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Investigating the predictive power of LDL subfractions in ischemic stroke risk - a clinical correlation study

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Abstract
A key health concern, being a major cause of functional dependence and the 2nd leading cause of death globally, is Stroke. Stroke survivors often face long-term disability and other illnesses, while treatments have advanced. High levels of Low-Density Lipoprotein Cholesterol (LDL-C) are a key and well-established risk factor for Ischemic Stroke (IS). But, the LDL-C sub-factors' impact on IS risk is unclear. Besides, better tools and responses to therapies are required to recognize LDL-C's specific role in IS. Also, detailed subclass analysis is desirable to develop personalized prevention and treatment strategies. Thus, the study investigates the link betwixt the LDL subfractions and IS. A total of 172 eligible participants are organized under the IS group (n=43) and the non-IS group (n=129). The analytical data are collected from the study participants by using the clinical cross-sectional studies. Moreover, the quantitative methods are used to evaluate the data. The results proved that the IS patients showed increased health hazards than the non-IS patients. The regression analysis showed that LDL-C4 significantly influenced (OR, 95% CI: 1.22, 1.08–1.38) the IS risk in patients. The Pearson Correlation (PC) outcomes also proved positive and negative correlations between the IS risk and LDL fractions. Furthermore, the predictive value assessment also exhibited the larger area under the curves and ROC curves of LDL-C4 (0.847) and LDL-C3 (0.813), which also confirmed the influence of LDL on IS risks.

Keywords: Low-density Lipoprotein, Ischemic Stroke, Cerebrovascular Events, and Exploratory Risks

1. Introduction
The Low-Density Lipoprotein (LDL) is termed the bad cholesterol, which is composed of a combination of fat and protein. The range of LDL differs significantly from person to person and can stimulate various health hazards. The cerebral vascular accidents, cardiovascular diseases, osteoarthritis, diabetes, and cancer are associated with the LDL pathology (Yang *et al.*, 2024; Costamagna *et al.*, 2023; Zhang & He, 2021) [14, 2, 15]. Besides, as per epidemiological studies, elevated LDL levels are one among the major reasons for IS development. Globally, stroke is estimated as the 2nd chief cause of death as well as the 3rd leading cause of disabilities (Qiao *et al.*, 2022; Puig *et al.*, 2023) [10, 8]. The Red Blood Cells (RBCs), fibrin, platelets, leukocytes, etc, are associated with IS and influence the bile acid metabolism and cholesterol homeostasis (Xia *et al.*, 2025) [13]. Yet, various therapies and laboratory methods are being established to lower the LPL-associated risks like inflammation, oxidative stress, cell proliferation, apoptosis, and mutations (Singh & Prabhakaran, 2024; Puig *et al.*, 2023a; Stanciulescu *et al.*, 2023) [11, 8, 12]. Moreover, the biomarker-based clinical practices also help to assess the early risk of cerebral IS along with its progression (Licari *et al.*, 2023) [5]. The LDL impacts on IS risks are displayed in Figure 1.

Priorly, various standard clinical measures were used to assess the LDL-associated IS risks. (Banach *et al.*, 2022) [1] evaluated LDL levels and lipid-lowering agents' impact on the diverse sorts of stroke. The highest risk of IS was induced by the highest category of LDL. Next, the Atherogenic Index of Plasma (AIP) predicted results in Acute IS (AIS) patients, which were evaluated by (Liu *et al.*, 2021) [7].

