓52,9	0,018
	VILDAGLIPTIN	215,7∓91,5	182,4∓61,2	0,005
Postprandial glucose	SAXAGLIPTIN	344,3∓112,3	277,7∓99,5	0,001
	SITAGLIPTIN	275,9∓114,6	251,7∓108,7	0,098
	VILDAGLIPTIN	327,9∓126,7	271,9∓109,6	0,001
HbA1C	SAXAGLIPTIN	9,4∓2,0	8,1∓1,5	0,001
	SITAGLIPTIN	8,9∓1,6	7,8∓1,4	0,001
	VILDAGLIPTIN	9,3∓1,9	8,6∓1,6	0,007
Amylase	SAXAGLIPTIN	60,7∓21,5	63,3∓22,7	0,400
	SITAGLIPTIN	56,8∓22,7	67,1∓20,4	0,001
	VILDAGLIPTIN	56,0∓21,7	62,0∓27,2	0,174
Lipase	SAXAGLIPTIN	19,7∓12,8	35,4∓18,2	0,360
	SITAGLIPTIN	25,3∓17,0	42,2∓20,9	0,001
	VILDAGLIPTIN	21,8∓14,3	34,1∓18,7	0,090
Creatinin	SAXAGLIPTIN	0,9∓0,3	0,9∓0,2	0,564
	SITAGLIPTIN	0,9∓0,3	0,9∓0,3	0,564
	VILDAGLIPTIN	0,9∓0,3	0,9∓0,2	0,564
ALT	SAXAGLIPTIN	23,1∓10,9	26,0∓10,3	0,037
	SITAGLIPTIN	24,6∓10,5	25,1∓11,7	0,789
	VILDAGLIPTIN	22,6∓9,4	25,5∓13,4	0,096