

Vitamin D Low-Levels and Silent Myocardial Ischemia in Type 2 Diabetes: Clinical Correlations and Prognostic Significance

Rosario Rossi (✉ rosario.rossi@unimore.it)

University of Modena and Reggio Emilia <https://orcid.org/0000-0001-9065-1808>

Marisa Talarico

University of Modena and Reggio Emilia <https://orcid.org/0000-0003-2523-7963>

Cristina Rolando

Università degli Studi di Modena e Reggio Emilia

Roberto Minici

University of Magna Graecia: Università degli Studi Magna Graecia di Catanzaro

Giuseppe Boriani

Università degli Studi di Modena e Reggio Emilia

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Abstract

BACKGROUND: Vitamin D deficiency has a pathogenetic and prognostic role in coronary artery disease. Moreover, Vitamin D has a key role in pain modulation and transmission. Diabetic patients have a high risk of silent myocardial ischemia (SMI), due to diabetic neuropathy. We aimed to evaluate the correlation between SMI and Vitamin D serum levels in a population of type 2 diabetic patients and to assess whether SMI patients had a worse survival than their symptomatic counterpart.

METHODS: In this retrospective, cross-sectional, observational study, we enrolled 253 patients admitted in Policlinico of Modena Hospital and compared them with 50 healthy volunteers. We divided the 253 patients into three sub-groups: symptomatic MI group (125, 32.4%); SMI (78, 25.7%), and no-MI one (50, 41.9%). The entire population had a 25-hydroxyvitamin D (25(OH)D) measurement.

RESULTS: 25(OH)D levels (nmol/l) were lower in SMI group (34.9 ± 5.8) respect to symptomatic MI (49.6 ± 6.1 ; $p = 0.01$); no MI (53.1 ± 6.2 ; $p = 0.001$) and control group (62.1 ± 6.7 ; $p = 0.0001$). 25(OH)D level was the only independent variable able to influence the development of SMI in diabetic patients, with an inverted odd-ratio of 1.11 ($p = 0.01$). Symptomatic MI group had better survival than SMI one (6-year survival rate: 83 vs. 69%; $p = 0.01$)

CONCLUSIONS: Type 2 diabetic with SMI had a higher mortality risk and showed lower 25(OH)D levels than the symptomatic group. This suggests the crucial vitamin D role in the pathogenesis of SMI.

Background

Vitamin D or 25 (OH) D is a fat-soluble hormone obtained either by sunlight exposure (ultraviolet B, 290–320 nm) or through dietary source and supplements, absorbed by the intestine (1). The world is actually in a state of hypovitaminosis D, primarily due to less exposure to sunlight. Other factors that also influence vitamin D level are age, gender, ethnicity, skin color, season, and clothing (2). It is estimated that 1 billion people worldwide suffer from vitamin D deficiency (3): more than 40% of the USA and European and even 80% of the Italian population (4, 5).

Low 25 (OH) D levels were associated with cardiovascular disorders, including heart failure, stroke, and especially coronary heart disease (CAD) (6–10). Besides, 25(OH)D deficiency was associated with endothelial dysfunction, subclinical atherosclerosis, significant reduction of coronary flow reserve (11, 12), and sub-epicardial ischemia in patients hospitalized for the acute coronary syndrome (13).

CAD is a leading cause of death in type 2 diabetes patients. In the diabetic population silent, myocardial ischemia (SMI) is extremely common and carries a higher risk of severe complications than symptomatic MI (14–17). SMI is the result of an 'anginal warning system' failure, resulting in a delay in anti-ischemic therapy (17, 18). Moreover, the pathophysiology of SMI in diabetic patients is poorly understood (19–21). In line with other previous large studies that demonstrated a potential correlation between 25 (OH)D

deficiency and subclinical myocardial injury and with sensitive neuropathy (22–25), we hypothesized that vitamin D deficiency is correlated to SMI.

Among the studies reporting a strong association between low 25(OH)D levels and MI, none differentiated between patients with silent myocardial ischemia (SMI) or symptomatic one, even if 25(OH)D may be related to one or another sub-type. There is thus a clear rationale for measuring 25(OH)D levels in carefully characterized type 2 diabetic patients with CAD and MI.

The purpose of the present study is two-fold. First, we established the importance of vitamin D levels on SMI development; and second, we compared the prognosis of diabetic patients with silent to the prognosis of diabetic patients with symptomatic MI, to assess whether SMI patients had a worse survival (as expected) than their symptomatic counterparts.

