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Comparative assessments of lipid profile in both the genders of diabetic tribal and non-tribal populations of south-east Rajasthan

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Abstract
The patho-physiology of the link between diabetes and cardio-vascular disease is complex and multi-factorial. The present study was carried out to compare the cardio-vascular risk among tribal womens, tribal men, non-tribal women and non-tribal diabetic subjects of south-east Rajasthan. The lipid values and lipid ratios i.e atherogenic index, atherogenic coefficient, atherogenic lipid profile, cardiac heart disease ratio and cardiac risk ratio of the studied subjects reveal highest cardio-vascular risk among non-tribal men followed by non-tribal women, tribal men and tribal womens. The least cardio risk among tribal women is attributed their non-sedentary dual work including domestic work and rigorous job work. In non-tribal women the domestic work though resembled tribal womens profile but their work was less rigorous and included less physical activity due to which the cardio-vascular risk indices were higher as compared to tribal subjects.In between womens and men subjects the mens are more prone to cardiac maladies as they perform single tone work which goes with sedentary type of life style making them more prone to cardio-vascular risk.

Keywords *- Southeast Rajasthan, Atherogenic index, Atherogenic coefficient, Atherogenic lipid profile, Cardiac heart disease Ratio, Cardiac Risk Ratio*

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I. Introduction

Diabetes type 2 has invaded the lives as an epidemic and has been evaluated as an outcome of sedentary lifestyle. Reduced physical activity, imbalanced and metabolic inhibitory junk food and increased stress have became the non-avoidable parts of modern society and hence given rise to various maladies. In addition, to life style factors Type 2 diabetes is also a genetical disease and therefore its outbreak becomes multi-factorial[1].

Type 2 diabetes is led by a pro-longer period of impaired glucose tolerance or milder disturbances in glucose metabolism. These glycemic disturbances and resistance to insulin leads to increased risk not only for type 2 diabetes but also for cardiovascular morbidity and mortality. According to The DECODE Study Group[2] vascular disorders include both nephropathy and retinopathy, stroke, peripheral vascular disease (PVD) and coronary artery disease (CAD). It also affects the heart muscle, causing both diastolic and systolic heart failure. Though, the etiology of this excess cardiovascular morbidity and mortality is not completely clear. Evidence suggests that hyperglycemia, contributes to myocardial damage after ischemic events, it is clearly not the only factor, because both pre-diabetes and the presence of the metabolic syndrome, even in normo-glycemic patients, increase the risk of most types of CVD[3-4].Therefore, the assessment of cardio vascular risk becomes o primary pre-requisite in diabetic patients. Lipid values i.e total cholesterol (TC), total triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (low density lipoproteins (VLDL) and their ratios i.e Atherogenic index (AI), Atherogenic coefficient (AC), Atherogenic lipid profile (ALP), Castelli‟s risk index I or Cardiac heart disease Ratio (CHDR)and Castelli‟s risk index II or Cardiac Risk Ratio (CRR) provide a pre-alarming signals for the existing cardiac risk and therefore are important parameters for the diabetic patients[5].

Diabetes type 2 has already tolled many lives in India and unfortunately, a very small amount of population is aware about this malady and its complications. India is preoccupied by various communities depending on their origin, occupation and practices which have been classified in population census. In the same lineage, Rajasthan is a state in which south east Rajasthan predominantly harbors many tribes such as Bhil, Bhil Garasia, Dholi Bhil, Dungri Bhil, Dungri Garasia, Mewasi Bhil, Rawal Bhil, Tadvi Bhil, Bhagalia, Bhilala, Pawra, Vasava, Vasave. Bhil Mina. Damor, Damaria. Dhanka, Tadvi, Tetaria, Valvi. Garasia which reside in different pockets of Aravallis[6]. During pre-historic times these tribes were nature dependent and had non-sedentary life style but the entire scenario changed with time. Rajasthan has two entirely different scoops of population one being tribal and other non-tribal. These two populations differ genetically, nutritionally and on

life style scale. Despite differences, the prevalence of diabetes type 2 at alarming rate between both the populations cannot be brushed off and therefore forms the thrust of comparative study.

II. Material and Methods

Diabetic population belonging to age group of 40-50 residing in tribal and non-tribal area of south-east Rajasthan were used in present study.The subjects were studied from primary health centers, general government hospitals and private hospitals with capacity of more than 50 beds from Bhilwara, Chittorgarh and Udaipur districts. The relevant information was obtained in prescribed format and blood samples were obtained through the patients consent.

