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Title: **Clinical Biomarkers of Dyslipidemia in Diabetes Mellitus Type II Patients**

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Clinical Biomarkers of Dyslipidemia in Diabetes Mellitus Type II Patients

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ABSTRACT

Background. This study aims to comparatively investigate the clinical biomarkers of diabetic dyslipidemia in type II diabetes mellitus (TIIDM) patients and healthy controls with no clinical diabetic history.

Method. A cross-sectional study was conducted on 100 TIIDM patients age-matched with 100 control subjects. They were evaluated from January 2021 to July 2022 in Narowal, Pakistan. Various biomarkers including total cholesterol (TL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides were measured for each subject.

Results. The results showed significantly higher levels of TC (238 ± 30 mg/dl), triglycerides (201 ± 72 mg/dl), and LDL (141 ± 47 mg/dl) in TIIDM patients as compared to control groups' levels of TC (167 ± 23 mg/dl), triglycerides (175 ± 75 mg/dl), and LDL (95 ± 30 mg/dl). However, HDL level was significantly lower (46 ± 21 mg/dl) in TIIDM patients as compared to control group (54 ± 27 mg/dl).

Conclusion. The clinical biomarkers of dyslipidemia are effective risk predictors for cardiovascular diseases in low-income countries such as Pakistan.

Keywords: biomarkers, cholesterol, dyslipidemia, lipids, type II diabetes mellitus (TBDM)

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Highlights

- TIIDM patients had significantly higher TC, TRG, and LDL levels.
- Gender-specific differences were observed in lipid profiles of TIIDM.
- TIIDM patients showed significantly lower HDL levels

1. INTRODUCTION

Diabetes is one of the major metabolic diseases caused by abnormal insulin action and secretion. In 2019, about 463 million people were reported to be living with diabetes. This figure constitutes 9.3% of the global population and the number is projected to rise by 25% in 2030 and by 51% in 2045 [1]. According to the Second National Diabetic Survey of Pakistan, 26.3% of the population is affected by diabetes including newly and previously diagnosed patients, with the second highest prevalence in Punjab, Pakistan [2].

Diabetes is primarily categorized into three types, namely (1) TIDM (pancreas don't produce insulin), (2) TIIDM (body cells develop resistance to insulin or less expression of insulin over time), and (3) gestational diabetes (happens during pregnancy) [3,4]. About 49.7% of people around the world are reported to be living with undiagnosed TIIDM, with an average life expectancy decreased by approximately 10 years. In low-income countries, diabetes prevalence peak was found in age group 55-64 years as compared to high-income countries where the prevalence peak occurs in age group 75-79 years (Cho et al., 2018). Risk factors for TIIDM include obesity, sedentary lifestyle, hypertension, smoking, sex, family history, and ethnicity [5-7].

Along with other potential health complications associated with TIIDM (neuropathy, kidney, eye, skin diseases, dementia, and hearing illness), cardiovascular morbidity and mortality are serious risk factors [8-10]. Most of the risk is associated with increased hypertension and dyslipidemia [11]. TIIDM causes dyslipidemia through insulin deficiency or insulin resistance that may co-occur. Diabetic dyslipidemia is characterized by abnormalities in serum lipid profiles of plasma and lipoproteins, such as an increase in LDL, small dense LDL particles, triglycerides, and a decrease in HDL-cholesterol [12]. Changes in lipid profile related to TIIDM are attributed to an increase in fatty acid flux, secondary to insulin resistance, and subsequently, in increased plasma lipids [13]. Lipid abnormalities are two-fold more common in TIIDM patients as compared to the general population. Coronary artery disease (CAD) is broadly reported to be 2-3 times more prevalent in TIIDM male and female patients than in nondiabetic patients [14].

Pakistan is a developing country struggling to cope with the socioeconomic burden of diabetes. This study intends to investigate dyslipidemia biomarkers in TIIDM patients in comparison with the nondiabetic population of District Narowal, Pakistan.

2. MATERIAL AND METHODS

2.1 Sampling Design, Duration, and Location

This cross-sectional study was conducted in the District Headquarters (DHQ) Hospital Narowal, Pakistan from January 2021 to July 2022. District Narowal is located in northeast Punjab, with 1.7 million population reported in the

2017 census. The percentage of men and women in the district is 49.96% and 50.04% (GOP, 2017). Samples were selected from the clinical laboratory of DHQ, Narowal, Pakistan. Ethical approval from the ethical review board and consent from the subjects was taken before sampling.

