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High density lipoprotein (HDL) level may be a risk factor of lower extremities amputation (LEA) in patients with diabetic foot ulcer (DFU).

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ABSTRACT

Diabetes related lower extremity amputation (LEA) is associated with high incidence of morbidity and mortality various risk factors of diabetic foot ulcers (DFU). Dyslipidemia including low HDL levels is a major risk factor of PVD in diabetic patients. The aim of this study was to evaluate HDL cholesterol level as a risk factor and predictor for LEA and major cardiac events (MACE) in patients with DFU. In this study, 112 diabetic patients had (DFU). The patients were divided into two subgroups: Group (A) which included 55 high-risk patients with a long history of DFU more than six month duration and group (B) of 57 low-risk patients with a short history of DFU less than six month duration.Lipid profile including HDL was measured in all patients. Peripheral pulsations, protective sensation and ankle/brachial index (ABI) were also evaluated in both groups.All patients were followed for one year for major cardiac events (MACE) and lower extremity amputation.The overall results revealed that HDL was significantly lower (31.47 ± 8.86 mg/dl) in high risk group compared to low risk group (36.72 ± 5.45 mg/dl; *P*< 0.03).Patients of high risk group had a significantly lower ankle pressure (105.72 ± 30.23 mmHg) and ABI (0.77 ± 0.24) compared to low risk group (136.75 ± 33.49 mmHg and 1.07 ± 0.22, respectively; *P* < 0.001).HDL was (31.34 ± 1.91& 40.38 ± 8.26; *P*< 0.001) for amputated and non-amputated patients, respectively. MACE were significantly higher in high risk group compared to low risk group (69.1% vs. 31.6%; *P*< 0.001) .Furthermore amputations were significantly more frequent among high risk patients compared to those with low risk (78.2% vs. 28.1%; *P*< 0.001).HDL had 100% sensitivity and 91% specificity for prediction of amputation at cutoff point < 35mg/dl with area under the curve was 0.91.The observed independent risk factors for lower limb amputation in patients with diabetic foot ulcer were HDL < 40 mg/dl (OR= 19.1, 95%CI= 12.9- 34.46; *P*= 0.01), previous amputation (OR= 4.68, 95%CI= 1.63- 13.44; *P*= 0.01), ankle brachial index (OR= 5.67, 95%CI= 3.45- 12.45; *P*= 0.03), and sever infection (OR= 17.8, 95%CI= 2.13- 18.89; *P*= 0.01).So, HDL cholesterol level may be an important independent predictor for LEA and MACE in patients with DFU.

Keywords:HDL cholesterol, Diabetic foot ulcer, Lower extremity amputation.

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INTRODUCTION

Diabetic foot ulcers (DFU) often result in amputation which is one of the worst outcomes of diabetes [1]. Diabetes-related lower extremities amputation (LEA) remains a source of significant morbidity and mortality, due to the high incidence of DFU [2].DFU occur as a result of various factors, including mechanical changes in conformation of the bony architecture of the foot, peripheral neuropathy, and atherosclerotic peripheral arterial disease [3].

Dyslipidemia, is a major risk factor for cardiovascular disease .Patients with diabetes frequently have impaired lipoprotein metabolism in the form of an increase in very low density lipoprotein (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) and a decrease in high-density lipoprotein cholesterol (HDL-C) [4].

HDL has various pleiotropic effects such as anti-oxidant and anti-inflammatory properties in addition to promoting the efflux of cholesterol from cells [5,6]. According to the European Study Group on Diabetes and the Lower Extremity (EURODIALE) study, 6% of patients with DFU died before healing within the 1-year follow-up period [7].

MACE should be prevented during treatment for limb-threatening DFU in high-risk patients.Erli Pei, et al.[8] reported that a decreased HDL-cholesterol was associated with diabetic footand measures to prevent diabetic foot should include attempts to increaseHDL-cholesterol levels.Ikura K. et al.[9] were the first report to show that lower HDL cholesterol levels might be an independent predictor for LEA and wound-related death in patients with DFU.There for, this present study was designed to evaluate HDL-c levels as a predictor of MACE and LEA in patients with DFU during one year follow up.

MATERIALS AND METHODS

The present study was a prospective observational longitudinal nonrandomized study in which 112 with DFU patient were included after obtaining their written informed consent and the approval of Ethics Committee of Assuit University.The patients attended Assiut university hospital as outpatients from the diabetic and diabetic foot care clinics, presented with diabetic foot ulcer from July 2016 and July 2018. They were divided into two groups: group A of 55 high-risk patients with a long history(more than 6 months duration) of diabetic foot ulcers and group B of57low-risk patients with a short history(less than 6 months duration) of diabetic foot ulcers.

Inclusion criteria included: patients withtype 2DM. ,age>18 years , with diabetic foot ulcers, history of amputation, history of ischemic heart disease, history of admission C.C.U for cardiac events (e.g. acute pulmonary edema, cardiogenic shock, cardiac arrest).

All patients were subjected to:

1-Full history, physical examination& laboratory investigations (HbA1c, lipid profile including HDL-c and kidney function test).
2-Sensory neuropathy assessment by 10 g-Semmes-Weinstein Monofilament.
3- Ankle Brachial Index (ABI) was measured by using a handheld Doppler. Patients with absent or reduced pedal pulses or ABI <0.9were considered to have peripheral arterial disease.
4- MACE included follow up for occurrenceof stroke, myocardial infarction and cardiac death during one year of follow up.

