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ESTIMATION OF SERUM LIPOPROTEIN(a) AND ROLE OF ABNORMAL THYROID PROFILE (T3,T4,TSH) AS PROGNOSTIC DETERMINANT IN YOUNG INDIVIDUALS (<45 YEAR) OF CORONARY ARTERY DISEASE

Physiology

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ABSTRACT

Background: Thyroid hormones modulate every component of the cardiovascular system necessary for normal cardiovascular development and function. When cardiovascular disease is present, thyroid function tests are characteristically indicated to determine if overt thyroid disorders or even subclinical dysfunction exists. As hypothyroidism, hypertension and cardiovascular disease all increase with advancing age monitoring of TSH, the most sensitive test for hypothyroidism, is important in this expanding segment of our population. A better understanding of the impact of thyroid hormonal status on cardiovascular physiology will enable health care providers to make decisions regarding thyroid hormone evaluation and therapy in concert with evaluating and treating hypertension and cardiovascular disease. The goal of this review is to access contemporary understanding of the effects of thyroid hormones on normal cardiovascular function and the potential role of overt and subclinical hypothyroidism and hyperthyroidism in a variety of cardiovascular diseases.

KEYWORDS

Thyroid dysfunction, cardiac output, heart failure, peripheral vascular function, atrial fibrillation, coronary artery disease

INTRODUCTION

Atherosclerosis has emerged as the most important factor for the production of coronary blockade. The risk factors discussed above in someway or other is related to the production of coronary atheroma. Recently genetic predisposition with various gene polymorphism like ACE (Angiotensin converting enzyme) gene, AGT (Angiotensinogen) gene and deranged endothelial nitric acid synthase, elevated level of GP-III etc, have been attributed for atherogenesis. Hyperhomocysteinemia, increased fibrinogen level, infection and inflammatory markers, like high sensitivity C-reactive protein (hs CRP) have also been participated in the etiopathogenesis of atherosclerosis.

Lipoprotein (a) is composed of LDL like particle, to which the lipoprotein (a) specific apolipoprotein (a) is bounded by a disulfide bond. This can be estimated in the serum of patient in the laboratory now.

Abnormal thyroid hormone metabolism especially hypothyroidism is a well known cause of accelerated coronary atherosclerosis. Low T3 syndrome (low total and free T3, normal T4 and TSH) has been emerged as strong prognostic determinant in CAD. CAD patient have increased mortality if associated with low T3 syndrome.

The present study is aimed to evaluate the followings –

i) To assess the level of lipoprotein (a) in the Indian population.
ii) The role of lipoprotein (a) in premature CAD patient.
iii) To assess the level of thyroid hormone (T3, T4 and TSH) level in the Indian population.
iv) The importance of thyroid hormone level in premature CAD patient.

MATERIAL AND METHODS

This study was conducted in the Department of Physiology, data collected from Medicine Department of Patna medical college and hospital, Patna. This hospital is a tertiary referral centre managed by the Health Ministry, Government of Bihar, housing all major specialties. It provides health care services to patients from North Bihar.

CASES

Cases were selected from the wards and outpatient department of the Medicine Patna medical college and hospital, Patna.

Selection criteria for cases were as follows –

A. *Inclusion Criteria -*

1. Age 45 years or less
2. Residents of the area demarcated as Bihar for not less than 20 years.
3. Evidence of ischaemic heart disease from any of the following –
   a. Resting ECG,
   b. Previous Myocardial Infarction,
   c. Previous coronary angiographic evidence of more than 70% narrowing in any major coronary artery, or,
   d. Positive Exercise Treadmill (Stress) Test.

B. *Exclusion Criteria –*

1. Recent intercurrent illness.
2. A known alteration in weight of more than 1.5 kg in the previous month.
3. Congestive cardiac failure.
4. Recent or on-going therapy with lipid altering drugs like clofibrate or statins.
5. Recent or on going therapy with thyroid hormone altering drug like amiodarone or radioactive iodine
6. Any major illness in the last three months.

CONTROLS

Controls were selected from the normal population and from those healthy adults who presented for routine health check-ups at the Medicine Department. These controls had a fulfill the same age and residence criteria as cases, and were carefully screened for any underlying medical, surgical or psychiatric illness. The absence of ischaemic heart disease in the control population was confirmed by a negative exercise treadmill test, after a normal resting ECG. Same exclusion criteria, as the cases, were applied to the control population.