Methods

Study population, setting, and data collected

In this single-center, cross-sectional, retrospective, observational study, we enrolled from 01 January 2000 and 31 December 2013, 2407 diabetic patients with suspected CAD who underwent coronary angiography at the Catheterization Laboratory of the Policlinico di Modena Hospital. The current study protocol is in accordance with the Declaration of Helsinki and obtained the approval of the Ethics Committee of Modena University the 20/05/2020 with protocol number 0014071/20.

Patients with primary valvular, congenital, myocardial and pericardial disease, as well as patients with previous surgery or percutaneous revascularization, previous acute coronary syndromes and/or EKG pathological Q waves, were excluded. Among the eligible patients, 960 patients also had an exercise test within 6 weeks of catheterization. In this group of patients, 203 were found to have significant CAD ($\geq 70\%$ diameter stenosis in at least one of the main coronary branches; or $\geq 50\%$ in the left main) on coronary angiography and had ≥ 0.10 mV exercise-induced ST-segment depression at EKG. The last patients, together with a group of 50 patients with type II diabetes and no MI, and 50 healthy, age- and gender-related non-diabetic control subjects, constituted the total selected population. All enrolled patients had at least one 25(OH)D serum determination until 12 months before catheterization in the year of catheterization. At six and 12 months after catheterization, and then yearly, follow-up information was obtained by clinic visit, telephone interview, or a combination. Follow-up was obtained for 95% of the studied population.

Assessments of vitamin D status

Vitamin D is a steroidal substance that is mainly produced in the skin by direct exposure to sunlight. The principal forms of vitamin D are cholecalciferol (D3) and ergocalciferol (D2). The human body cannot produce D2, but it may be ingested in the form of supplements. Vitamine D is not biologically active as D3. It undergoes successive hydroxylations in the liver and kidney, to form the active hormone 1,25-

dihydroxyvitamin D (calcitriol). The major storage form of vitamin D is D₂ (25-hydroxyvitamin D [25(OH)D]), with plasma levels more than 1000-fold greater than the active 1,25-dihydroxyvitamin D. Hence, 25(OH)D measurement is considered to be adequate to calculate overall vitamin D status. We used a chemiluminescence immunoassay method for the quantitative determination of total serum 25(OH)D (DiaSorin, Stillwater, MN, USA). People on vitamin D oral supplementation were excluded, as were those using sunbeds.

Statistical analysis

All analyses were performed using the statistical package SPSS, version 22.0 (IBM Corp., Armonk; NY, USA). Baseline characteristics were expressed as mean \pm one standard deviation (SD) or standard error of the mean (SEM) when specified. Categorical variables were described as percentages. One-way analysis of variance (ANOVA) was used to compare the mean of baseline characteristics and post hoc analysis to make comparisons between the group means; and χ^2 test for dichotomous variables. Analysis of covariance (ANCOVA) was performed to examine differences in mean serum vitamin D levels between the study groups using age, gender, BMI, and season of vitamin D measurement as covariates. Direct logistic regression was executed, and odds ratios were calculated to assess the impact of various potentially independent factors on SMI. Survival probabilities were estimated using the Kaplan-Meier method. Breslow's formulation of the Cox proportion hazard model was used to test for a significant association between survival time and the presence of SMI. Differences were considered statistically significant when the p-value was < 0.05 .

Results

Table 1 summarize demographic details and study assessments performed for each group. Patients with SMI had a higher body mass index (BMI) and were older compared to patients with symptomatic MI. The SMI group demonstrated a longer duration of diabetes, but there were no significant differences in HbA1c between them. After adjusting for age, gender, BMI, and season of vitamin D measurement, 25(OH)D levels (nmol/l) (SE) were significantly lower in patients with SMI (34.9 ± 5.8) respect to patients with symptomatic MI (49.6 ± 6.1 ; $p = 0.01$); no MI (53.1 ± 6.2 ; $p = 0.001$); and healthy volunteers (62.1 ± 6.7 ; $p = 0.0001$). Pairwise comparisons revealed the main group significance between patients with silent respect to symptomatic MI (see Table 1). Direct logistic regression was performed to assess the impact of all potential independent variables on the presence of SMI. The full model that emerged was statistically significant ($\chi^2 = 27.3$, $p = 0.001$). As shown in Table 2, vitamin D was the only independent variable that made a statistically significant contribution to the model, with an inverted odd ratio of 1.11 ($p = 0.01$). For each unit reduction in vitamin D, the likelihood of SMI increased by 11%.