Statistical analysis

Results wereanalyzed by using SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Continuous data was expressed in form of mean ± SD or median (range) while nominal data was expressed in form of frequency (percentage). Chi²-test was used to compare the nominal data of different groups in the study while student t-test was used to compare mean of different two groups and ANOVA test for more than two groups. Multivariate regression analysis was used to determine the independent risk factors for prediction of amputation and major cardiac events (MACE) in the current study. Person correlation was used to determine the correlation between HDL and other continuous variables while

ROC curve was used to determine the diagnostic accuracy of HDL for prediction of amputation. P value was considered significant if < 0.05.

RESULTS

Mean age of those with high risk was 54.7 ± 11.5 years and 37 (67.3%) of them were males while mean age of those with low risk was 51.6 ± 13.8 years and 29 (50.9%) of them were males.

As regarding management of DM in studied patients, majority (83.6% of those with high risk and 77.2% of those with low risk) of studied patients used insulin while oral agents were used in 10.9% of those with high risk and in 15.8% of those with low risk group. Both insulin and oral agents were used in 5.5% and 7% patients of high risk and low risk group respectively. Age, sex, and type of therapy had no significant differences between both groups.

Out of those patients with high risk, 27 (49.1%) patients had history of previous amputation while 7 (12.3%) patients of those with low risk had history of previous amputation (*P*< 0.001).

It was observed that patient of high risk group had a significantly higher glycated hemoglobin, LDL, triglyceride, cholesterol, blood urea and serum creatinine compared to those of high risk. On the other hand, the high risk group showed a significantly lower HDL in comparison to low risk group**(Table 1)**.

Table 1: Baseline laboratory data in both groups

Variables	High risk group (n= 55)	Low risk group (n= 57)	*P* value
HbA1c (%)	11.25 ± 1.23	9.63 ± 1.51	< 0.001
LDL (mg/dl)	162.82 ± 37.15	140.21 ± 37.59	< 0.001
HDL (mg/dl)	31.47 ± 8.86	36.72 ± 5.45	0.03
TG (mg/dl)	290.49 ± 93.81	245.02 ± 75.56	0.04
Cholesterol (mg/dl)	255.38 ± 48.79	226.19 ± 45.09	0.01
Urea (mg/dl)	7.05 ± 3.70	5.77 ± 1.25	0.03
Creatinine (mg/dl)	104.29 ± 96.22	77.48 ± 19.66	0.04

Data was expressed in form of mean (SD). *P* value was significant if < 0.05. **HbA1c**, glycated hemoglobin; **LDL,** low density lipoprotein; **HDL,** high density lipoprotein; **TG,** triglyceride

Patients of high risk group had significantly lower ankle pressure and ankle/ brachial index in comparison to those of low risk group **(Table 2 and Figure1)**.Peripheral pulsations were either weak or absent 44(80%) of patients with high risk compared to 18(31.6%) of those with low risk (*p*<0.001) (**Figure2**).Protective sensations was either diminished or lost in 51(92.7%) of high risk group compared to 33(58%) of low risk group (*p*<0.001)

Table 2: Baseline ankle pressure, brachial pressure and ankle/ brachial index

Pressure (mmHg)	High risk group (n= 55)	Low risk group (n= 57)	*P* value
Ankle pressure	105.72 ± 30.23	136.75 ± 33.49	< 0.001
Brachial pressure	137.18 ± 14.52	128.85 ± 16.03	< 0.001
ABI	0.77 ± 0.24	1.07 ± 0.22	< 0.001

Data was expressed in form of mean (SD). *P* value was significant if < 0.05. **ABI**, ankle/ brachial index

It was observed that the frequency of major cardiac event (MACE) and amputations were significantly higher in high risk group than in low risk group (*P*< 0.001) **(Table 3 , Figure3 and Figure 4)**.The observed independent risk factors for lower limb amputation in patients with diabetic foot ulcer were HDL < 40 mg/dl (OR= 19.1, 95%CI= 12.9- 34.46; *P*= 0.01), previous amputation (OR= 4.68, 95%CI= 1.63- 13.44; *P*= 0.01), ankle brachial index (OR= 5.67, 95%CI= 3.45- 12.45; *P*= 0.03), and sever infection (OR= 17.8, 95%CI= 2.13- 18.89; *P*= 0.01) **(Table 4 and 5)**.

The observed independent risk factors for MACE in patients with diabetic foot ulcer were HDL < 40 mg/dl (OR= 7.5, 95%CI= 1.69- 33.85; *P*= 0.03), Male gender (OR= 1.19, 95%CI= 0.40- 3.03; *P*= 0.01), ankle

brachial index (OR= 11.46, 95%CI= 3.11- 36.74; P= 0.02), and age >60 years old (OR= 7.23%CI= 2.18- 23.89; P= 0.01) **(Table 6 and 7)**.

It was noticed that HDL had a significant negative correlation with HbA1c (r= - 0.44; P< 0.001), age (r= - 0.30; P= 0.04), and size of ulcer (r= -0.21; P= 0.02 but significant positive correlation with ankle/ brachial index (r= 0.54; P< 0.001) **(Table 8).**

Diagnostic accuracy of HDL for prediction of amputation has been presented in (**Table 9 and Figure 5**) with sensitivity 100% and specificity 91% at cutoff point < 35 mg/dl.