CLINICAL EVALUATION

All cases and control were subjected to the following clinical protocol –

1. A detailed history with special reference to effort angina, exertional breathlessness, weight gain, smoking, appetite, bladder and bowel habit, contraceptive pill use, and past medical events.
2. A thorough scrutiny of available medical records.
3. Detail clinical examination with particular attention to blood pressure, evidence of cardio-respiratory discomfort, dependent oedema and waist-hip ratio (WHR).
4. A biochemical work-up including fasting blood sugar (FBS), blood urea, serum creatinine, serum total and direct bilirubin and lipid profile including total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL cholesterol and triglycerides.
5. Chest X-ray (PA view) and resting ECG for all cases and controls, followed by exercise treadmill test for all control subjects and those cases without frank ischaemic changes on the resting ECG.

MEASUREMENT OF WAIST HIP RATIO (WHR)

The common measuring tape was used to measure the WHR. The waist circumference was measured at the smallest diameter between the costal margin and the iliac crest. The hip circumference was measured at the greatest diameter over the buttocks. The ratio obtained by dividing the waist circumference by the hip circumference was the value of the WHR.

MEASUREMENT OF FASTING BLOOD SUGAR (FBS)

Blood samples were obtained after an overnight fast and ultracentrifuged to obtain plasma. These were subjected to the orthotoluidine test, which is the only chemical method widely used till today. It is based on the condensation of aldosaccharides, such as glucose, with an aromatic amine and glacial acetic acid. The stable green color that develops is measured spectrophotometrically.

The lack of accuracy with this method stems from the reactivity of galactose and mannose, as well as lactose, maltose, sucrose and fructose, to a lesser extent, with ortho-toluidine. Hence values with this method are slightly higher than for more specific enzymatic methods.

MEASUREMENT OF THYROID HORMONE

Serum T3,T4 and TSH measured by Radioimmuneassay technique. Blood samples were obtained from vein after overnight fasting.

ESTIMATION OF SERUM LIPOPROTEIN (a)

In our analysis we used immunonephelometric assay for the quantitative in vitro determination of lipoprotein(a) in human serum.

Test Principle

Immunonephelometric assay consists of a suspension of polystyrene particles coated with murine monoclonal antibodies to lipoprotein(a). The concentration of suspended particles is optimal for agglutination measurement by nephelometry. When reagents are mixed with samples containing lipoprotein (a), the intensity of the scattered light in the nephelometer depends on the lipoprotein content of the sample. In this way, the lipoprotein concentration was determined by comparison with dilutions of a standard of known concentration.

The instrument used was Dade Behring BN II Nephelometer.

CUT OFF VALUES FOR DIAGNOSES

1. **Smoker -**

Only present smokers were categorized as smokers, while both former smokers and never smokers were grouped under non-smokers.

2. **Hypertension**

Patients on antihypertensive medications with a documented history of hypertension were classified as hypertensives. Those not in this category were clinically examined, and a systolic blood pressure 140 mmHg or a diastolic blood pressure 90 mmHg, were also classified as hypertensives, as per the Seventh Report of The Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC VII criteria).

3. **Abdominal Obesity**

The diagnosis of abdominal or truncal obesity was made at WHR 0.92, a value advocated by Pais et al (1996) from south India.

4. **Diabetes Mellitus**

The diagnosis of diabetes mellitus was made with a FBS 126 mg/dl, in accordance with the 1997 American Diabetes Association Criteria.

5. **Hypercholesterolemia**

Total cholesterol > 200 mg/dl.

6. **Hypertriglyceridemia**

Estimated triglycerides > 150 mg/dl.

7. **Low HDL cholesterol**

HDL cholesterol < 40 mg/dl.

8. **Elevated LDL cholesterol**

Estimated LDL cholesterol > 130 mg/dl.

9. **Lipoprotein (a)**

The cut off value in our study is > 30 mg/dl (>0.30 g/l).

DATA PROCESSING

The data collected was tabulated and analysed to asses the differential prevalence of Lipoprotein (a) [Lp(a)] as a major risk factor among cases and controls.