2.2. Sample Size and Selection Criteria

A total of 200 subjects including 100 diagnosed TIIDM patients and 100 non-diabetic controls in the age group 40-70 years were included. Participants with conditions, such as renal failure, acute cerebrovascular problems, myocardial infections, trauma, acute burns, post-surgery, and pregnancy were excluded. Additionally, individuals currently taking blood pressure or lipid-lowering medications were also excluded.

2.3. Blood Sample Collection and Processing

Blood samples were collected from the TIIDM patients and non-diabetic controls, following the overnight fasting criteria for lipid profile testing. About 3-5 ml of blood from both groups was taken using the vein puncture technique in a yellow gel tube. Figure 1 shows the schematic diagram of sample processing using commercial kits and a Gcare Lipid Analyzer (Green Cross Medical Science).

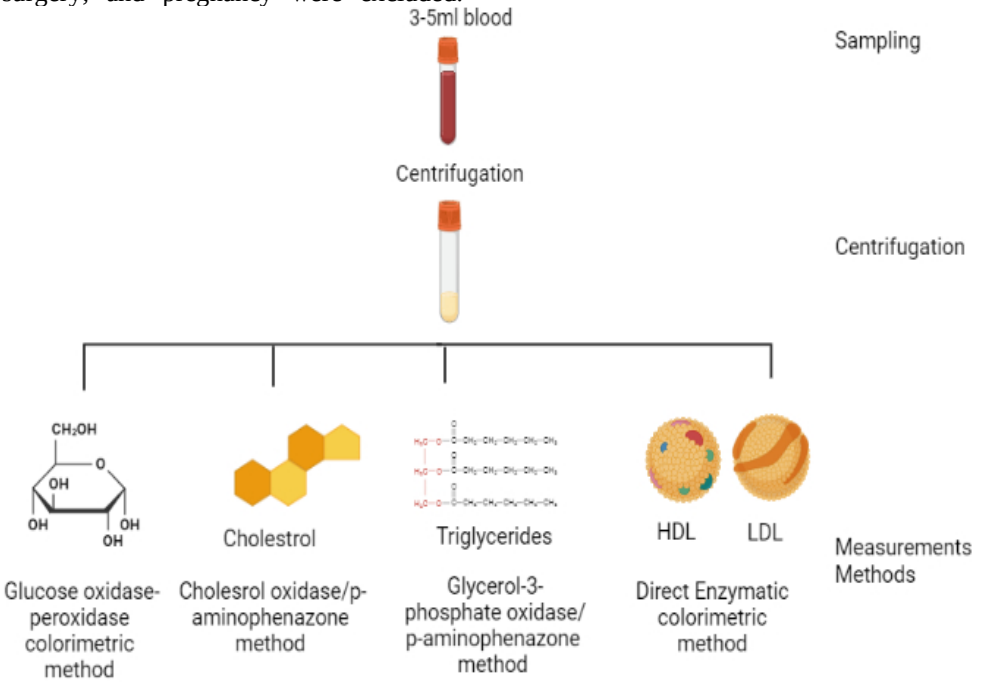

Figure 1. Sample Processing for Lipid Profile Analysis

2.4. Data Analysis

Mann Whitney U test was used for the comparative analysis of non-parametric variables including TC, TRG, HDL, and LDL. The software employed was IBM SPSS (version 25).

3. RESULTS

In this study, the lipid profile of 200 patients was analyzed. Figure 2 presents the percentage of control. Among TIIDM patients participating in the study, about 53% were women and 47% were men. Serum levels of TC, TRG, HDL, and LDL were found to be higher in TIIDM patients as compared to control (Table 1). Figure 3 presents data dispersion and skewness in each group.