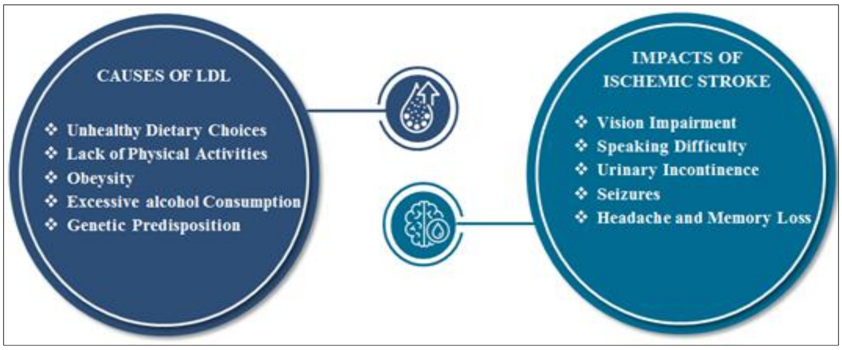


Fig 1: Role of LDL in ischemic stroke risks

The outcomes proved that API was a key as well as an independent forecaster of dysfunction outcome in AIS patients. (Gkantzios *et al.*, 2023) [3] demonstrated the significant progression of stroke patients' health using the National Institutes of Health Stroke Scale (NIHSS). Also, (Licari *et al.*, 2021) [5] highlighted the molecular mechanisms allied to patient functionality as well as mortality. Yet, only a few preceding studies addressed the link betwixt LDL subclass distribution and IS. Especially, traditional LDL measurement was an insufficient predictor of IS onset. Also, unfavorable functional outcomes allied with augmented risk of recurrent cerebral-vascular events in IS patients remained unknown. Still, the privation of predictive assessment of the LDL subfractions may significantly expand the ischemic risk and influence the prevention strategies. Therefore, the present clinical correlation study assesses the prediction of IS risk with LDH sub-fractions.

1.1 Objectives

- To analyze the impacts of medical conditions and the LDL subfractions in control as well as IS patients.
- To evaluate the logistic relation betwixt the LDL subtypes as well as patients with and without IS onset.
- To assess the correlation relationship between clinical characterizations and IS risks.
- To examine the predictive values of LDL subtypes in IS patients.

The remaining part is arranged as: the adopted materials as well as methods are detailed in Section 2, the analysis results are displayed in Section 3, the analysis findings are deliberated in Section 4, as well as Section 4 outlines the conclusion with future scope and the limitations of this research.

2. Research Methodology

Here, a clinical cross-sectional analysis was wielded for appraising LDL subfractions' predictive power in IS risk. This section describes the details of the population and sampling techniques, study design, data collection and examination, analysis methods, along with ethical considerations. In Figure 2, the study process is outlined.

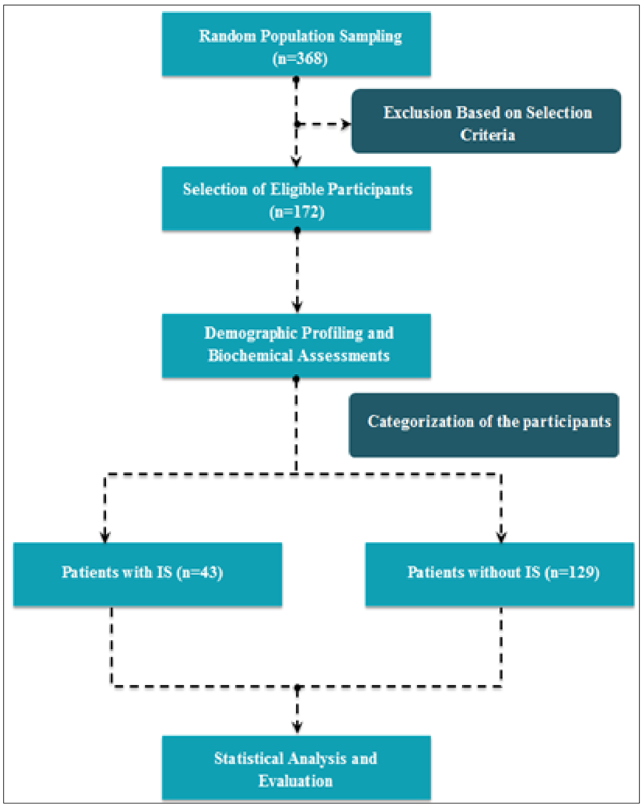


Fig 2: Framework for the present research

2.1 Study population

368 hospitalized patients with dizziness and headaches from the Department of Neurology were randomly selected as the study population. Based on the eligibility criteria, 172 patients were further comprised. The patients who were aged ≥45 years for both genders with an IS were included in the study. But, the exclusion standards comprised: (i) the time as of onset to admission should be more than 24hours, (ii) age should be <18 years, and (iii) patients with a history of cancer, hematologic disease, immunosuppressant usage, severe renal diseases, inflammatory diseases, and major surgery should be excluded from the study.