1. Collection of Blood Samples

Blood samples were collected through venipuncture procedure from subjects of all groups. 10-14 ml blood was drawn and blood collection tubes were arranged in a specific order to avoid cross-contamination of additives between tubes. The tubes were ordered as-

1. First - blood culture bottle or tube (yellow or yellow-black top)
2. Second - coagulation tube (light blue top).
3. Third - non-additive tube (red top)
4. Last draw - additive tubes in this order:

- SST (red-gray or gold top). Contains a gel separator and clot activator.
- Sodium heparin (dark green top)
- PST (light green top). Contains Li-heparin anticoagulant and a gel separator.
- EDTA (lavender top)
- Oxalate/fluoride (light gray top) or other additives

All specimens were legible labeled containing at least two unique identifiers. Tubes were filled to the stated draw volume to ensure the proper blood-to-additive ratio and were followed by centrifugation to separate serum before coagulation.

2. Biochemical Estimation :

2a. Glycated Hemoglobin

The estimation of glycosylated hemoglobin was carried out using Glycosylated hemoglobin kit (Accurex biomedical Pvt. Ltd., Mumbai). Haemolysate was prepared by mixing 0.25 ml lysing reagent (Triton x 100) with sample 0.05 ml and allowed to stand room temperature (25-30˚C) for 5 minutes. For GHb separation and assay the resin tube (CM Sephadex, Sodium Hyroxide) was bought to assay temperature (30˚ C ± 10˚C) and 0.1 ml of haemolysate was added to it. Further, it was positioned in a resin separator in the tube such that the rubber sleeve was approximately 3 cms above the resin level and the contents were mixed on vortex mixer continuously for 5 minutes. The resin was allowed to settle at assay temperature (30˚ C ± 10C) for 50 minutes. The resin separator was further pushed down in the tube until the resin was firmly packed. The supernatant was poured directly into a cuvette and the absorbance was measured at 415 nm against deionized water.

Calculation:

$$\textbf{GHb \%} = \frac{\textbf{Absorbance of GHb}}{\textbf{Absorbance of THb}} \textbf{ X } 4.61(\textbf{Assay factor})$$

(GHb-Glycosylated hemoglobin; THb- Total hemoglobin)

2b. Triglycerides (TG)

Triglycerides are calculated by[7] enzymatic method using *Accurex* diagnostic kit. Sets of test tubes were labelled as ‘test’ (T), ‘standard' (S) and ‘blank' (B).Serum (0.01 ml) was added to T, while standard solution (200 mg %, 0.1 ml) was added to `S'. Enzyme solution (1.0 ml) containing lipoprotein lipase, glycerol phosphate oxidase, glycerol kinase and peroxidase was added to all the tubes.; mixed well and incubated at 37˚C for 3 minutes. The assay mixture was incubated for 10 minutes at 37˚C. After incubation the absorbance was measured of assay mixture against blank at 510 nm (500-530 nm).

Calculation:

$$\textbf{Total triglyceride in mg\%} = \frac{\textbf{Absorbance of sample}}{\textbf{Absorbance of Standard}} \textbf{ X 200}$$

2c. Total cholesterol (TC)

Total cholesterol was determined according to enzymatic method using cholesterol esterase, cholesterol oxidase and peroxidase through *Accurex* diagnostic kit[8]. Sets of test tubes were labelled as `test' (T) ‘standard'

(S) and 'blank' (B). Serum (0.01 ml) was added to T', while standard solution (200 mg %, 0.1 ml) was added to `S'. Enzyme solution (1.0 ml) containing cholesterol esterase, cholesterol oxidase, peroxidase was added to all the tubes.; mixed well and incubated at 37˚C for 3 minutes The assay mixture was incubated for 5 minutes at 37˚C. After incubation the absorbance was measured of assay mixture against blank at 510 nm.

Calculation:-

$$\textbf{Total cholesterol in mg\%} = \frac{\textbf{Absorbance of sample}}{\textbf{Absorbance of Standard}} \textbf{ X 200}$$

2d. High Density Lipoproteins (HDL)

High-density lipoprotein was calculated according to enzymatic method using Accurex diagnostic kit[9]. Three test tubes were labelled as `Test' (T), `Standard' (S) and `blank' (B). Test contains serum supernatant (0.05 ml), while standard solution (50 mg %, 0.5 ml) in 'S'. Enzyme solution (1.0 ml) was added in all test tube *i.e.* test, standard and blank. The supernatant was assayed for HDL cholesterol within 2 hours after centrifugation using working solution of autozyme cholesterol reagent. Assay mixture was incubated for 10 minutes at 37˚C. After completion of incubation, absorbance was measured of assay mixture against blank at 510 nm.