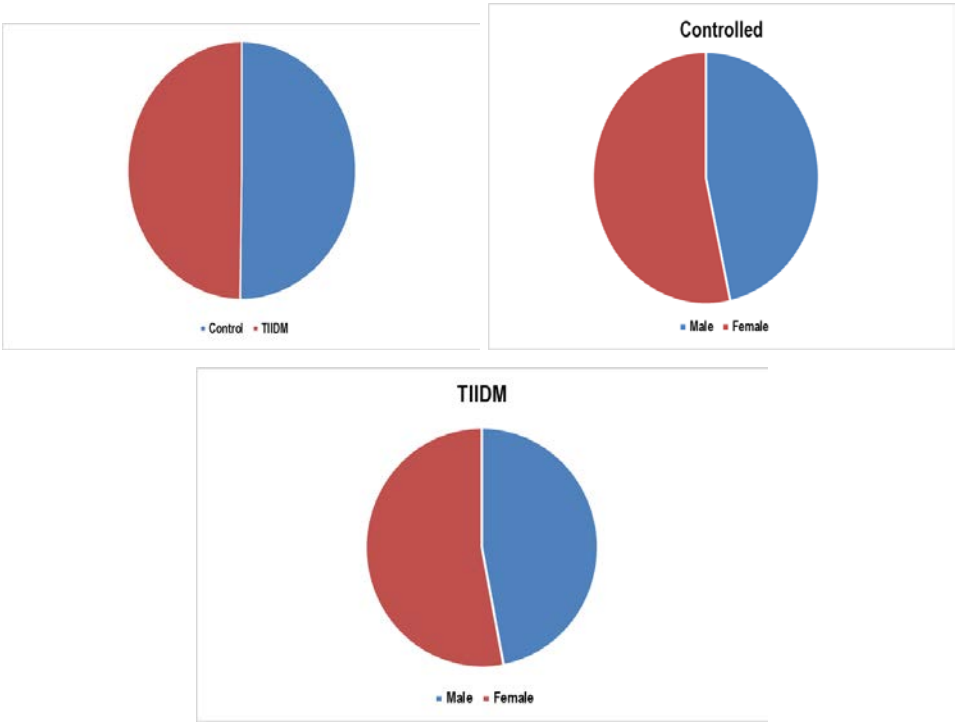

Figure 2. Percentage of Patients a. Control and TIIDM Subjects, b and c. Gender Characterization between Control and TIIDM Subjects

Table 1. Average Concentration of Lipid Profile in TIIDM Patients and Control

Clinical Biomarkers	Group	Sample Mean ± SD	*p* values
TC	TIDM	238 ± 30	0.003
	Control	167 ± 23	
TRG	TIDM	201 ± 72	0.01
	Control	175 ± 75	
HDL	TIDM	46 ± 21	0.002
	Control	54 ± 27	
LDL	TIDM	141 ± 47	0.004
	Control	95 ± 30	