2.2 Study design

The IS-experienced patients were owed to the stroke group (n=43). The outstanding population was organised under the non-stroke group (n=129). By using a standard questionnaire, the demographic details were collected, and the laboratory tests were used to assess the medical condition of the study populations.

2.3 Data collection and examination

The baseline data, like (1) age, (2) gender, (3) Body Mass Index (BMI), (4) previous medical history, and (5) medical records like blood pressure, heart rate, along with biochemical indexes, were recorded upon visiting the hospital. The lab tests, including Tri-Glycerides (TG), HDL-Cholesterol (HDL-C), Total Cholesterol (TC), LDL-Cholesterol (LDL-C), glycosylated haemoglobin (HbA1c), Fasting Blood Glucose (FBG), High-sensitivity C-Reactive Protein (H-CRP), Uric Acid (UA), along with Creatinine (Cr), were collected from the intravenous blood of the patients.

2.3.1 MRI acquisition

It was done on a 1.5 T MRI scanner (Ingenia - Philips Medical Systems, Netherlands) with an 8-channel receiver array head coil. Higher-resolution T1-weighted axial images MRI scanning covering the entire brain, were attained by a 3D magnetization.

2.3.2 Low-density lipoprotein analysis

For LDL sub-fraction analysis, the gathered blood samples were wielded. LDL subgroups were categorized as well as gauged with the Lipoprint System (Quantimetrix Corporation, Redondo Beach, CA, USA). The polyacrylamide gel electrophoresis was used to separate the LDL subfractions. Each lipoprotein subfraction's area was scanned after electrophoresis. The LDL subfractions were categorized grounded on the net charge, size, along with movement.

2.4 Statistical analysis

The descriptive statistics, such as percentages (%), mean, along with standard deviation, were wielded to determine the study variables. Clinical variables were first correlated with the risk of IS using PC analysis. By employing Student's t- along with chi-square tests, Group differences in clinical data were assessed. Subsequently, a binary Logistic Regression (LR) was conducted to further discover the relation betwixt variables as well as IS risk. Optimal cut-off values were determined using ROC curves, based on maximizing diagnostic accuracy. All statistical tests were appraised at an importance level of $p<0.05$. All the obtained data were visually represented through tables and graphs.

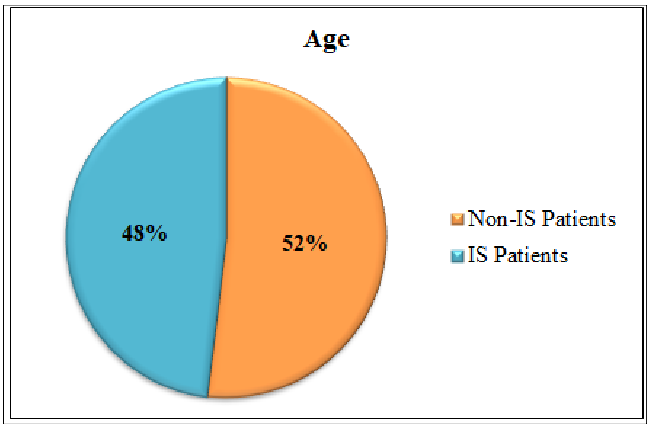
2.5 Ethical considerations

The study followed the ethical protocols and obtained ethical approval as of the Human Research Ethics Committees. Every patient was offered a written informed agreement for the study follow-up, and each patient signed the informed consent.