Calculation:

$$\textbf{HDL in mg\%} = \frac{\textbf{Absorbance of sample}}{\textbf{Absorbance of Standard}} \textbf{ X 100}$$

2e. Low Density Lipoproteins (LDL) and very low density lipoproteins (VLDL)

Very low density lipoprotein is approximately one fifth of the sample triglyceride (reference). It is determined from actual content, if a triglyceride level in a samples is 400mg / dL [10].

(VLDL) = Triglyceride / 5

The low density lipoprotein was estimated using the Friedewald method (Friedewald *et al.,* 1972)

Estimated LDL= (total cholesterol) – (total HDL) – (estimated VLDL)

or

LDL-cholesterol = Total cholesterol - (HDL + VLDL)

2e. Lipid ratios- Lipid ratios was obtained to evaluate cardio-vascular risk. Following lipid ratios were obtained as-

- Atherogenic index (AI) = $\log_{10}$ (TG)/HDL,
- Atherogenic coefficient (AC) = (TC-HDL) /HDL),
- Atherogenic lipid profile (ALP) = TG/HDL
- CRI-I or Cardiac heart disease Ratio (CHDR) = (TC/HDL)
- CRI-II or Cardiac Risk Ratio (CRR) = (LDL/HDL)

3 Statistical Analysis

Statistical analysis was carried out using using Erba Transasia auto analyzer. The results were expressed as mean ± SD. The data was analyzed by one-way ANOVA followed by Dunett test. The minimum level of significance was fixed at $p < 0.05$, 0.01 and 0.001.

III. Result and Discussion

Sedentary behaviors cum physical inactivity are among the prominent modifiable risk factors for cardiovascular disease and all-cause mortality specifically maximum in diabetic patients. Physical inactivity is also associated certain cancers, osteoporosis, obesity, type 2 diabetes and hypertension[11].

Sedentary behaviors are associated with cultural linkages. Southeast Rajasthan chiefly occupied by both tribal and non-tribal population's forms the distinct geographical area for the comparative study for lifestyle-mediated diseases as tribals peruse different lifestyle as compared to non-tribal population. In present study, the diabetic patients with HbA1c in between 10.5 to 11.5 % from four different groups i.e. tribal women (TW), tribal men (TM), non-tribal women (NTW) and non-tribal men (NTM) from Bhilwara, Chittaurgarh and Udaipur were selected for the comparative cardio-vascular risk studies. All the subjects were of 40 to 50 years age group and had 5 to 10 years of diabetic history.

The average HbA1c among studied was found to be 11.4% in TW, 11.87% in TM, 10.86 % in NTW and 11.32% in NTM which was regarded to be nearly equivalent in all the groups. Total cholesterol among non-tribal was found to be more as compared to tribal subjects. TC i.e. 196.91 mg/dL was found in NTM followed by NTW (194.12), TM (175.21) and TW (169.36).Though the values of TC in none of the subject was 200.00 mg/dL their TC levels were marginal. The same lineage was also observed in triglyceride values of the studied subjects. HDL values were nearly similar in all the studied groups and were at par to the prescribed standard