Table 1
Demographic, clinic and catheterization characteristics of our study population.

c	Healthy volunteers	No myocardial ischemia	Symptomatic myocardial ischemia	Silent myocardial ischemia	Post hoc p-value, comparing silent and symptomatic ischemia
n	50	50	125	78	
Demographic and anthropometric parameters					
Age, years	62.0 ± 9.0	55.4 ± 8.2	61.9 ± 9.4	64.1 ± 10.3	0.03
Male gender, %	72.0 (n = 36)	80.0 (n = 40)	72.0 (n = 90)	71.8 (n = 56)	0.7
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	
Coronary risk factors					
BMI, kg/m ²	26.1 ± 4.6	30.1 ± 6.7	31.1 ± 5.5	33.8 ± 6.6	0.04
Diabetes duration, years		6.5 ± 6.0	13.5 ± 7.0	15.5 ± 9.0	0.03
HbA1c, mmol/mol		62 ± 16	62 ± 15	65 ± 17	0.2
HbA1c, %		7.9 ± 1.9	7.9 ± 1.6	8.1 ± 1.6	
Total cholesterol, mmol/L		4.1 ± 1.4	4.2 ± 1.5	4.0 ± 1.6	0.6
MAP, mmHg	91.1 ± 12.8	101.5 ± 11.4	100.9 ± 11.0	102.0 ± 12.7	0.1
eGFR, ml/min/1.73 m ²	90 ± 25	82 ± 21	67 ± 23	65 ± 24	0.5
Urine ACR, mg/mmol		0.6 ± 0.9	4.1 ± 7.2	3.3 ± 30.1	0.1
25(OH)D, mmol/L (± SEM)	62.1 (6.7)	53.1 (6.2)	49.6 (6.1)	34.9 (5.8)	0.01
Summer determination of 25(OH)D, % (n)	28.0 (n = 14)	30.0 (n = 15)	28.8 (n = 36)	29.5 (n = 23)	0.6
LVEF, median (iqr), %	65 (60–70)	60 (50–70)	58 (50–66)	59 (52–66)	0.1

Legend: ACR, albumin creatinine ratio; BMI, body mass index; CAD, coronary artery disease; eGRF, estimated glomerular filtration rate; LVEF, left ventricle ejection fraction; iqr, interquartile range; MAP, mean arterial blood pressure; n, number; 25(OH)D, 25-hydroxy-vitamin D.

c	Healthy volunteers	No myocardial ischemia	Symptomatic myocardial ischemia	Silent myocardial ischemia	Post hoc p-value, comparing silent and symptomatic ischemia
Exercise test characteristics					
Exercise time (iqr), min		8.5 (7.8–9.2)	4.5 (3.9–5.1)	4.9 (3.9–5.9)	0.1
Exercise heart rate, median (iqr), bpm		128 (112–138)	112 (105–119)	118 (109–127)	0.3
ST depression, median (iqr), mm			0.17 (0.15–0.20)	0.20 (0.12–0.28)	0.2
Catheterization characteristics					
1-vessel CAD, % (n)			20.0 (n = 25)	19.3 (n = 15)	0.9
2-vessel CAD, % (n)			29.6 (n = 37)	26.9 (n = 21)	0.4
3-vessel CAD, % (n)			47.2 (n = 59)	50.0 (n = 39)	0.5
Left main, % (n)			3.2 (n = 4)	3.8 (n = 3)	0.8
<i>Legend: ACR, albumin creatinine ratio; BMI, body mass index; CAD, coronary artery disease; eGRF, estimated glomerular filtration rate; LVEF, left ventricle ejection fraction; iqr, interquartile range; MAP, mean arterial blood pressure; n, number; 25(OH)D, 25-hydroxy-vitamin D.</i>					

Table 2
Logistic regression predicting likelihood of silent myocardial ischemia.

parameter	B	SE	Wald	p	Odds ratio	95% C. I.
Age	0.10	0.06	2.8	0.09	1.11	0.98–1.25
Body mass index	0.02	0.06	1.3	0.22	1.02	0.92–1.20
Diabetes duration	0.08	0.05	2.5	0.11	1.08	0.97–1.19
Male gender	0.27	0.40	0.9	0.8	1.25	0.51–2.87
Summer determination of 25(OH)D	0.34	0.20	1.0	0.6	1.50	0.74–2.12
25(OH)D	-0.06	0.03	5.9	0.01	1.11	1.06–1.17
constant	-11.28	6.9	2.2	0.10	0.00	

At the follow-up, the majority of our patients underwent coronary revascularization: 93.6% (117 of 125) of symptomatic MI patients vs 94.9% (74 of 79) in the SMI group (p = 0.3, inter-group comparison). Detailed

therapeutic options are shown in Table 3. We observed that the two studied groups were comparable to the type of revascularization. The median follow-up time for the study population was 6 years. Symptomatic MI patients had a better survival rather than SMI once (6-year survival rate: 83 vs. 69%; $p = 0.01$; Fig. 1). It can be observed that during the first years after revascularization the survival curves appear approximately coupled. The divergence of the curves occurred from the third year onwards.