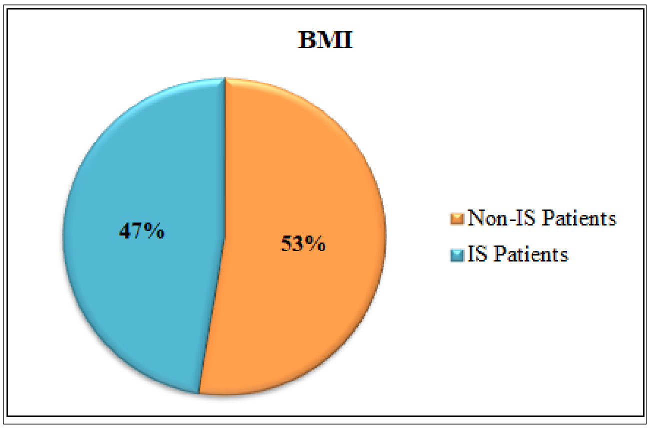
3. Results

3.1 Baseline characteristic studies

The impacts of demographic features, medical conditions, and LDL sub-fractions (LDL-C1 to LDL-C7) on the patients were evaluated. In the present analysis, the demographic studies, including age, BMI, and gender of the patients, were assessed separately. The overall age percentage of IS patients was calculated as 48% and the non-IS group was 52%. The IS patients showed a 47% BMI range, and the non-IS patients showed a 53% BMI range. Furthermore, male participants were higher (56%) in the non-IS group, whereas female participants were higher (55%) in the IS group. The obtained demographic details are presented in Figure 3.



(a)



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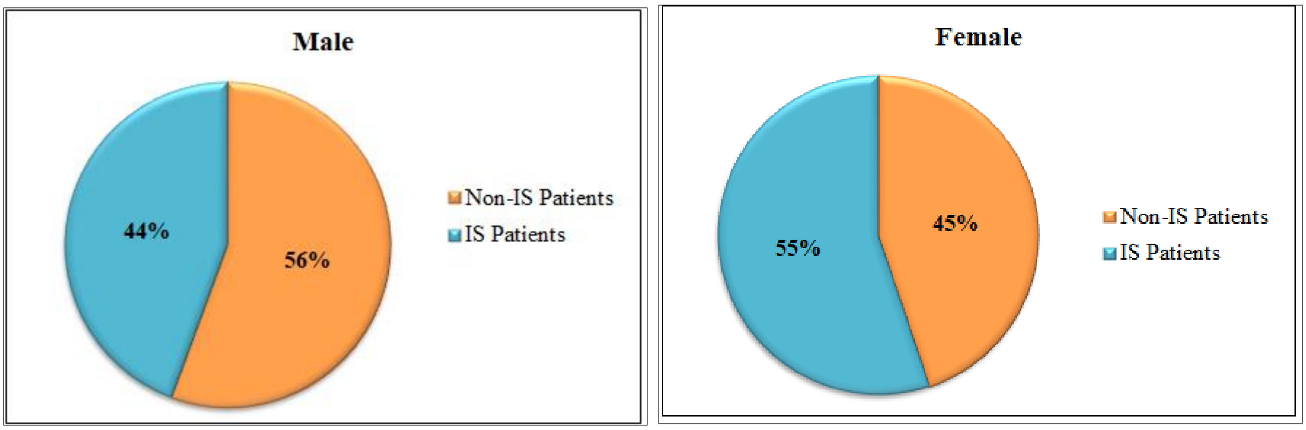

(c) (d)

Fig 3: Demographic details regarding a) Age, b) BMI, c) Gender - Male, and d) Gender - Female

Further, the gathered medical conditions and the impacts of LDL subfractions were analysed amongst the study participants and were documented. In Table 1, the results are displayed.

Table 1: Baseline analysis between IS and non-IS patients

Variables	Obtained data (% and mean ± SD)		*p*-value
	IS Patients (*n* = 43)	Non-IS Patients (*n* = 129)	
Hypertension	37.25	63.91	0.000
Diabetes	17.69	24.64	0.011
Previous cerebral hemorrhage	3.31	0.27	0.126
Previous ischemic stroke	37.42	11.22	0.000
Statin therapy	44.78	50.42	0.248
Pulse pressure (mmHg)	45.70 ± 21.47	51.67 ± 20.75	0.004
CRP (mg/L)	4.01 ± 17.03	4.06 ± 22.07	0.981
FPG (mmol/L)	5.34 ± 1.44	5.81 ± 2.09	0.018
HbA1C	5.59 ± 0.88	5.99 ± 1.26	0.002
Creatinine (μmol /L)	60.07 ± 14.28	64.95 ± 20.57	0.011
Uric acid (μmol /L)	294.61 ± 80.56	311.94 ± 85.93	0.040
Total cholesterol	4.24 ± 1.17	4.40 ± 1.14	0.136
Triglyceride	2.31 ± 1.96	1.98 ± 1.67	0.062
LDL-C (mmol/L)	2.39 ± 0.84	2.59 ± 0.84	0.018
LDL-C1 (mg/dl)	30.14 ± 13.92	24.30 ± 13.48	0.000
LDL-C2 (mg/dl)	23.04 ± 11.19	23.73 ± 10.35	0.498
LDL-C3 (mg/dl)	8.40 ± 6.50	14.02 ± 8.11	0.000
LDL-C4 (mg/dl)	2.69 ± 4.05	7.55 ± 6.99	0.000
LDL-C5 (mg/dl)	0.56 ± 1.73	2.94 ± 4.82	0.000
LDL-C6 (mg/dl)	0.08 ± 0.39	0.45 ± 1.57	0.006
LDL-C7 (mg/dl)	0.06 ± 0.34	0.10 ± 0.57	0.440
HDL-C (mmol/L)	3.25 ± 16.12	1.12 ± 0.28	0.005