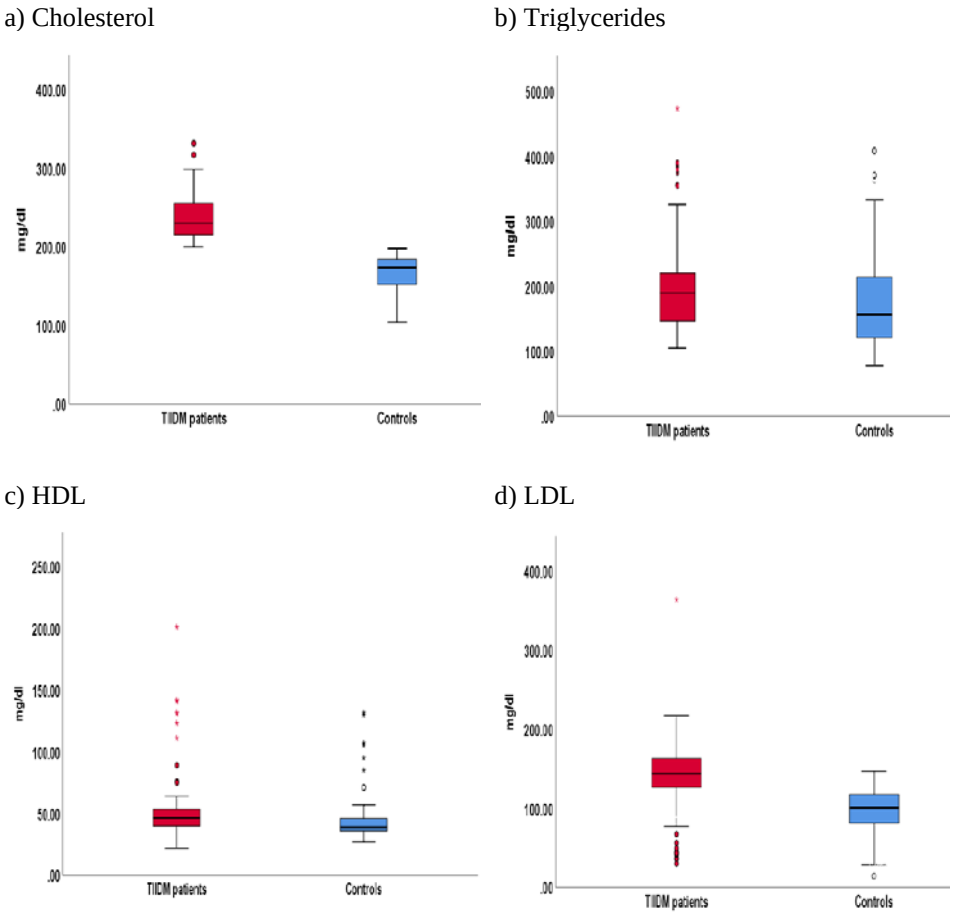

Figure 3. Summary Statistics of Various Lipid Profile Variables **(a)** Cholesterol, **(b)** Triglycerides, **(c)** HDL, and **(d)** LDL in TIIDM Patients and Control

3.1 Serum Cholesterol

According to National Cholesterol Education Program-Adult Treatment Panel III (NCEP, ATP III) defined range of lipid profile, 40% and 60% of TIIDM patients were found to have very high cholesterol and borderline high cholesterol serum concentrations, respectively. The serum concertation levels in TIIDM patients and control ranged from 200 to 332 mg/dl and 104 to 198 mg/dl, respectively (Figure 3). TIIDM patients were found to have statistically higher TC concentrations as compared to control (p= 0.001).

In terms of sex, in TIIDM and control males, serum cholesterol levels ranged from 202 to 299 mg/dl and 104 to 195mg/dl, respectively. Whereas, in TIIDM and control females the levels ranged from 200 to 332 mg/dl and 117 to 198mg/dl, respectively (Figure 4 and Table 2).