Statistical functions that were calculated include the mean, standard deviation and Odds Ratio. The Chi square test was employed to calculate the acceptability of the Null Hypothesis from the derived p value.

Prevalence percentage were plotted on bar graphs. Statistical analysis and graphical representations were obtained from the Microsoft Office Program on an IBM compatible computer.

OBSERVATION

RISK FACTOR DISTRIBUTION (TABLE - 1)

Though the main emphasis in this study was on Lipoprotein(a) [Lp(a)] and thyroid profile but other risk factors also analysed in this study were increased waist hip ratio (WHR), smoking habit, hypertension, diabetes mellitus and lipid abnormality.

Of these an increased Lp(a) and an increased WHR (a marker of abdominal obesity), were found to be the most determinative of coronary risk. While 60% had abdominal obesity and 80% had an elevated Lp(a) in the case group, the values were 10% and 22.3% among controls, respectively. Calculation of Odds Ratio (OR) yield markedly high values, while the Chi Sqaure test, at one degree of freedom proved these observations to be valid at very high levels of statistical significance (P<0.001).

Low Plasma T3 level were found to be more commonly associated with CAD.53% among cases and 20% among controls had decreased plasma T3 level. No significant relationship of plasma T4 and TSH level with CAD. Normal plasma free T3 level taken as 2.0-4.4pg/ml.

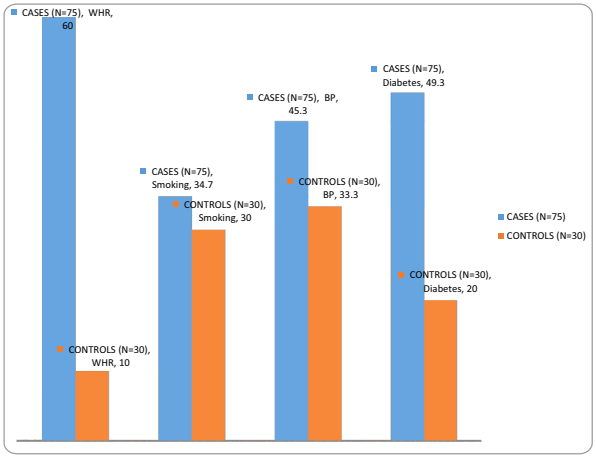
Diabetes mellitus emerged as an important risk factor, predisposing our subjects to CAD. With 64% of cases and 15.2% of controls confirming a diagnosis of diabetes, the calculated p value for this risk factor was < 0.001, documenting a substantially high level of statistical significance. Diabetes mellitus is now being considered a coronary risk equivalent according to the National Cholesterol Education Programme, Adult Treatment Panel – III (ATP - III) guidelines 2001, leading to a 10 year risk of CAD equal to 20%.

Hypertensive status and smoking habit were less important predictors of coronary risk in the population studied. Elevated blood pressure was seen in 45.3% of cases and 33% of controls, though the prevalence of hypertension was high among cases, but the number of controls showing an elevated blood pressure was also large thereby making hypertension a less important predictor of coronary risk in this study. Nevertheless, hypertension is an important risk factor for CAD as approved by various other studies. Smoking habit was seen in 35% cases and 30% controls and seemed to be unrelated with this excess risk, with comparable prevalence values.

TABLE – 1

CLINICAL CHARACTERISTICS CASES VERSUS CONTROLS

	CASES (N=75)	CONTROLS (N=30)	OR	P
↑ WHR	45 (60.0%)	3 (10%)	8.27	< 0.001
Smoking	26 (34.7%)	9 (30%)	1.73	NS
↑ BP	34 (45.3%)	10 (33.3%)	1.66	NS
Diabetes	48 (49.3%)	5(20.0%)	8.9	< 0.001



LIPID ANALYSIS (TABLE – 2)