Table 3: Outcome in both studied groups

	High risk group (n= 55)	Low risk group (n= 57)	*P* value
MACE	**38 (69.1%)**	**17 (31.6%)**	**< 0.001**
Amputation	**43 (78.2%)**	**16 (28.1%)**	**< 0.001**

Data was expressed in form of frequency (percentage).*P* value was significant if < 0.05.**MACE**, major cardiac event

Table 4: Univariate analysis of risk factors associated with amputation

	Amputation (n= 59)	No amputation (n= 53)	*P* value
Age (> 60 years)	**29 (49.2%)**	**8 (15.1%)**	**< 0.001**
Male sex	**41 (69.5%)**	**25 (47.2%)**	**0.01**
Type of therapy **Inulin** **Oral agents** **Both**	 **49 (83.1%)** **8 (13.6%)** **2 (3.4%)**	 **41 (77.4%)** **7 (13.2%)** **5 (9.4%)**	**0.42**
HbA1c (%)	**11.58 ± 0.67**	**9.22 ± 1.47**	**0.03**
LDL (mg/dl)	**177.4 ± 16.37**	**122.23 ± 36.37**	**< 0.001**
HDL (mg/dl)	**31.34 ± 1.91**	**40.38 ± 8.26**	**< 0.001**
TG (mg/dl)	**325.49 ± 60.03**	**232.62 ± 65.34**	**< 0.001**
Cholesterol (mg/dl)	**255.20 ± 27.38**	**221.92 ± 37.61**	**< 0.001**
Urea (mg/dl)	**6.79 ± 1.46**	**5.96 ±3.74**	**0.45**
Creatinine (mg/dl)	**98.01 ± 21.43**	**82.45 ± 22.98**	**0.31**
ABI	**0.67 ± 0.18**	**1.20 ± 0.13**	**0.02**
Previous amputation	**28 (47.5%)**	**6 (11.3%)**	**< 0.001**
Absent peripheral pulsation	**10 (16.9%)**	**0**	**< 0.001**
Lost protective sensation	**26 (44.1%)**	**22 (41.5%)**	**0.03**

Data was expressed in form of mean (SD) and, frequency (percentage). *P* value was significant if < 0.05. **HbA1c**, glycated hemoglobin; **LDL,** low density lipoprotein; **HDL,** high density lipoprotein; **TG,** triglyceride

Table 5: Multivariate regression analysis for prediction of amputation

Risk factors	OR	Coefficient	95%CI	*P* value
Sever infection	**17.8**	**1.5**	**2.13- 18.89**	**0.01**
Previous amputation	**4.68**	**1.6**	**1.63- 13.44**	**0.02**
HDL < 40 mg/dl	**19.1**	**1.65**	**12.90- 34.46**	**0.01**
ABI < 0.9	**5.67**	**1.01**	**3.45- 12.45**	**0.03**

Pvalue was significant if <0.05.**HDL,** high density lipoprotein; **OR,** odd's ratio; **CI,** confidence interval; **ABI,** ankle brachial index

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Table 6: Univariate analysis of risk factors associated with MACE

	No MACE (n= 54)	MACE (n= 58)	P value
HDL (mg/dl)	37.89 ± 5.76	33.50 ± 8.11	< 0.001
ABI	1.12 ± 0.20	0.75 ± 0.27	0.01
Previous amputation	6 (11.1%)	28 (48.3%)	< 0.001
Absent peripheral pulsation	3 (5.6%)	7 (12.1)	0.04
Lost protective sensation	7 (13%)	22 (37.9%)	< 0.001

Table 7: Multivariate regression analysis for prediction of MACE

Risk factors	OR	Coefficient	95%CI	*P* value
Age (> 60 years)	7.23	1.90	2.18- 23.89	0.01
Male sex	1.19	0.14	0.40- 3.03	0.01
HDL < 40 mg/dl	7.5	1.22	1.69- 33.85	0.03
ABI < 0.9	11.46	2.42	3.11- 36.74	0.02

Pvalue was significant if <0.05.**HDL,** high density lipoprotein; **OR,** odd's ratio; **CI,** confidence interval; **ABI,** ankle brachial index, **MACE**, major cardiac events

Table 8: Correlation of HDL with different variables in the current study

	P	r
Age	0.04	- 0.30
Glycated hemoglobin	< 0.001	- 0.44
Low density lipoprotein	< 0.001	- 0.45
Triglyceride	< 0.001	- 0.61
Cholesterol	< 0.001	- 0.45
Ankle/ brachial index	< 0.001	0.54
Duration of ulcer	0.87	0.01
Size of the ulcer	0.02	- 0.21
Urea	0.22	0.34
Creatinine	0.43	0.09

Data was expressed in form of *P* that indicated to significance of correlation and r that indicated to strength of correlation. *P* value was significant if < 0.05.

Table 9: Diagnostic accuracy of HDL for prediction of amputation

Indices	Value
Sensitivity	100%
Specificity	91%
Positive predictive value	92%
Negative predictive value	100%
Accuracy	0.91
Area under the curve	95.5%
Cutoff point	< 35 mg/dl
P value	< 0.001