The IS patients showed increased health hazards than the non-IS patients based on the population number. In particular, the blood pressure, glucose, previous IS, pulse pressure, FPG, HbA1C, creatinine, and uric acid levels showed Statistical Significance (SS) ($p<0.05$). The CRP, Lp(a), TG, and TC outcomes of both groups expressed more similarity with one another. However, the highest HDL-C concentration and Lp-PLA2 mass mean ± SD values were reported in IS patients (3.25 ± 16.12 and 143.26 ± 15.81, respectively) than in the non-IS patients (1.12 ± 0.28 and 138.26 ± 21.83, respectively).

3.2 Relationship of LDL subtypes with ischemic stroke risk

For analyzing the relation betwixt LDL subtypes along with IS risk, the LR was used. In this evaluation, the IS is the dependent variable, along with the risk features were wielded as the independent variables. In Table 2, the univariate LR outcomes are detailed.

Table 2: Univariate regression analysis

Variables	β-value	WALS-value	OR (95%CI)	p-value
LDL-C1	0.010	0.659	1.01 (0.99–1.03)	0.453
LDL-C2	-0.049	3.704	0.97 (0.91–1.00)	0.062
LDL-C3	-0.083	1.053	0.92 (0.76–1.09)	0.312
LDL-C4	0.196	8.791	1.17 (1.06–1.32)	0.002
LDL-C5	0.276	3.586	1.34 (0.99–1.73)	0.072
LDL-C6	-0.104	0.137	0.91 (0.51–1.60)	0.784
LDL-C7	-0.357	0.249	0.89 (0.45–1.61)	0.694

***OR- Odds Ratio, CI- Confidence Interval**

The LDL-C4 was higher in the IS patients than the non-IS patients (OR (95% CI): 1.17 (1.06–1.32), p-value: 0.002). Yet, the other LDL subtypes didn't depict any SS ($p< 0.05$) between the IS as well as the non-IS patients. Also, various modifications were made for (1) LDL subtypes, (2) sex, (3) age, (4) pulse pressure, (5) hypertension, (6) previous IS, (7) diabetes, along with (8) statin therapy in the multivariate LR evaluation. In Table 3, the results are displayed.

Table 3: Multivariate regression analysis

Variables	β-value	WALS-value	OR (95%CI)	p-value
LDL-C1	0.037	5.842	1.04 (1.01–1.06)	0.013
LDL-C2	-0.058	4.376	0.98 (0.90–1.00)	0.035
LDL-C3	-0.083	0.783	0.93 (0.75–1.11)	0.383
LDL-C4	0.195	10.023	1.22 (1.08–1.38)	0.002
LDL-C5	0.294	3.724	1.16 (1.00–1.80)	0.053
LDL-C6	-0.116	0.136	0.89 (0.47–1.70)	0.768
LDL-C7	0.013	0.002	1.03 (0.54–1.92)	0.954
Gender	-0.483	4.087	0.64 (0.40–0.99)	0.043
Age	0.049	12.578	1.07 (1.02–1.08)	0.000
Pulse pressure	0.006	1.921	1.02 (1.00–1.02)	0.178
Hypertension	0.725	8.417	2.06 (1.26–3.31)	0.004
Previous ischemic stroke	0.796	7.421	2.23 (1.25–3.86)	0.006
Diabetes	0.463	2.164	1.64 (0.85–3.00)	0.184
Statin therapy	-0.272	1.269	0.79 (0.48–1.22)	0.256