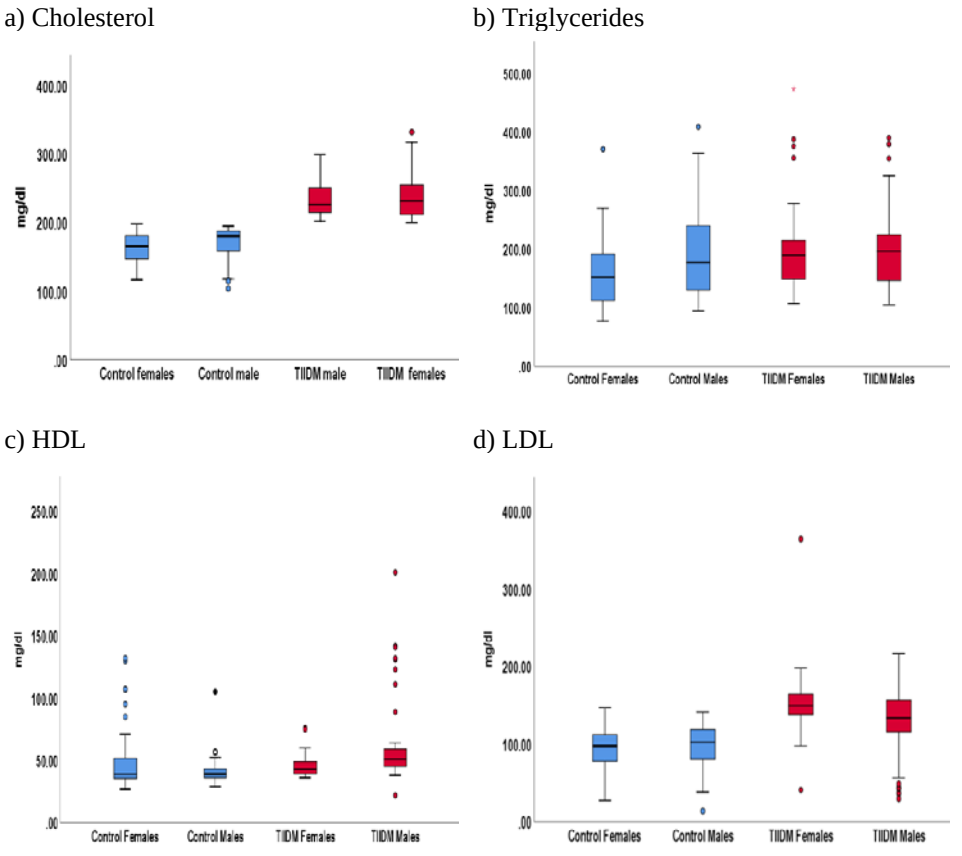

Figure 4. Summary Statistics of Various Lipid Profile Variables **(a)** Cholesterol, **(b)** Triglycerides, **(c)** HDL, **(d)** and LDL in TIIDM Patients and Control

Table 2. Average Concentration of Lipid Profile in Male and Female TIIDM Patients and Control

Clinical Biomarkers	Group	Male	Female
		Mean ± SD	Mean ± SD
TC	TIDM	237 ± 30	237 ± 30
	Control	170 ± 24	164 ± 22
TRG	TIDM	204 ± 73	198 ± 72
	Control	193 ± 82	164 ± 69
HDL	TIDM	63 ± 36	45 ± 09
	Control	42 ± 12	49 ± 25
LDL	TIDM	127 ± 51	152 ± 40
	Control	99 ± 28	92 ± 31

3.2 Serum Triglycerides

Serum triglycerides (TRG) were observed to be high in 41%, borderline in 32%, and desirable in 27% of TIIDM patients. The serum concertation levels in TIIDM patients and control ranged from 105 to 447 mg/dl and 104 to 198 mg/dl, respectively (Figure 3). TIIDM patients were found to have a statistically higher TRG as compared to control (p= 0.002).

In TIIDM and control males, TRG levels ranged from 105 to 390 mg/dl and 95 to 409 mg/dl, respectively. Whereas, in TIIDM and control females the levels ranged from 107 to 474 mg/dl and 78 to 371 mg/dl, respectively (Figure 4 and Table 2).

3.3 Serum HDL

Serum HDL levels were low in 24% of TIIDM patients. The serum concertation levels in TIIDM patients and control ranged from 27 to 132 mg/dl and 22 to 201 mg/dl, respectively (Figure 3). TIIDM patients were found to have statistically lower HDL levels as compared to control (p= 0.001).

In TIIDM and control males, HDL levels ranged from 29 to 105 mg/dl and 22 to 201 mg/dl, respectively. While, in TIIDM and control females the levels ranged from 36 to 76 mg/dl and 132 to 27 mg/dl, respectively (Figure 4 and Table 2).

3.4 Serum LDL

Serum LDL concertation levels were found to be very high in 4%, high in 27%, borderline in 43%, and near-optimal in 14% of TIIDM patients. Moreover, 14% of patients had LDL below the optimal level of 100 mg/dl. The levels in TIIDM patients and control ranged from 30 to 364 mg/dl and 14 to 147 mg/dl, respectively (Figure 3). TIIDM patients were found to have statistically higher LDL levels as compared to control (p= 0.001).

In TIIDM and control males, LDL levels ranged from 30 to 217 mg/dl and 14 to 141 mg/dl, respectively. Whereas, in TIIDM and control females the levels ranged from 41 to 364 mg/dl and 28 to 147 mg/dl, respectively (Figure 4 and Table 2).

4. DISCUSSION

Dyslipidemia is an efficient biomarker of cardiovascular risk and endothelial dysfunction in TIIDM patients. In this study, the clinical biomarkers of TIIDM patients and control were comparatively measured to evaluate the cardiovascular risk to patients. Table 3 shows the ranges of clinical biomarkers defined by NCEP, ATP III. In a study conducted in the KPK province of Pakistan, Khan et al. (2022) recorded a comparatively lower level of 208 + 150 mg/dl [15]. However, this level exceeded the normal range. This study found that TIIDM patients had significantly higher cholesterol levels than control, with 40% of TIIDM patients having higher TC. This finding is inline with a recent study conducted in Islambad, Pakistan where 36% of TIIDM patients were reported to have high risk cholestrol levels [16].