P value was significant if < 0.05. **HDL**, high density lipoprotein

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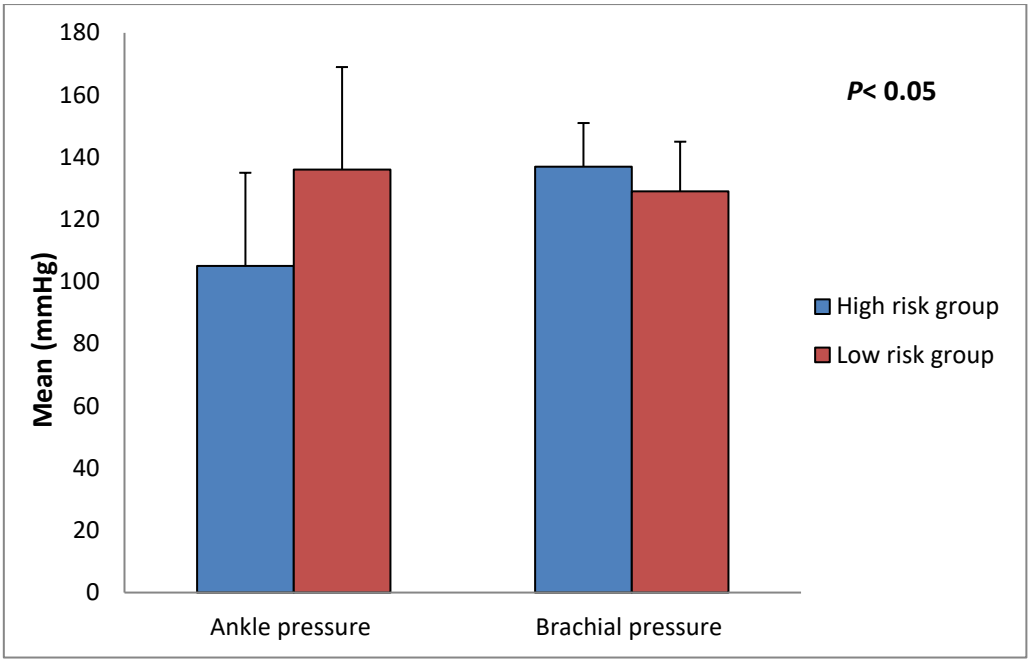

Figure 1: Baseline ankle and brachial pressure in both studied groups

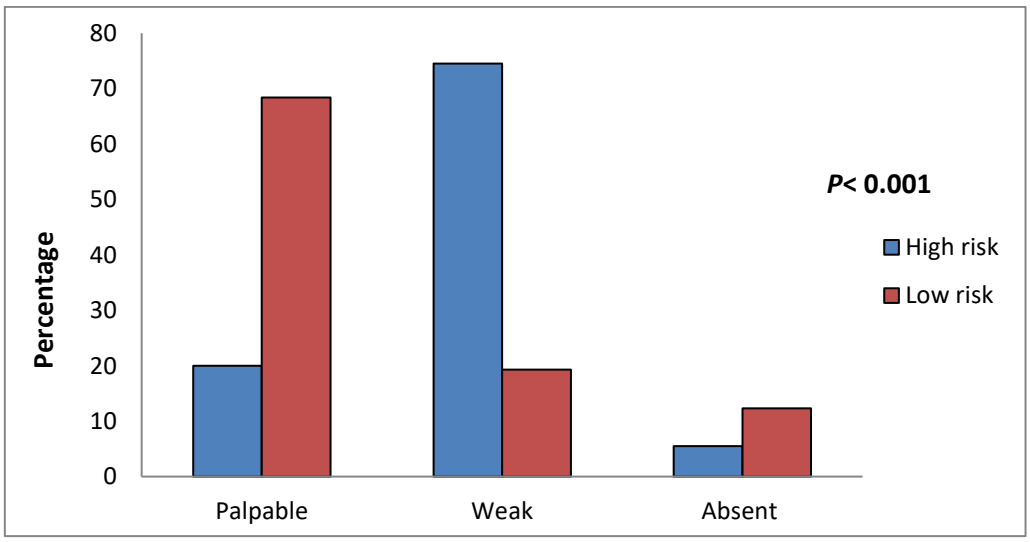

Figure 2: Peripheral pulsation in both groups

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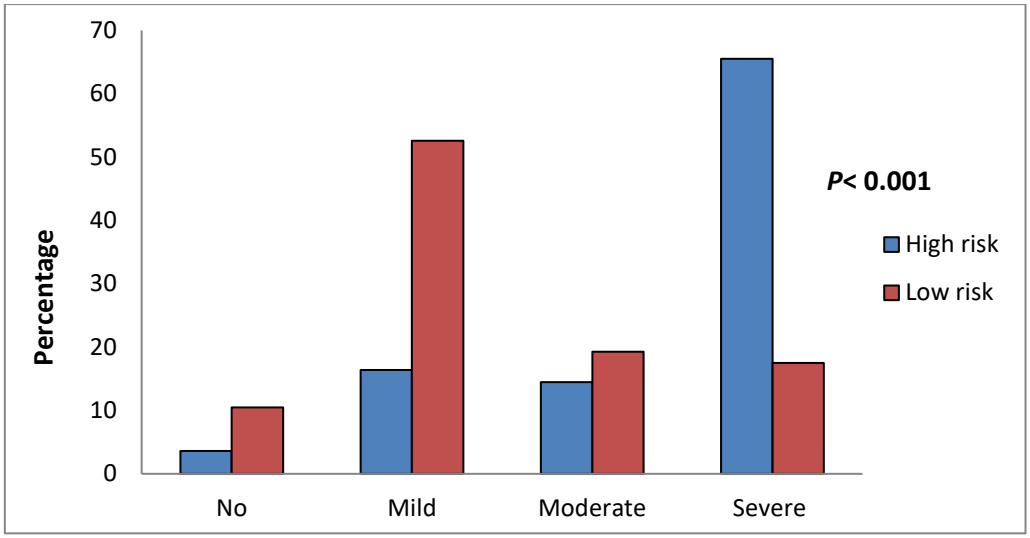

