Study of lipid profile in type -2 diabetes mellitus patients in Agra city"

K.P. Mishra¹, Alok Mawar², Suchi Singh³ and Pawan Kare⁴

1-2-3-4 Department of Biochemistry, S.N. Medical College& Hospital, Agra (U.P)

(Received : July, 2013 : Revised : August, 2013; Accepted : October, 2013)

Abstract

The present study on "lipid profile among type 2 diabetes mellitus patients in Agra population" was conducted. For this, 40 clinically established hyperglycemic patients suffering from type-2 diabetes mellitus, attending the O.P.D of S.N. Medical College & Hospital, Agra were selected under study. The result was compared with 50 age matched healthy, non-obese, non- diabetic and normotensive control subjects. The aim of the present study was to evaluate the lipid profile and assess the risk of C.V.D complications in the type-2 diabetic patients in Agra city. It was found that total- cholesterol, triglyceride and LDL cholesterol were significantly higher and HDL cholesterol was significantly lower in diabetic patients than in non diabetic control subjects. A significant increase in fasting blood sugar, total- cholesterol and triglyceride and LDL levels was observed in diabetic male patients when compared with the healthy non diabetic control males except VLDL and HDL levels. However, in diabetic females, a significant increase in fasting blood sugar, totalcholesterol, LDL cholesterol, HDL cholesterol and triglyceride level was observed when compared with the healthy non diabetic female control subjects without any change in VLDL level. No significant change was observed in fasting blood sugar, total-cholesterol, HDL, triglyceride and VLDL levels in diabetic male patients when compared with the diabetic female patients, except for HDL levels which were significantly higher in female diabetic patients as compared to male diabetic patients. From this study, it is evident that diabetes mellitus has a real impact on lipid metabolism. This was substantiated by the fact that all the lipid fractions were elevated in diabetics when compared to the healthy non diabetic control subjects.

Key words:

Type-2 Diabetes Mellitus, Fasting Blood Sugar, Hyperglycemia, hyperlipidemia and hyper triglyceridemia

Inroduction

Diabetes Mellitus is a clinical syndrome associated with an abnormally high plasma glucose concentration. There are two main types of diabetes mellitus, (i) type-1, Insulin dependent diabetes mellitus, which occurs when there is severe lack of insulin due to the destruction of most or all of the betacell in the islet of langerhans. (ii) type -2, Non-insulin dependents diabetes mellitus, which occurs when the body does not produce enough insulin or the insulin that is produced is less effective. Type-2 DM usually appears in people over the age of 40 years (maturity onset). In most cases, glucose level in the blood can be controlled by diet and drugs. About 90% of diabetes are non- insulin dependent *(Chatterjee M N et.al 2005)*.

Corresponding author's e-mail : kpmishraindian@gmail.com

Published by Indian Society of Genetics, Biotechnology Research and Development, 5, E Biotech Bhawan, Nikhil Estate, Mugalia Road, Shastripuram, Sikandra, Agra 282007

Onlince management by www.isgbrd.co.in

Diabetes mellitus arises when insufficient insulin is produced, or when the available insulin does not function properly .Without insulin, the amount of glucose in the bloodstream is abnormally high, causing unquenchable thirst and frequent urination. The body's inability to store or use glucose causes hunger and weight loss (*Chatterjee CC et.al*, 1992).Insulin deficiency causes excessive metabolism of free fatty acid. This may lead to the disorders in lipid metabolism. Insulin also affects lipid metabolism (*WHO*, 1995).

There are probably 100 millions people in the world with diabetes, and the figures are on the rise. As diabetes progresses, lipid metabolism is affected and the patients are on increased risk of developing coronary artery diseases. (Godkar P et. al, 2003).Coronary artery disease represents a wide spectrum from angina pectoris, myocardial infarction and sudden death to silent myocardial ischemia (Mozaaffrian D et.al, 2006). Silent myocardial ischemia has a reported prevalence of 10-20% in diabetic population as compared to 1-4% in non- diabetic population (Ronald M et.al, 2004).

Cardiovascular disease is the major cause of morbidity and mortality in both men and women with diabetes (approximately 60-70% of death). The risk of cardiovascular disease is increased approximately 2 fold in men and 3-4 fold in women (*Hong D, Chris R et. al, 2005*).

It has been concluded that silent myocardial events are quite common in diabetes (*Samia J et.al 2000*). In patients with type-1 diabetes in good glycemic control, the lipid profiles is very similar to lipid profiles in general population in contrast, In patients with type-2 diabetes, even when in good glycemic controls, there are abnormalities in lipid level (*Ali A, et.al, 1999*). HDL cholesterol concentration is abnormally low in type -2 diabetic patients, which is unrelated to control of diabetes (*Bogus law L et.al, 2001*).