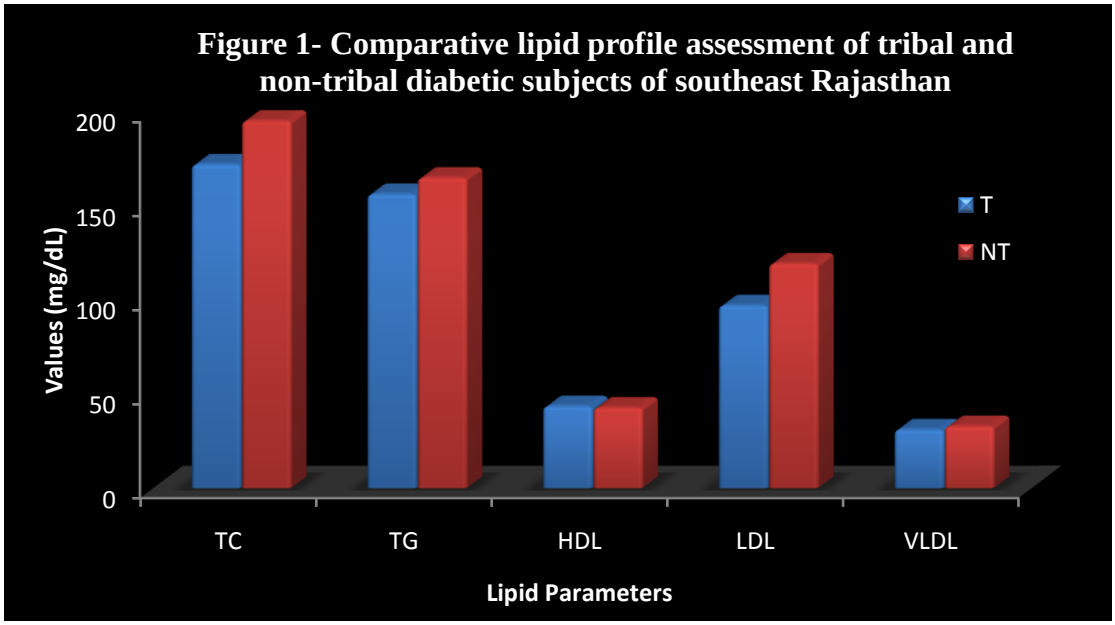
values as about 40 mg/dL but none of the subject had 60 mg/dL. The LDL values in non tribal subjects was found to be 94.9 and 99.73 in TW and TM respectively which was below than a optimal range inscribing lower cardiac risk. VLDL values were nearly equal in TW (31.06), TM (31.54), and NTW (31.88) while in NTM the values was comparatively higher i.e. 34.21 mg/dL. In all the subjects, the VLDL was at borderline of 35.0 mg/dL (Table-1).

Table 1: Comparative assessments of lipid profile in both the genders of diabetic tribal and non-tribal populations of southeast Rajasthan

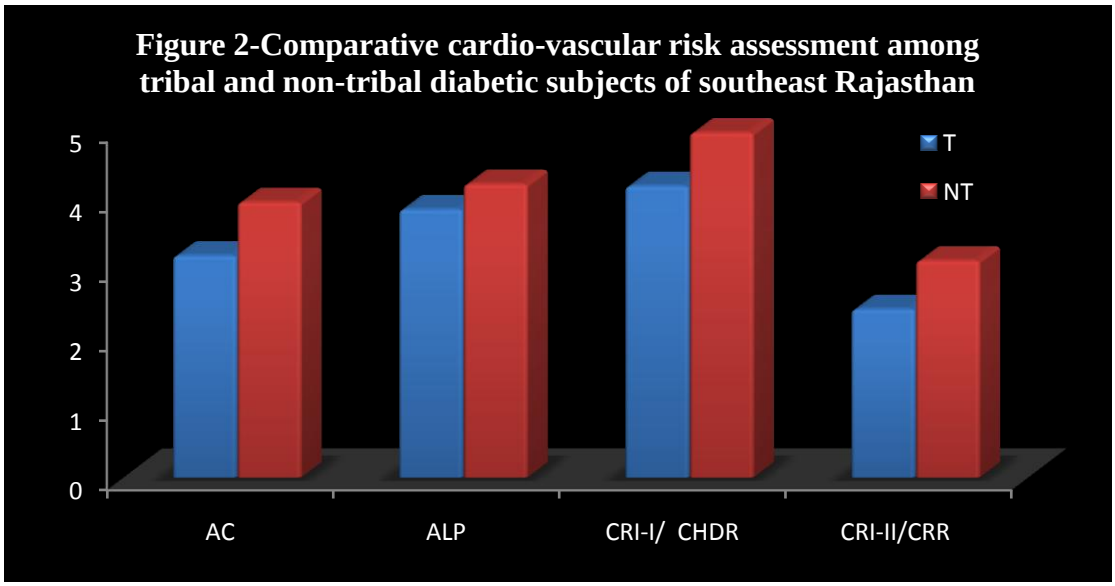
Parameters	TW	TM	NTW	NTM
HbA1c	11.4 ± 0.80**	11.87 ± 0.66*	10.86 ± 0.33***	11.32 ± 0.33**
TC	169.36 ± 0.33	175.21 ± 1.20	194.12 ± 1.00	196.91 ± 1.10
TG	155.33 ± 0.66	157.72 ± 1.66	159.4 ± 1.33	171.07 ± 1.20
HDL	43.4 ± 0.64*	43.94 ± 0.24**	43.24 ± 0.66**	42.58 ± 0.20*
LDL	94.9 ± 1.20	99.73 ± 1.10	119.01 ± 1.10	120.11 ± 0.66*
VLDL	31.06 ± 0.70**	31.54 ± 0.33	31.88 ± 0.80	34.21 ± 0.64
AI	0.05 ± 0.03	0.05 ± 0.01***	0.05 ± 0.01*	0.06 ± 0.01
AC	3.12 ± 0.24	3.27 ± 0.24*	3.85 ± 0.30	4.08 ± 0.14
ALP	3.85 ± 0.54***	3.86 ± 0.80	4.01 ± 0.10*	4.43 ± 0.54***
CRI-I (CHDR)	4.12 ± 0.33*	4.27 ± 0.41	4.85 ± 0.66	5.08 ± 0.33
CRI-II (CRR)	2.35 ± 0.66	2.5 ± 0.54	3.05 ± 0.14	3.2 ± 0.33