Table 3

Therapeutic options chosen for the patients of our study, divided according to the presence of angina or silent myocardial ischemia.

Therapeutic options *	Patients with symptomatic myocardial ischemia (n = 125)	Patients with silent myocardial ischemia (n = 78)	p**
Optimal medical treatment	3.2% (n = 4)	3.8% (n = 3)	0.5
Percutaneous revascularization	68.8% (n = 86)	68.0% (n = 53)	0.3
Surgical revascularization	28.0% (n = 35)	28.2% (n = 22)	0.8

*Legend: *All patients were affected by significant coronary artery disease. **All comparisons resulted not statistically significant.*

Discussion

Modern research revealed a new horizon and function for 25(OH)D, beyond its proven role in the treatment of rickets and osteoporosis. 25(OH)D deficiency is believed to be associated with multiple sclerosis, respiratory diseases, many types of cancers, metabolic disorders, diabetes, and cardiovascular disease (7–9, 26–28).

Our results demonstrated that 25(OH)D deficiency was significantly associated with SMI in our population of well-selected type 2 diabetic patients, all with significant CAD. After adjusting for age, gender, body mass index, and season of 25(OH)D measurement, we demonstrated significantly lower serum levels in patients with SMI vs. patients with symptomatic MI, no MI, and even in healthy volunteers.

Pain is a distressing sensation, as well as an emotional experience that is linked to actual or potential tissue damage, with the sole purpose of notifying the mechanism to react towards a stimulus to avoid further tissue damages. The sensation of pain is associated with the activation of the receptors in the primary afferent fibers, which is inclusive of the unmyelinated C-fiber and myelinated A σ -fiber.

Because pain is mediated by small sensory fibers, our findings allow us to hypothesize that vitamin D deficiency is possibly related to the presence of SMI; or promoting an abnormal transmission of pain, or thought-provoking a degenerative neuropathy of the small nociceptive nerve fibers.

This view is supported by the experimental studies on the effects of 25(OH)D on the nervous system. 25(OH) D deficiency was associated with small nervous fibers neuropathy, involved in the transmission of pain (22, 24). Experimental data leading to the evidence that 25(OH)D have a pivotal role in the regulation of synthesis of neurotrophic factors, conductance velocity of motor neurons, neuronal plasticity processes, and the neuroprotective actions (29). Vitamin D receptors are widely expressed by the central and peripheral nervous systems (30). Particularly, in the peripheral nervous system, the vitamin D receptors are found in predominantly nociceptive neurons of the dorsal root ganglia (31). The vitamin D receptors tend to rearrange itself in the neurons of diabetic rats (32), allowing us to suppose the role of low levels of vitamin D in the abnormal transmission of pain in diabetics.

Besides, v25(OH)D has an important role in promoting nerve growth factor (NGF) secretion. NGF is a target-derived protein that regulates the phenotype and sensibility of nociceptor fibers, and its deficiency may lead to the development of clinical diabetes small fibers neuropathy (33). A recent study has shown a positive correlation between 25(OH)D and serum NGF in diabetes patients (33, 34). A study in rodents reported deactivation of 25(OH)D in the presence of hyperglycemia (35). This may result in decreased vitamin D-mediated NGF secretion, which in turn could lead to a predominantly small nerve fibers neuropathy. In other words, it seems reasonable to assume a pathophysiological link between diabetes, low level of vitamin D, and compromising nociceptor fibers.