Lipid abnormalities in diabetic patients with type-2 are described as increased serum Triglycerides, VLDL, LDL and lowering of HDL*(Anebela RPet.al* 2006).Blood insulin level may remain normal or high yet hyperglycemia may develop and may be accompanied with glycosuria. These perturbation are accompanied with change in a variety of biochemical processes and are exacerbated by overweight and obesity, altered lipid profile, degree of hyperglycemia, smoking and/or genetic profile(*Nessar A et.al* 2005, Haffner SM et.al 2004and Krolewski S et.al 1977).

DM often co-exists with obesity, hypertension and dyslipidemia. Dyslipidemia is common in DM ,as both insulin deficiency and resistance affects enzyme and pathways of lipid metabolism (Folsom AR et.al 1997).Complication of atherosclerosis and lipid abnormalities have been reported to account for the majority of death and disproportionate amount of morbidity in people with diabetes and other conditions characterized by insulin resistance. It has been suggested that diabetic patients have 2-4 times higher risk of cardiovascular disease compared to those non-diabetic counter part (George P et.al 2000).

The aim of our study is to evaluate the lipid profile and assess the risk of C.V.D complications in the type-2 diabetes patients in Agra city.

Material & Methods

Patient attending OPD of Department of Medicine at S.N Medical College and Hospital Agra, were taken for the present study. Clinical examination and personal history of patients were recorded by physician in the OPD of Department of Medicine and biochemical investigations were conducted in the Clinical Laboratory of Department of Biochemistry, S.N. Medical College and Hospital, Agra.

In the present study, a total numbers of 90 human subjects of age ranging from 30 to 65 years were selected to study the lipid profile in diabetic patients. Out of 90 subjects, 50 non obese, non diabetic and normotensive volunteers (30 males and 20 females) were in control group (group 1) and other 40 subjects (23 males and 17 females) were diabetic group (group 2). Criteria for inclusion and exclusion of the patient under study were as follows:

Criteria of type-2 DM: - as per W.H.O. criteria

- Fasting (110-126 mg/dl)
- PP (140-200 mg/dl)

Inclusion Criteria

- Newly diagnosed type-2 DM patients.
- · Patients with impaired glucose tolerance test.
- Known diabetic cases taking oral hypoglycemia drugs.
- Managed with diet for the control of diabetic.
 Age group 30 to 65 years of both sexes

Exclusion criteria

patients using insulin, using cardiovascular, hypertension medication, having previous history of angina, severe vascular disease, nephropathy or other complications, patients taking cartico steroid or any drugs affecting lipid profile or diabetic status etc.

Sample collection

Methods

Blood sample were obtained by puncture of anticubital vein. 5ml blood was collected in the glass vial from each subject. The blood samples were centrifuged at 5000rmp for 10 minutes for separation of serum or plasma

Determination of fasting blood sugar by the GOD-POD

method. (Trinder P. et al, 1969 and Kaplan L.A. 1984).

Determination of serum Total Cholesterol by the CHOD-PAP method.

Determination of serum Triglycerides by the GOP-PAP method.

Determination of serum HDL by the CHOD-PAP method.

RESULTS

The present study was conducted to study the lipid profile in diabetic patients .The demographic data of the subjects under study are presented in Table -1 .The study was undertaken on 50 non- diabetic, nonobese, healthy control subjects (30 males and 20 females). Out of these, 15 subjects belonged from rural area and 35 from urban area. In diabetic subjects, 40 subjects (23 males and 17 females) were selected. Out of these, 35 patients belonged to urban area and only 5 patients belonged to rural area. The mean value of age of control subjects was 48±9.0 years (range 30-65 years) and that of diabetic patients was 53±9.2 years (range 30-70 years). The body mass index (BMI) of diabetic patients under study was 25 ±3.2 kg/m² while the BMI of matched control group was 23±0.9kg/m².

Table 1. Age, Sex and Anthropometric parameters in control and diabetic groups

Variables	Control (Group -1) (n=50)	Diabetic(Group -2) (n=40)		
Male	n=30	n=23		
Female	n=20	n=17		
Rural	n=15	n=5		
Urban	n=35	n=35		
Age (35-60 years)	40±9.0	53±9.2		
Weight (kg)	63.8±9.5	61.9±8.8		
Height (cm)	165±10.5	164±9.7		
BMI(kg/m ²)	23±0.9	25±3.4		
Duration of diabetes	-	5.8±3.7		
(4months-10 years)				

The fasting blood sugar level was significantly higher in diabetic patients 154 ± 67.4 mg/dl (range 73-293 mg/ dl) as compared to the control (group-1) 80 ± 7.0 mg/ dl (range 70-110 mg/dl) (p<0.001) (Table-2).