Figure 3: Frequency of MACE in both studied groups

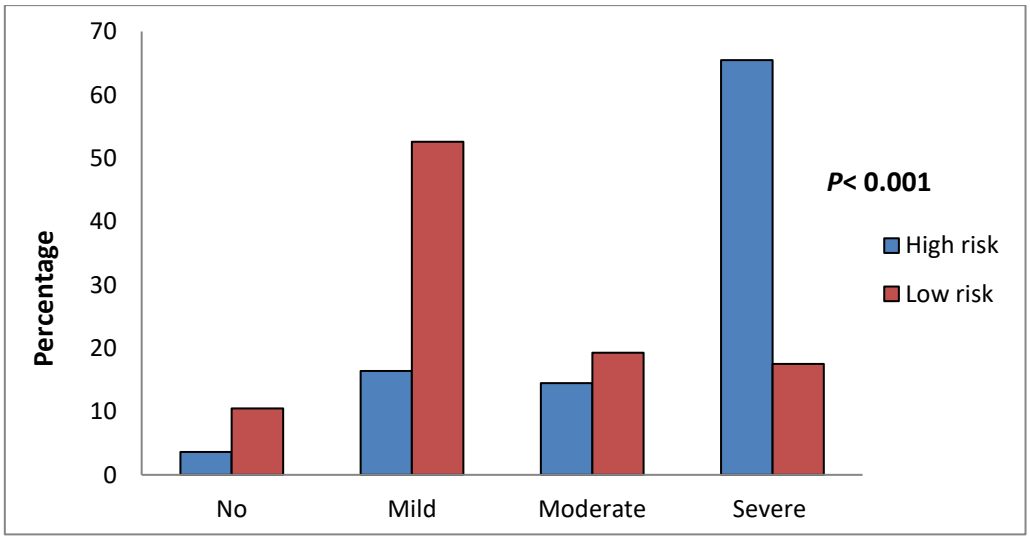

Figure 4: Frequency of amputation in both studied groups

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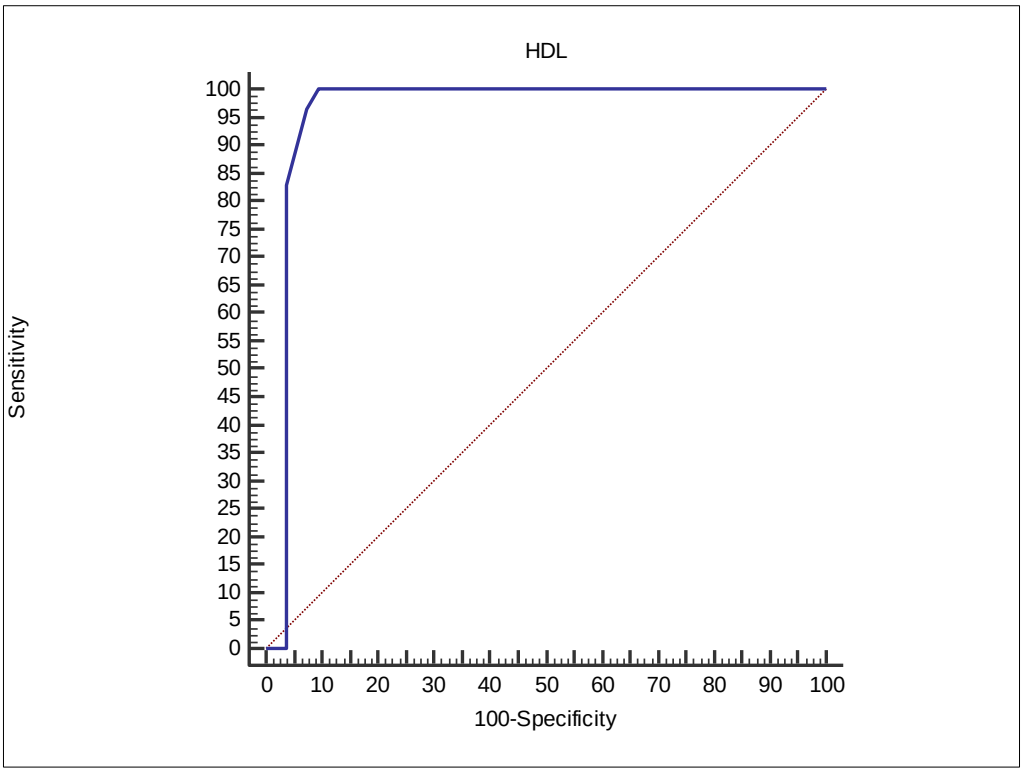

Figure 5: Diagnostic accuracy of HDL for prediction of amputation

It was noticed that HDL had 100% sensitivity and 91% specificity for prediction of amputation at cutoff point < 35mg/dl with area under the curve was 0.91.

DISCUSSION

Group (A) high-risk patientsand Group (B) low-risk patients showed no significance difference as regarding age, sex. Or type of therapy between the two groups.While with comparing between the two groups in previous history of amputation was significantly higher in high risk group (*P*< 0.001).Interestingly, Musa et al.[10] reported that patients with a history of previous amputation had a higher risk for further amputation among patients with diabetic foot ulcers in a Saudi population.

In our study, we observed that patient of high risk group had significantly HbA1c, LDL, triglyceride, cholesterollevels but those a significantly lower HDL compared to those with low risk. In our study we reported also a significant positive correlation between HbA1c level and foot amputation. Furthermore, we found that high cholesterol patients had high risk for amputation because of ours patients had bad dietary style and low compliance of treatment.In contrast Musa et al. [10] observed no relation between HbA1c, LDL, triglyceride, and cholesterol and the risk for amputation.Pemayunet al.[11] reported that HbA1c above 8% was a significant risk factor for LEA (OR 20.47, 95% CI 3.44_134.31; *p*=0.002).However,Al-Wakeel et al. [12] observed that there was a good relationship between poor glycemic control and the risk of amputation (*p*<0.006).Miyajima et al. [13] showed no correlation between HbA1c levels and amputations , conversely Yekta et al. [14] found that HbA1c level was a significant risk factor for diabetic foot ulceration and that HbA1c level ≥8% was statistically significant predictor risk for amputation. Golinkoet al.[15] reported no difference in total cholesterol between patients with foot amputation and those without.