Table 3. Range of Clinical Biomarkers Reported by NCEP, ATP III

TC	
< 200	Desireable
200-239	borderline high
≥ 240	High
TRG	
< 150	Desireable
150- 199	borderline high
200- 499	High
>500	very high
HDL	
< 40	Low
≥ 60	High
LDL	
<100	Optimal

100-129	near optimal
130-159	borderline high
160-189	High
≥190	very high

The current study found triglyceride levels to be significantly higher in TIIDM patients with an average concentration of 201 ± 72 mg/dl, as compared to control with an average concentration of 175 ± 75 mg/dl. Similar levels were reported by [15]. They reported 202 ± 82 mg/dl triglyceride level in TIIDM patients in KPK, Pakistan. Moreover, mean triglyceride levels were found to be higher in men than women in this study, similar to what was reported previously [17] (Table 2). They also reported that 18% of TIIDM patients have desirable levels of triglycerides as compared to the current study (27%). On the contrary, Dalling-thie et al. (2006) reported higher triglycerides in women. Higher triglycerides are associated with coronary artery diseases in TIIDM patients [18].

HDL levels were found to be significantly lower in TIIDM patients as compared to control (Table 2). HDL is regarded as good cholesterol for metabolism in liver. Low levels of HDL are indicative of higher cardiovascular risk in diabetic patients [19,20]. The average observed level (46 ± 21mg/dl) was similar to [15]. They reported HDL level of 42.48 mg/dl in TIIDM patients [15]. Artha et al. (2019) also reported a low HDL level (39.33 ± 4.03) in poorly controlled glycemia [21]. In this study, 24% of patients were found to have a low level of HDL (<40 mg/dl), which is considerably lower than 54% of TIIDM patients in UAE [22]. Similarly, 46% of Algerian diabetic patients were found to have low HDL levels [23]. The above study observed higher mean HDL levels in men (63 ± 36 mg/dl) than women (45 ± 09 mg/dl). However, [24] and [17] reported higher levels in female as compared to male diabetic patients [24].

In the current study, LDL levels were found to be significanlty higher (141± 47 mg/dl) in TIIDM patients than control (95± 30 mg/dl). Compraritively similar levels (148± 113 mg/dl) were reported by Khan et al. (2022) in TIIDM patients [15]. The primary function of LDL is to transport cholestrol from liver to tissues. Biadgo et al. (2015) also reported higher LDL levels (113 mg/dl) in TIIDM patients than control (100 mg/dl) [25]. Serum LDL levels in the current study showed 4% patients as prone to very high risk, 27% to high risk, and 43% to borderline risk. This is line with Chehade et al. (2013) who reported 25% of diabetic patients to be at high risk [26]. On the contrary, Borle et al. (2016) reported 0% patients as prone to very high risk, 6% to high risk, and 22% to borderline risk in India. In this study, male TIIDM patients were found to have higher LDL levels (204 ± 73 mg/dl) as compared to female patients (198 ± 72mg/dl). This is also in line with Borle et al. (2016), who reported higher LDL levels in male patients (116 ± 24 mg/dl) than female patients (111 ± 27 mg/dl) [17]. Diabetetic dyslipidemia is one of the major risk factors of heart diseases caused by increased levels of cholestrol, triglycerides, LDL, and HDL.

4.1 Conclusion

To conclude, the clinical biomarkers of dyslipidemia including TC, TRG, LDL, and HDL serve as effective risk predictors for cardiovascular diseases. The current study revealed a significant dyslipidemia prevalence of approximately 36% among patients with TIIDM. It is imperative to emphasize the importance of routine lipid profile examinations as a preventive measure against cardiovascular diseases in

individuals with TIIDM. Potential advancements in diagnostic technologies, innovative treatment strategies, and lifestyle interventions aimed at mitigating dyslipidemia-associated cardiovascular risks are warranted. By delving into these areas, future research can contribute valuable insights for the development of more effective preventive measures and personalized interventions to maintain cardiovascular health for individuals with TIIDM.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

CONFLICTS OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

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