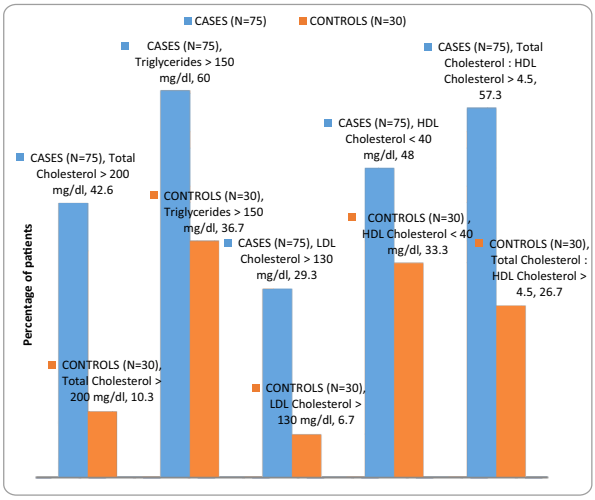
All abnormalities were very significantly associated with CAD. While multiple abnormalities produced p values of statistical significance, the values of decreased HDL cholesterol could not achieved statistical significance, through it was decreased in 48% of cases. This was probably due to the fact that a large number of controls 33.3% had a low HDL value, showing its importance in people without CAD and predisposing them to it.

The commonest lipid abnormalities among the cases were hypertriglyceridemia (60%) and an accentuated ratio of total

cholesterol : HDL cholesterol (57.3%). This was followed by decreased HDL cholesterol (48%), increased total cholesterol (42.6%) and increased LDL cholesterol (29.3%). All the above parameters were significantly associated with CAD (Table - 5).

TABLE – 2
LIPID ABNORMALITIES : CASES AND CONTROLS

	CASES (N=75)	CONTROLS (N=30)	OR	P
Total Cholesterol > 200 mg/dl	32 (42.6%)	4 (10.3%)	4.84	<0.01
Triglycerides > 150 mg/dl	45 (60.0%)	11 (36.7%)	2.59	<0.05
LDL Cholesterol > 130 mg/dl	22 (29.3%)	2 (6.7%)	5.81	<0.02
HDL Cholesterol < 40 mg/dl	36 (48.0%)	10 (33.3%)	1.84	N.S.
Total Cholesterol : HDL Cholesterol > 4.5	43 (57.3%)	8 (26.7%)	3.69	<0.01



DISCUSSION

This study is prospective case control study aimed at identifying the relationship of Lp(a) and abnormal thyroid profile with CAD (Coronary Artery Disease).

DISCUSSION OF METHODOLOGY

This study was conducted at a tertiary referral hospital serving a large population block, dominated by rural residents. Diagnosis of CAD was done by fresh ECG/Enzymes/Stress Tests, so the problem of previous misdiagnoses avoided. Moreover, our biochemical work up was based on data obtained after six weeks of any acute event to avoid the possibility of altered serum values during stress episodes.

The case selection was based on strictly to the inclusion and exclusion criteria, detailed earlier. A long permanent residence in the area of today's Bihar is selected as a case. Much evidence exists on the role of genetics and environment on risk of CAD, and a 30 years residence in this area will have the desired impact on life-style and other factors.

The categorization of subjects (cases and controls) into risk factor positive or negative was based on appropriate internationally accepted cut off values. For the diagnosis of the abdominally obese, the upper limit of a normal WHR was < 0.92, a value more suited to the Indian population. However, the values for hypertension (SBP 140, DBP 90 mmHg), diabetes (FBS 126 mg/dl), hypercholesterolemia (>200 mg/dl), hypertriglyceridemic (> 150 mg/dl) and decreased HDL (< 40 mg/dl) are all accepted worldwide. The cut off value of < 4.5 in the diagnosis of elevated total cholesterol: HDL cholesterol ratio has been specifically set at a level to accommodate more than an average risk of developing CAD in either sex. Earlier workers have suggested that a value higher than 4.88 in males and 4.23 in females carried an excess risk of CAD than the average population risk.

The cut-off value for lipoprotein (a) [Lp(a)] was set at > 30 mg/dl, which is in accordance with the internationally accepted cut-off value. An immuno-nephelometric assay was used for the determination of Lp(a) values which were calculated by automated clinical chemistry analyzers.