TW-Tribal women, TM-Tribal men, NTW-Non-tribal women, NTW-Non-tribal men, HbA1c-Glycated hemoglobin (%), TC- Total Cholesterol (mg/dL), TG- Total Triglyceride (mg/dL), HDL- High density Lipoprotein (mg/dL), LDL- Low density lipoprotein(mg/dL), VLDL- Very low density Lipoprotein(mg/dL). AI- Atherogenic index, AC- Atherogenic coefficient, ALP- Atherogenic lipid profile, CR I(CHDR) -Castelli"s risk index I (Cardiac heart disease Ratio), CR II (CRR)- Castelli"s risk index II (Cardiac Risk Ratio), Values are mean ± SD, level of significance *P* *<0.05; **<0.01; ***<0.001.

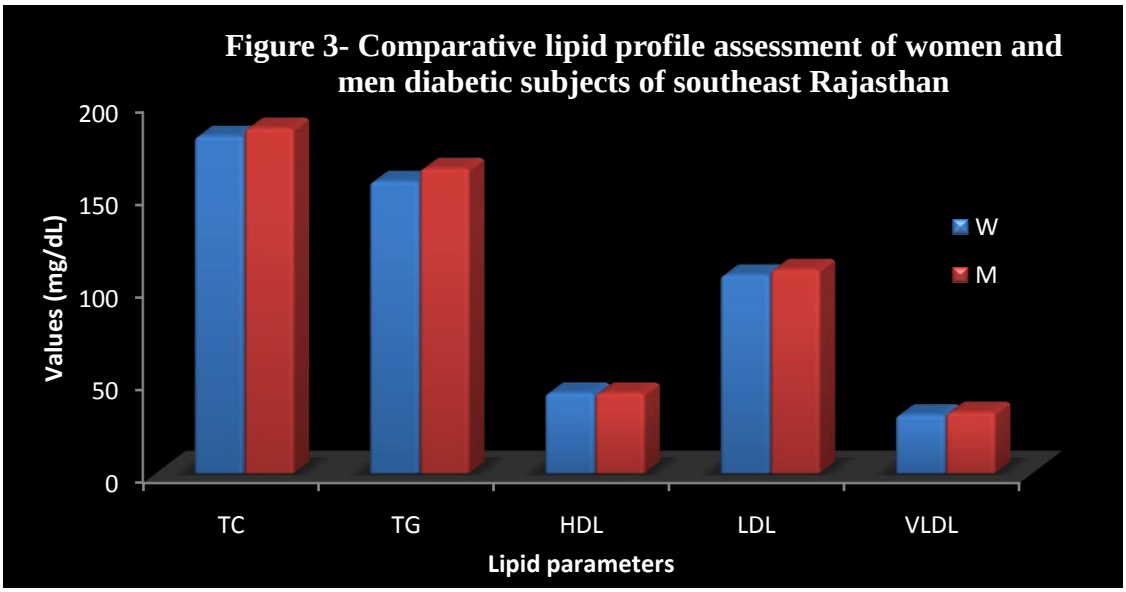
Irrespective of gender, the comparison of all parameters of lipid profile between tribal and non-tribal reveals bad lipid profile in non-tribal subjects as compared to tribal subjects. TC,TG, LDL and VLDL was higher by 13.47%, 5.56 % , 22.87% and 5.56% respectively in non-tribals whereas the good cholesterol i.e HDL was less by 1.74%. Non-tribal subjects were at border line values with respect to TC and VLDL while their LDL values exceeded the normal range. Though tribal subjects bear comparatively better lipid profile still in respect to LDL and VLDL they were at border line (Figure 1).