Our study revealed that ankle pressure, brachial pressure and ankle/ brachial index in patients of low risk group had significantly been lower in comparison to those of high risk group.Similar to our study Ikura K et al.[9] found that presence of ankle brachial index (ABI) was considered significant value in undergone amputation (*P* values <0.05).

In our study, Foot examination in both studied groups showed that 80% of high risk patients had either weak or absent peripheral pulsation and 92.7% had either diminished or lost protective sensation compared to 31.6% and 58% of low risk patients, respectively (p<0.001) Ahmed et al.[16] in their study noticed that peripheral pulsation had no significance difference, but in agreement with us that peripheral neuropathy had significant difference that's due to most of patients of our study had amputated lower extremities after they suffered from PAD and they transferred to vascular surgery clinic.Hung, Shih-Yuan et al. [17] reported that MACE should be prevented in high risk patients with limb-threatening DFU liable for lower limb amputation.

In our study, MACE was significantly higher in high risk patients in compared to those with low risk (69.1% vs. 31.6%, respectively$P$< 0.001). And alsoamputation was significantly higher in case of high risk patients in comparison to those with low risk (78.2% vs. 28.1%; P< 0.001).Zubair et al.[18] reported that the levels of fasting triglycerides (>150 mg/dl), cholesterol (>150 mg/dl), LDL-cholesterol (>100 mg/dl), and HDL cholesterol (<40 mg/dl) were associated with increased risk of amputation On the other hand, Ikura k et al. [9] eported that neither triglycerides levels nor LDL cholesterol levels were a predictor of the future amputation, but HDL was a risk factor for amputation.Manda et al. [19] also found no difference observed in levels of total cholesterol, LDL, VLDL and triglycerides between control and patientsbut only HDL low levels which were in patient group.Assaad-Khalil et al.[20] in their study PAD was based on the assessment of Ankle–Brachial Index (ABI) ≤0.9. Presence of PAD showed a high statistical significance with the occurrence of diabetic foot amputation and ulceration (27.9% in patients with ulceration and amputation vs. 11.9%diabetics without ulceration and amputations, O.R. = 2.65) (p< 0.001) there for, we studied ABI for our patients and we found that ABI ≤ 0.9 had significance value as a risk factor in LEA (p value =0.02).As regard pervious amputation Crawford et al.[21] his study revealed the same results of our study that history of previous amputation is a highly significant risk factor for LEA (p value <0.001).

As regard that the absence peripheral pulsation is a risk factor for LEA, we found it is a highly significant risk factor for LEA in DFUs patients p value <0.001 this was previously reported byPemayun et al.[11] presence of peripheral arterial disease (PAD) (OR 12.97, 95% CI 3.44_48.88; p<0.001) is risk factor of LEA.

In our study we found that lost protective sensation is significant difference between those with amputation and those without amputations p value = 0.03.

This, in disagreement with Pemayun et al. [11] that found neuropathy alone were not independently associated with LEA. It has been suggested that neuropathy may precipitate an ulcer through decreased foot protective sensation because all their patients in the study were with sever PAD and were hospitalized for intensive surgical management but in our study patients were observed and treated in our diabetic foot care of internal medicine department for diabetic foot ulcers if they developed PAD they transferred to vascular surgery clinic.

In our study we found that the size of the foot ulcer had a highly significant risk factor for LEA (p value <0.001).In disagreement with Akha et al.[22] ulcer size had no significant value (p value=0.84) that's may be the earlier treatment for DFUs, good compliance, more diabetic foot caring centers.Pemayun et al. [11] disagreed with our study that more severe infection is associated with higher rates of LEA than milder one (45.7 vs. 39.3%, p=0.138). If compared to mild infection obviously the more severe infection only shows a step-up increase of OR which was not statistically significant. However their data did not reveal a strong association between severity of infection and LEA but our study showed that infection in DFUs had a highly significant risk factor for LEA (p value <0.001).Also, Treece et al. [23] agree that foot infection is a risk factor for diabetic foot amputation.Akha et al [22] found that Ulcer's duration had significant value (p value=0.01) with foot amputation in their results and we found in our study the same results that the duration of foot ulcer is significant risk factor for LEA (p value =0.03) that's why we suggested to divide the patients into two groups (high risk group & low risk group) according to the duration of the ulcer.

In our study we didn't report a significant value to foot deformity for LEA (p value =0.47).While, Ahmed et al.[16] that foot deformity is the most common risk factor for diabetic foot ulcers (DFUs) detected in their study.Also, in our study we found that the site of the foot ulcer has no significant value for LEA (p value

=0.26).In disagreement with Akha et al.[22] found that the most common amputation site was the right foot at the fingers level.

All this will explain the rational of the study that we found the independent risk factors for lower limb amputation in patients with diabetic foot ulcer were HDL < 40 mg/dl (OR= 19.1, 95%CI= 12.9- 34.46; *P*= 0.01), previous amputation (OR= 4.68, 95%CI= 1.63- 13.44; *P*= 0.01), ankle brachial index (OR= 5.67, 95%CI= 3.45- 12.45; *P*= 0.03), and sever infection (OR= 17.8, 95%CI= 2.13- 18.89;*P*= 0.01).