Moreover, the results confirmed that LDL-C4 significantly influenced IS risk in patients. The data exposed the OR (95% CI) value as 1.22(1.08–1.38). The reports also proved that the LDL-C4's impact was higher in the IS patients than in the non-IS patients. In addition, gender, age, hypertension, along with preceding IS, depicted differences betwixt the '2' groups. The obtained OR (95% CI) values of these variables were 0.64 (0.40–0.99), 1.07 (1.02–1.08), 2.06 (1.26–3.31), and 2.23 (1.25–3.86), respectively.

3.3 Correlation between clinical characteristics and ischemic stroke onset: Here, for assessing the link betwixt medical conditions as well as IS onset, the PC coefficient was wielded. In Table 4, the results are given.

Table 4: Correlation analysis

Variables	Ischemic Stroke Risk		Result
	Pearson r-value	Significance	
Age	0.194	0.000*	Significant
Gender	-0.093	0.027	Non-Significant
BMI	0.061	0.309	Non-Significant
Hypertension	0.216	0.000*	Significant
HbA1C	0.139	0.004**	Significant
Pulse Pressure	0.114	0.002**	Significant
Creatinine	0.109	0.004**	Significant
Diabetes	0.172	0.012**	Significant
FPG	0.093	0.173	Significant
Uric Acid	0.084	0.052**	Significant
Previous IS	0.18	0.000*	Significant
Previous Cerebral Hemorrhage	0.068	0.083	Significant
TC	0.053	0.129	Non-Significant
TG	0.036	0.074	Non-Significant
CRP	0.001	0.000*	Significant
HDL-C	-0.118	0.000*	Significant
LDL-C	0.098	0.063	Significant
LDL-C1	-0.168	0.000*	Significant
LDL-C2	0.023	0.482	Non-Significant
LDL-C3	0.287	0.000*	Significant
LDL-C4	0.294	0.000*	Significant
LDL-C5	0.226	0.000*	Significant
LDL-C6	0.13	0.008**	Significant
LDL-C7	0.032	0.432	Non-Significant

°Correlation is significant at the level of 0.001* and 0.05** (2-tailed)

The IS risk was positively associated with higher levels of (1) LDL-C3, (2) LDL-C4, (3) LDL-C5, (4) glucose, (5) age, (6) hypertension, and (7) prior ischemic events, with statistically significant correlations ($p < 0.001$). In contrast, IS risk was harmfully allied with lower levels of LDL-C1, along with HDL-C. In addition, HbA1C, pulse pressure, creatinine, uric acid, glucose, and LDL-C6 also showed SS ($p<0.05$).

3.4 Predictive power of LDL-C sub-fractions in ischemic stroke risk

LDL-C sub-fractions' predictive values (LDL-(C1, C2, C3, C4, C5, C6, C7)), in IS risk, were evaluated. The Receiver Operating Characteristic curve (ROC) was created based on the Area Under the Curve (AUC) and was depicted in Figure 4.