Several limitations must be recognized. First of all, this is an observational study, so it is not possible to establish causality, but only associations. An additional limitation regards the sample size, which was not calculated but derived from the number of patients who matched the strict inclusion criteria and the relatively small cohort size and the cross-sectional design. To minimize the impact of the sample size, we carefully characterized and matched patients in different cohorts. The only statistical significance between subgroups (patients with SMI vs. patients with angina) was higher body mass index and age, for patients in the SMI group. This was expected, like age, duration of diabetes, and features of metabolic syndrome (including obesity) are well-known risk factors for SMI (40). To address this, we included age, body mass index, duration of diabetes as covariates in the ANCOVA, and still found mean serum 25(OH)D levels were significantly lower in patients with SIM. Another limitation is that 25(OH)D plasma level is influenced by various factors: age, gender, ethnicity, skin color, season, and clothing. To overcome this limitation, we studied whether there was an imbalance regarding the season in which the dosage of 25(OH)D was affected. The studied groups, including that of healthy controls, resulted statistically comparable for this parameter. Furthermore, summer (from July to September) 25(OH)D dosage was inserted as a covariate in the statistical analysis, together with age and gender, and these parameters were not able to modify the final result. Moreover, all enrolled patients belonged to the same ethnic group and came from the same geographical area, so we postulated equal skin color and similar habits regarding sun exposure and clothing.

Several prognostic studies have shown that SMI is associated with an unfavorable outcome in clinical patients affected by acute myocardial infarction, unstable angina, and chronic stable CAD (34–37). In type 2 diabetic patients, SMI is an issue of public health a condition significantly related to mortality rate

(14, 17, 38, 39). Survival analysis in our study confirmed an increased mortality risk in SMI type 2 diabetic patients compared to patients with angina, despite the same extent of CAD and the optimal revascularization obtained.

In conclusion, we conducted a carefully designed study that involved detailed clinical and invasive/non-invasive procedures, to accurately stratify the presence of CAD and MI in all enrolled patients. Our study included an appropriately matched disease control group (type 2 diabetic patients without MI) and healthy volunteers. The findings suggest a role of 25(OH)D in MI. Our results also suggest that low levels of 25(OH)D may contribute to the development of diabetic-related neuropathy. Further long-term prospective studies are needed to examine causality, i.e. if low 25(OH)D levels make silent the MI, or if they are a risk factor/surrogate marker for the development of SMI. It may be reasonable to obtain plasma levels of 25(OH)D in all diabetic patients with significant CAD and to consider hypovitaminosis D as a factor potentially associated with SMI. It is also confirmed that SMI is a significant negative prognostic factor for mortality in type 2 diabetic patients. Whether hypovitaminosis D will represent a therapeutic target for the treatment of neuropathy, or if it will be able to improve prognosis in diabetic patients with MI, it will have to be evaluated in subsequent randomized trials.

The current study provided a novel point of view on the role of low Vitamin D levels in SMI development in type 2 diabetes, never investigated before, to the best of our knowledge. Moreover, to remove possible confounders and considering vitamin D physiology, we excluded patients on vitamin D oral supplementation and those using sunbeds and recorded the season of vitamin D measurement. Then, we did not consider vitamin D deficiency in our analysis but low vitamin D levels. The reason is that low vitamin D levels that do not fulfill the strict definition of vitamin D deficiency may have a pathogenetic role in SMI development.

Several limitations must be recognized. First of all, this is an observational study, so it is not possible to establish causality, but only associations. An additional limitation is the relatively small cohort size and the cross-sectional design. To minimize the impact of the sample size, we carefully characterized and matched patients in different cohorts. The only statistical significance between subgroups (patients with SMI vs patients with angina) was higher BMI and age, for patients in the SMI group. This was expected, like age, duration of diabetes, and features of metabolic syndrome (including obesity) are well-known risk factors for SMI (50). To address this, we included age, BMI, duration of diabetes as covariates in the ANCOVA, and still found mean serum 25(OH)D levels were significantly lower in patients with SIM.

Another limitation is that the vitamin D plasma level is influenced by various factors: age, gender, ethnicity, skin color, season, and clothing. To overcome this limitation, we studied whether there was an imbalance regarding the season in which the dosage of vitamin D was effected. The studied groups, including that of healthy controls, resulted statistically comparable for this parameter. Furthermore, summer (from July to September) determination of vitamin D was inserted as a covariate in the statistical analysis, together with age and gender, and these parameters were not able to modify the final result. Moreover, all enrolled patients belonged to the same ethnic group and came from the same

geographical area, so we postulated equal skin color and similar habits regarding sun exposure and clothing.

Conclusions

In the current study, we established the importance of vitamin D levels on SMI development in the population of type 2 diabetic patients. Moreover, we demonstrated that SMI patients with low vitamin D levels had a worse prognosis than their symptomatic counterparts. These findings may open new horizons in risk-stratification, management, and prevention of cardiovascular complications in type 2 diabetes. Further prospective and larger studies are needed to better investigate and confirm the current study novel findings.