Outcome of our study we had seen 55 patients of total 112 patients had MACE. In our study we found that age had a significant risk factor for major cardiac events (MACE) (*p* value =0.03).In agreement with our study Huang, Yu-Yao et al., [24] that found age had a moderate significant risk in MACE and mortality (*p* value =0.01).
Glycemic control may have a role in occurring MACE as we found that HbA1c had a moderate significance risk factor for MACE (*p* value =0.01).In disagreement with Huang, Yu-Yao et al., [24] that found HbA1c had no significance risk in MACE and mortality (*p* value =0.71).

Dyslipidemia play a great role in cardiac events as CAD and so we found that LDL, HDL, TG & cholesterol had highly significant differences between those with major cardiac events (MACE) and those without (MACE) considered as risk factors for (MACE) *p* value <0.001.

In our results we found that ABI ≤ 0.9 had moderate significance value as a risk factor in MACE (*p* value =0.01).In disagreement with Hung, Shih-Yuan et al., [17] that found ABI had no significance value as a risk factor for MACE (p value =0.29) in their study the only risk factor associated with MACEs was previous myocardial infarction and also identified a causal relationship between major procedures during treatment for limb-threatening DFUs and MACEs. Among these cases, 70% were related to major amputation.

Major amputation is usually performed in cases of overwhelming infection or failed wound healing following revascularization but our study observed patients with DFUs undergo MCAEs though their follow up over the duration of the study were 55 patients.We found in our study that history of previous amputation is a highly significant risk factor for MACE (*p* value <0.001) this will explain our aim to find the relationship between MACE and amputation.

As regards the absence of peripheral pulsation one of the vascular complication of diabetes we found that absent peripheral pulsation is significant risk factor for MACE (*p* value =0.04) this was previously reported by Prompers et al. [7] that foot ulcers in diabetic patients indicate coexisting peripheral arterial disease and diffuse atherosclerosis that lead to MACE.

Diabetic neuropathy is one of the most complications of diabetes so; we found that lost protective sensation is highly significant difference between those with MACE and those without MACE *p* value < 0.001.We found that the duration of foot ulcer is highly significant difference between those with MACE and those without MACE *p* value < 0.001.In our study we found that the size of the foot ulcer is highly significant difference between those with MACE and those without MACE *p* value < 0.001.We noticed that site of the foot ulcer had significant difference between those with MACE and those without MACE *p* value =0.02.In our study found that infection in DFUs had a highly significant risk factor for MACE *(p* value <0.001).In agreement with Hung, Shih-Yuan et al.[17] found that major adverse cardiac events (MACE) were the most common complications, followed by nosocomial infection.

Thus, we found that the independent risk factors for MACE in patients with diabetic foot ulcer were age> 60 year (OR= 1.19, 95%CI= 0.40- 3.03; *P*= 0.01), ankle brachial index < 0.09 (OR= 11.64, 95%CI= 3.11- 36.74; *P*= 0.02), HDL< 40 mg/dl (OR= 7.5, 95%CI= 1.69- 33.85; *P*= 0.03), and age >60 years (OR= 7.23, 95%CI= 2.18- 23.89; *P*= 0.01).

As regard the degree of infection we found it was affected by the level of HDL in a moderate significance value (*p* value =0.02) this was previously reported by Canturk et al. [25] that lower HDL cholesterol levels were associated with the development of in-hospital infections.Also, we agreed with Ikura k, et al.[9] found that HDL might play a role in the suppression of acute phase conditions such as infections and sepsis caused by DFUs.We found in our study that peripheral pulsation had a high significantly affection by the level

of HDL in all patients *p* value <0.001. In our study we found that protective sensation had a high significantly affection by the level of HDL in all patients *p* value <0.001.

Lastly, we found that HDL had significant negative correlation with HbA1c (r= - 0.44; *P*< 0.001), LDL (r= - 0.45; *P*< 0.001), TG (r= - 0.61; *P*< 0.001), cholesterol (r= - 0.45; *P*< 0.001), age (r= - 0.30; *P*= 0.04), and size of ulcer (r= -0.21; *P*= 0.02 but had significant positive correlation with ankle/ brachial index (r= 0.54; *P*< 0.001).

We concluded that the Diagnostic Accuracy of HDL for prediction of Amputation ;It was noticed that HDL had 100% sensitivity and 91% specificity for prediction of amputation at cutoff point < 35mg/dl with area under the curve was 0.91 as this will explain the rational of the study.

CONCLUSIONS

We could conclude that HDL cholesterol levels are clinical predictor for the incidence of LEA and MCAE with patients have DFU.

REFERENCES

[1] DjokomoeljantoR. TinjauanUmumTentang Kaki Diabetes. KAKI Diabetik: Patogenesisdanpenatalaksanaan. Semarang: Diponegoro University. 1997:1-0.

[2] Abbas ZG. Reducing diabetic limb amputations in developing countries. Expert review of endocrinology & metabolism. 2015 Jul 4; 10(4):425-34.

[3] American Diabetes Association. Diabetes care in the school and day care setting. Diabetes care. 2014 Jan 1;37(Supplement 1):S91-6.

[4] Hirano T. Abnormal lipoprotein metabolism in diabetic nephropathy. Clinical and experimental nephrology. 2014 Apr 1; 18(2):206-9.

[5] Kontush A, Chapman MJ. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacological reviews. 2006 Sep 1; 58(3):342-74.