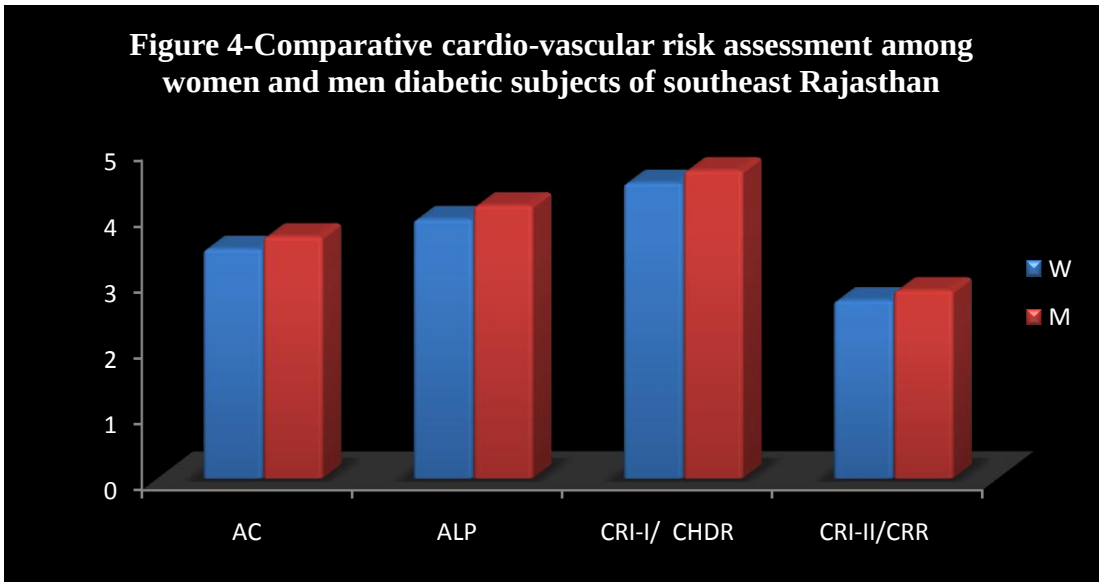
Comparison of cardio-vascular risk indices between tribal (T) and non-tribal (NT) subjects reveals that atherogenic index was equal in both tribal and non-tribal subjects. All the cardio-vascular risk indices were high in non-tribal subjects as compared to tribal subjects. AC of NT subjects was greater by 24.13% while ALP of NT exceeded over tribal subjects by 9.46%. CRI-I/ CHDR of NTS was found to be 4.96 which was 18.37% higher as compared to tribal subjects and CRI-II/CRR of was 28.92% higher as compared to that of TS (Figure 2).



The lipid profile as compared on gender basis reveals better results in women subjects as compared to men. The average TC and TG values were normal in both the subjects as values were far below 200 mg/dL but when both the genders were compared TC in men was higher by 2.38% and TG by 4.47%. HDL was nearly same and differed only by 0.16% though it was 33.33% below the optimal values.LDL and VLDL vales in men subject were higher by 2.78 and 4.45% respectively. LDL values were above optimal range and VLDL at margin to optimal range, revealing disposition for CVD (Figure 3).



Comparison of CVD indices among women(W) and men(M) reveals that except AI, all the CVD indices were higher in men subjects as AC,ALP, CRI-I/ CHDR and CRI-II/CRR by 5.46%, 5.34%, 4.24% and 5.56% respectively. AI was equal in both women and men subjects (Figure 4).



The findings of present study concludes lowest cardiac risk among tribal women's. These subjects were indulged in dual work processes including domestic work and non-sedentary job work as none of the tribal lady was engaged in setting office work. It indicates that non sedentary life style or physical activity is protective against CVD risk[12] and that less-active non-tribal women's chiefly engaged in setting office jobs have a greater associated risk of cardio-vascular diseases[13-16] thus resulting in increased mortality[17-19].

Women's involved in dual activity with special reference to Indian culture protects her from CVD as compared to men's. Among men's sedentary behavior is more obvious in non-tribal populations as compared to tribal's as the job profiles entirely differs in both the subject groups as non-tribal men subjects are more engaged in office work and use self vehicles for the transportation which expose them to lethargic life style[20] (Katzmarzyk et al., 2009). The current study's findings add to the cumulative evidence for the benefits of being physically active despite the presence of other potentially health-diminishing behaviors and conditions. Some of the mechanisms may include adverse alterations to cardiac function, glucose homeostasis, and lipid metabolism[21-23].

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