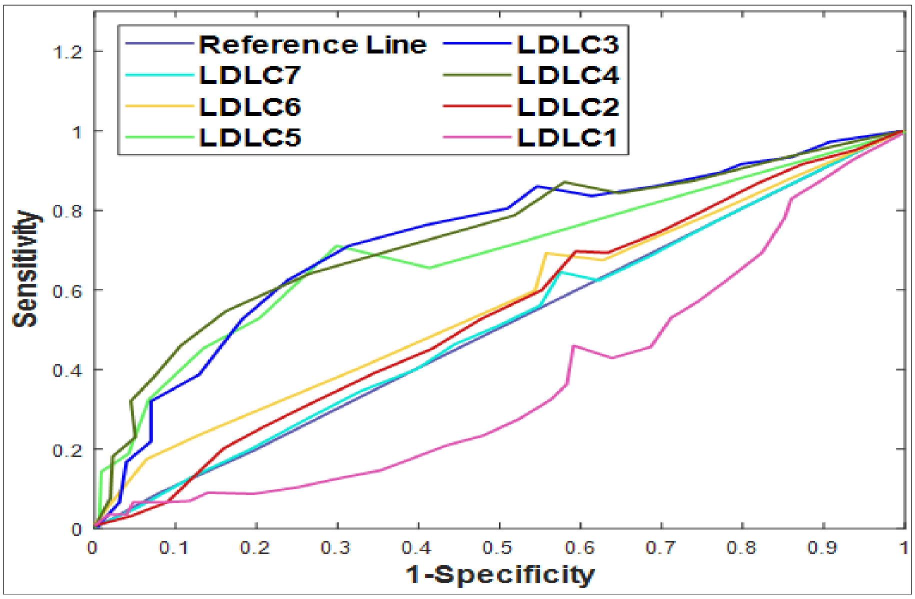

Fig 4: Predictive value of LDL-C sub-fractions for IS risk

Among the seven sub-fractions, the larger area under the curves was exhibited by LDL-C4 (0.847) and LDL-C3 (0.813). The LDL-C1 showed the smallest area under the curves (0.454), and the fractions like LDL-C5 (0.712), LDL-C6 (0.672), LDL-C2 (0.646), and LDL-C7 (0.611) showed moderate curves. In addition, the current analysis explored that the best LDL-C4 cut-off value to envisage IS risk was 7.2 mg/dL with 71.2% sensitivity as well as 90.4% specificity; also, the best cut-off value of LDL-C3 was 5.3 mg/dL with 61.3% sensitivity along with 76.7% specificity.

4. Discussion

Here, examining the predictive power of LDL subfractions in IS risk is the present study's goal. The baseline characteristic studies appraised the role of demographic features, medical conditions, along with LDL subfractions, and proved that the selected study features highly influenced the risk of IS patients than others. Similarly, (Banach *et al.*, 2022) [1] evaluated the relationship between IS risk and LDL-C and proved that LDL-C significantly enhanced IS risk. Furthermore, (Li *et al.*, 2021) [4] also examined the impacts of IS risk using an LR model and concluded that LDL-C, HDL-C, along with TG enhanced the possibility of IS onset. In the present analysis, the PC coefficient method explored the correlation between clinical characteristics and IS onset. Also, the seven LDL-C subfractions' predictive values in IS risk were appraised. The larger area under the curves with specificity and sensitivity was exhibited by LDL-C4 and LDL-C3. Likewise, (Xia *et al.*, 2025; Liu *et al.*, 2021) [13, 7] also assessed the predictive values and generated an ROC curve. The outcomes also exposed that the high levels of sub-fractions were associated with the IS risks and other related health hazards.

5. Conclusion

Here, the connection betwixt LDL subfractions and IS onset is examined. Also, to evaluate the relationship of LDL subtypes with IS risk, both univariate along with multivariate regression analyses were conducted. The LDL-C4 type exposed the highest significant ($p < 0.05$) in both univariate (OR, 95% CI: 1.17, 1.06–1.32) and multivariate studies (OR, 95% CI: 1.22, 1.08–1.38). Also, the PC studies assessed the relation betwixt clinical characteristics as well as IS onset. The positive correlation was exhibited by the (i) LDL-C3, (ii) LDL-C4, (iii) LDL-C5, (iv) glucose, (v) age, (vi) hypertension, and (vii) previous IS ($r > 0.15$, $p < 0.001$). Yet, the LDL-C1 and HDL-C levels depicted a negative correlation ($r = -0.168$ as well as $r = -0.118$). Also, the current analysis explored the LDL-C4 (7.2 mg/dL with 71.2% sensitivity as well as 90.4% specificity) best cut-off value and LDL-C3 to predict IS risk (5.3 mg/dL with 61.3% sensitivity as well as 76.7% specificity). Thus, the present study provided more potential advantages to generate a more precise along with cost-effective predictive tool to appraise the risk of IS in patients. Yet, limited study population selection, short-term data, and unreliable baseline details reduced the accuracy of the findings. Thus, Multiple Time Frame Analysis (MTFA), enriched database sources, and boundless participants should be included in the future to enhance the study effects.

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