To avoid inter-observer variation, the same researcher performed all clinical assessments and interpretations (measurement of blood pressure and WHR, ECG and ETT data analysis

DISCUSSION OF RESULTS

Our study shows that Lp(a) is a major contributor to CAD in this region, with a younger age of onset as other factors like abdominal obesity, diabetes mellitus , dyslipidemias and thyroid profile also analysed in this study. This is cleared from the recent findings that abdominal obesity, insulin resistance, dyslipidemia and hypothyroidism are also important predisposing risk factors in CAD. Moreover, the pattern of lipid abnormalities in our patients with hypertriglyceridemia, decreased HDL and increased total cholesterol : HDL ratio as the dominant abnormalities, are in accordance with the characteristic finding of diabetic dyslipidemia. These findings shows the typical lipid profile seen in the Indian population in other studies.

Lipoprotein (a) [Lp(a)] has come out as an important risk factor for CAD in this study and also contributing to the Indian paradox which revolves around the issue of paradoxically low rate of conventional risk factors, in the face of high rates of CAD in Indian worldwide. The serum Lp(a) levels were raised in a large number of cases with only a normal or slightly increased levels of other lipid parameters indicating the significance of Lp(a) in early onset of CAD. This also indicates that this genetically determined factor is more common in Indians and Asian Indian as is indicated in other studies on Lp(a). It also confirms that it is much more atherogenic than LDL.

The mean Lp(a) levels in our study among the cases were 61.18 mg/dl and that among controls were 28.1 mg/dl. Though the normal Lp(a) levels are 7 mg/dl, studies have shown that the Lp (a) levels in Asian Indians, vary between 15 mg/dl to 60 mg/dl which may go up to 100 mg/dl in a few cases, thus predisposing them to increased risk of CAD. In our study the Lp(a) levels ranged from 19 mg/dl to 172 mg/dl among cases and from 19 mg/dl to 62 mg/dl among controls, thus being consistent with other studies regarding the Lp(a) levels in Indians.

Only a few isolated cases had Lp(a) levels > 150 mg/dl. The interesting finding in such cases was the presence of multiple risk factors, like abnormal lipid profile, abdominal obesity and insulin resistance. One of the control's showed a value of 62 mg/dl, but this was an isolated case and apart from having diabetes mellitus, there was no other risk factors, thus confirming the genetically high levels in a few Indians without CAD, as has been shown previously. The higher levels of Lp(a) in a few controls when compared to other controls was associated with a decreased level of HDL in the controls with a higher value of Lp(a).

It was observed that the Lp(a) levels among cases with multiple risk factors were higher in comparison with those without them indicating that multiple risk factors potentiate the action of Lp(a) in causing CAD. A few cases which had Lp(a) levels in the normal range also showed higher HDL levels.

A large number of our cases had an isolated rise in Lp(a) levels, without altered lipid profile, though they had a low HDL level. This finding was also consistent with other studies which show that Lp(a) confers an increased risk of CAD and demonstrate that Lp(a) is an independent risk factor for this disease.

Most of the patients also present with abnormal thyroid profile, especially low fT3 level in this study.Multiple risk factors like abdominal obesity,hypertriglyceredemia and insulin resistance also associated in patients of low fT3 level.

LACUNAE

Among the many short comings of this study, a small size of the sample and absence of angiographic corroboration of our diagnosis, demand particular mention. Moreover, our lack of consideration for other newer risk factors like elevated homocysteine levels, hyperfibrinogenemia and our avoidance of multivariate analysis, affected the objectivity of our findings.

Closer to our data, the clumping together of former smoker and never smokers, in the same group was a drawback. The use of a chemical

method for glucose estimation is probably not representative of a modern methodology. The performance of a glucose tolerance test (GTT) could possibly have further sensitized our diagnostic protocol towards the detection of glucose intolerance.

SUMMARY AND CONCLUSION

From this study we have come to the following conclusion –

- Age of CAD onset in this area is lower than that in the Western world, but similar to the Indian trend.
- Lipoprotein (a) [Lp(a)] is the most significant risk factor in the present study conferring an increased risk of CAD.
- Hypothyroidism,especially with low T3 level is an important risk factor for CAD in this study.
- Abdominal obesity, abnormal lipid profile and diabetes mellitus are other important risk factor for CAD in this study.
- This study also proves that genetically determined risk factors are important predictors of CAD in Indians and studies regarding other new risk factors like elevated homocysteine levels, hyperfibrinogenemia should be carried out to put into proper perspective, preventive strategies and intervention priorities.