[6] Fruchart JC, Sacks F, Hermans MP, Assmann G, Brown WV, Ceska R, Chapman MJ, Dodson PM, Fioretto P, Ginsberg HN, Kadowaki T. The Residual Risk Reduction Initiative: a call to action to reduce residual vascular risk in patients with dyslipidemia. The American journal of cardiology. 2008 Nov 17; 102(10):1K-34K.

[7] Prompers L, Schaper N, Apelqvist J, Edmonds M, Jude E, Mauricio D, Uccioli L, Urbancic V, Bakker K, Holstein P, Jirkovska A. Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. Diabetologia. 2008 May 1; 51(5):747-55.

[8] ErliPei ,Jue Li , Chenhui Lu , Jichong Xu , Tao Tang , Meng Ye a, Xiaoping Zhang a,c, Maoquan Li ,J Diabetes Complications. 2014 Jul-Aug; 28(4):559-64. doi: 10.1016/j.jdiacomp.2014.04.002. Epub 2014 Apr 13.

[9] Ikura K, Hanai K, Shinjyo T, Uchigata Y. HDL cholesterol as a predictor for the incidence of lower extremity amputation and wound-related death in patients with diabetic foot ulcers. Atherosclerosis. 2015 Apr 1; 239(2):465-9.

[10] Musa IR, Ahmed MO, Sabir EI, Alsheneber IF, Ibrahim EM, Mohamed GB, Awadallah RE, Abbas T, Gasim GI. Factors associated with amputation among patients with diabetic foot ulcers in a Saudi population. BMC research notes. 2018 Dec; 11(1):260.

[11] Pemayun TG, Naibaho RM, Novitasari D, Amin N, Minuljo TT. Risk factors for lower extremity amputation in patients with diabetic foot ulcers: a hospital-based case–control study. Diabetic foot & ankle. 2015 Jan 1;6(1):29629.

[12] Al-Wakeel JS, Hammad D, Al Suwaida A, Mitwalli AH, Memon NA, Sulimani F. Microvascular and macrovascular complications in diabetic nephropathy patients referred to nephrology clinic. Saudi Journal of Kidney Diseases and Transplantation. 2009 Jan 1; 20(1):77.

[13] Miyajima S, Shirai A, Yamamoto S, Okada N, Matsushita T. Risk factors for major limb amputations in diabetic foot gangrene patients. Diabetes research and clinical practice. 2006 Mar 1; 71(3):272-9.

[14] Yekta Z, Pourali R, Nezhadrahim R, Ravanyar L, Ghasemi-rad M. Clinical and behavioral factors associated with management outcome in hospitalized patients with diabetic foot ulcer. Diabetes, metabolic syndrome and obesity: targets and therapy. 2011;4:371.

[15] Golinko MS, Margolis DJ, Tal A, Hoffstad O, Boulton AJ, Brem H. Preliminary development of a diabetic foot ulcer database from a wound electronic medical record: a tool to decrease limb amputations. Wound repair and regeneration. 2009 Sep; 17(5):657-65.

[16] Ahmed AA, Algamdi SA, AlzahraniAM. Surveillance of risk factors for diabetic foot ulceration with particular concern to local practice. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2015 Oct 1; 9(4):310-5.

[17] Hung SY, Huang YY, Hsu LA, Chen CC, Yang HM, Sun JH, Lin CW, Wang CC. Treatment for diabetic foot ulcers complicated by major cardiac events. Canadian journal of diabetes. 2015 Jun 1; 39(3):183-7.

[18] Zubair M, Malik A, Ahmad J. Plasma adiponectin, IL-6, hsCRP, and TNF-α levels in subject with diabetic foot and their correlation with clinical variables in a North Indian tertiary care hospital. Indian journal of endocrinology and metabolism. 2013 Sep; 16(5):769.

[19] Manda V, Sreedharan J, Muttappallymyalil J, Das R, Hisamatsu E. Foot ulcers and risk factors among diabetic patients visiting Surgery Department in a University Teaching Hospital in Ajman, UAE. International Journal of Medicine and Public Health. 2012;2(3).

[20] Assaad-Khalil SH, Zaki A, Rehim AA, Megallaa MH, Gaber N, Gamal H, Rohoma KH. Prevalence of diabetic foot disorders and related risk factors among Egyptian subjects with diabetes.Primary care diabetes. 2015 Aug 1;9(4):297-303.

[21] Crawford F, Cezard G, Chappell FM, Murray GD, Price JF, Sheikh A, Simpson CR, Stansby GP, Young MJ. A systematic review and individual patient data meta-analysis of prognostic factors for foot ulceration in people with diabetes: the international research collaboration for the prediction of diabetic foot ulcerations (PODUS) 2015.

[22] Akha O, Kashi Z, Makhlough A. Correlation between amputation of diabetic foot and nephropathy. Iranian journal of kidney diseases. 2010; 4(1):27.

[23] Treece KA, Macfarlane RM, Pound N, Game FL, Jeffcoate WJ. Validation of a system of foot ulcer classification in diabetes mellitus.Diabetic medicine. 2004 Sep;21(9):987-91.

[24] Huang YY, Lin CW, Yang HM, Hung SY, Chen IW. Survival and associated risk factors in patients with diabetes and amputations caused by infectious foot gangrene.Journal of Foot and Ankle Research. 2018 Dec;11(1):1.

[25] Canturk NZ, Canturk Z, Okay E, Yirmibesoglu O, Eraldemir B. Risk of nosocomial infections and effects of total cholesterol, HDL cholesterol in surgical patients. Clinical Nutrition. 2002 Oct 1; 21(5):431-6.