List Of Abbreviations

SMI (silent myocardial ischemia); MI (myocardial ischemia); 25-hydroxyvitamin D (25(OH)D); coronary heart disease (CAD); ergocalciferol (D2); cholecalciferol (D3); body mass index (BMI).

Declarations

Ethics approval

The current study protocol is in accordance with the Declaration of Helsinki and obtained the approval of the Ethics Committee of Modena University the 20/05/2020 with protocol number 0014071/20.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors have nothing to disclose.

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This study received no funds.

Authors' contributions

R.R. and M. T. provided analysis, and interpretation of data and wrote the manuscript. C.R. provided data acquisition. G. B. and R. M. provided critical analysis of data and discussion and performed the revision of the manuscript. All the authors gave a substantial contribution to the work and approved the final version of the manuscript.

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Figures

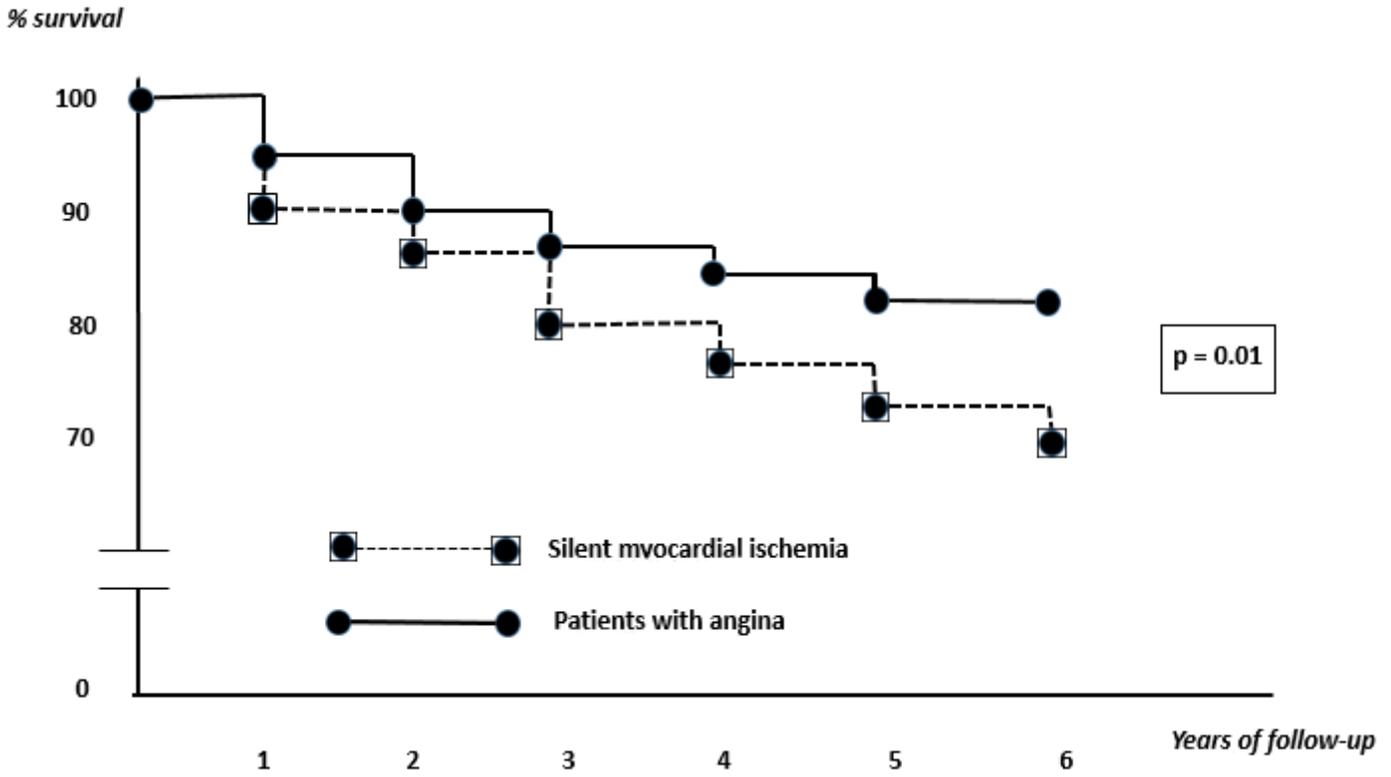


Figure 1

Six-year survival of diabetic patients with silent myocardial ischemia vs. symptomatic ischemia.