REFERENCES

1. Bard JM, Delattre-Lestavel S, Clavey V, Pont P, Derudas B, Parra Hj, Fruchart JC : Isolation and characterization of two subspecies of Lp(a), one containing apo E and one free of apo E. Biochem Biophys Acta 1992; 1127:124.
2. Boerwinkle E, Menzel G, Kraft HG, and G. Utermann. 1989. Genetics of the quantitative Lp(a) lipoprotein trait III. Contribution of Lp(a) glycoprotein phenotypes to normal lipid variation. Hum. Genet. 82:73-78.
3. Tracy RP, Psaty BM, Macy E, Bovill EG, Cushman M, Cornell ES, et al. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and sub-clinical disease in healthy elderly subjects. Arterioscler Thromb Vosc Bioi 1997; 17:2167-2176.
4. Tyrrell HJ, Cooke T, Reilly M, Colgan M, Moore D, Shanik DG, Bergin C, Freely J : Lipoprotein(a) and peripheral vascular disease. J Intern Med 1992; 232:349.
5. McCall MR, Van den Berg JJ, Kuypers FA, et al. Modification of LCAT activity and HDL structure. New links between cigarette smoke and coronary heart disease risk. Arterioscler Thromb 1994; 14:248.
6. Kannel WB. Blood pressure as a cardiovascular risk factor. JAMA 1996; 275 : 1571-1576.
7. Kurth HS : The fate of lipoprotein cholesterol entering the arterial wall. Curr Opin Lipidol 8: 246-252, 1997.
8. Strachan DP, Mendall MA, Carrington D, Butland BK, Yarnell JWG, Sweetnam PM, et al. Relation of Helicobacter pylori infection to 13-year mortality and incident ischaemic heart disease in the Caerphilly Prospective Heart Disease Study. Circulation 1998; 98 : 1286-1290.
1. 9.The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, JAMA May 21, 2003, Vol. 289, No. 19.
10. Ornek E,Murat S,Duran M,Turfan M,Kurtul A,Demircelik MB,et al:The relationship between lipoprotein(a) andcoronary artery disease,as well asits variable nature following myocardial infarction. Clin Investig Med.2011;34(1): E14-E20.
11. Koschinsky M,Marcovina SM. Lipoprotein(a) In: Ballantyne C, editor. Clinical lipidology: a companion to Braunwald's heart disease.Philadelphia: Saunders Elselvier;2009.pp.130-143.
12. Tsimikas S,Hall JL.Lipoprotein(a) as a potential causal genetic risk factor of cardiovascular disease.J Am Coll Cardiol.2012;60:716-721.doi:10.1016/j.jacc. 2012.04.038
13. Bruckert E,Labreuche J,Amarenco P.Meta-analysis of the effect of nicotinic acid alone or in combination on cardiovascular events and atherosclerosis. 2010;210:353-361.doi:10.1016/j.atherosclerosis.2009.12.023.
14. Report of the Expert Committee on Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2000; 23 (Suppl. 1) S4-S19.
15. Merki E,Graham MJ,Mullick AE,Miller ER,Crooke RM,Pitas RE,et al.Antisense oligonucleotide directed to human apo B-100 reduces lipoprotein(a) levels and oxidized phospholipids on human apolipoprotein B-100 particles in lipoprotein(a) transgenic mice. Circulation 2008;118:743-753.
16. Hak, A.E.et al. Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in eldery women: the Rotterdam Study. Ann. Intern. Med. 132,270-278(2000).
17. Razvi,S.,Weaver,J.U.,Vanderpump,M.P & Pearce, S.H. The incidence of ischaemic heart disease and mortality in people with subclinical hypothyroidism: reanalysis of the Whickham Survey cohort.j.clin. Endocrinol. Metab.95,1734-1740(2010).
18. Cappola, A.R. et al. Thyroid status, Cardiovascular risk, and mortality in older adults. JAMA 295,1033-1041(2006)
19. Park K. Epidemiology of Chronic Non-communicable diseases and conditions. In : Park K, Ed. Park's Textbook of Preventive and Social Medicine, 23rd Edition. Jabalpur : Banarsidas Bhnot Publisher, 2015:270-304.