For studying lipid profile in the diabetic patients under study, the level of total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were estimated. It was found that the total cholesterol level increased in diabetic group 193.8±18.31mg/dl (range 215-299 mg/dl) as compared to the control group 178.2±14.23 mg/dl (range 150-197mg/dl. (p<0.05), triglyceride level was also significant higher in diabetic group 147.3±58 mg/dl.(range 80-290 mg/dl) as compared to the control group 128.0±21.77 mg/ dl(range 83-164 mg/dl) (p<0.001). The LDL level was highly significantly increased in diabetic group 138.5 \pm 35.8 mg/dl (range 102-190 mg/dl), as compared to the control subjects 96.12 \pm 18.96 mg/dl (range 102-132mg/dl) (p<0.001).VLDL level of diabetic patients 29. 8 \pm 11.20mg/dl (range 52-60mg/dl) did not change as compared to the control group 26.9 \pm 5.09mg/dl (range 30-35mg/dl) (p>0.05). However HDL level was moderately decreased in diabetic patients 44.4 \pm 5.96mg/dl (range 30-58mg/dl) as compared to the control subjects 51.3 \pm 14.61mg/dl (range 35-55 mg/dl) (P<0.01) (Table-2).

The findings of the present study reveals that there is significant increased in fasting blood sugar level(hyperglycemia), Cholesterol, Triglyceride and LDL level(p<0.001), where as the level of HDL cholesterol was moderately decreased in diabetic subjects (Table-2).

Group	n	Fasting blood	Total	Triglyceride	HDL	LDL	VLDL
		sugar (mg/dl)	cholesterol	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
			(mg/dl)				
Control	50	80±7.0	178.2±14.23	128.0±21.77	51.3±14.61	96.12±18.96	26.9±5.09
(group1)							
Diabetic	40	154±67.4	193.8±18.31	147.3±58.0	44.4±5.96	138.5±35.8	29.8±11.20
(group 2)							
t-test		6.8207	4.4245	1.995	3.0384	6.7663	1.4814
p-value		P<0.001	P<0.001	P<0.05	P<0.01	P<0.001	p>0.05
Significance		HS	HS	significant	moderate	HS	NS
level							

Table 2. Serum fasting blood sugar level and lipid profile in diabetic and control group

HS= highly significant, NS= not significant

HS= highly significant, NS= not significant.

The comparison of fasting blood sugar level and lipid profile parameter between diabetic male and female patients are presented (Table-3). The blood sugar Fasting level in male and female diabetic groups was 161±65.6 mg/dl (range 72-289 mg/dl) and 148±69.9 mg/dl (range 82-275 mg/dl) respectively. Values between two groups was not significantly different (Table-3).

The level of cholesterol, triglyceride, LDL and VLDL

in diabetic males were 190.1 ± 16.79 mg/dl, 160.9 ± 60.38 mg/dl, 143.2 ± 38.9 mg/dl and 30.2 ± 11.9 mg/dl respectively, were not also significantly different from diabetic females in which the values of cholesterol, triglyceride, LDL and VLDL were 181.2 ± 23.5 mg/dl, 155.9 ± 53.4 mg/dl, 135.5 ± 34.0 mg/dl and 29.0 ± 9.99 mg/dl respectively. However, HDL level was significantly low in diabetic males 43.23 ± 5.77 mg/dl as compared to the HDL values in diabetic females 49.8 ± 4.58 mg/dl (Table-3).

Group	n	Fasting blood	Total	Triglyceride	HDL	LDL	VLDL
		sugar (mg/dl)	cholesterol	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
			(mg/dl)				
Males	23	161±65.6	190.1±16.79	160.9±60.38	43.23±5.77	143.2±38.9	30.2±11.9
Females	17	148±69.9	181.2±23.5	155.9±53.4	49.8±4.58	135.5±34.0	29.0±9.99
t-test		0.5968	1.3306	0.277	4.0122	0.6657	0.346
p-value		P>0.05	P>0.05	P>0.05	P<0.001	P>0.05	p>0.05
Significance		NS	NS	NS	HS	NS	NS
level							

Table 3	Serum	fasting	blood su	nar leve	l and linid	nrofile in	diabetic m	ales and females
Iable J.	Jerum	lasung	0000 30	yai ieve	i anu iipiu	prome in	ulabelic III	ales and lemales.

HS= highly significant, NS= not significant

The comparison of data between diabetic males and non diabetic males are presented in Table-4. It was found that fasting blood sugar level in diabetic males was significantly higher 161±65.6 mg/dl as compared to the value found in non-diabetic males 78±6.7 mg/ dl. Cholesterol level was significantly higher in diabetic males 190.1±16.79 mg/dl as compared to non diabetic males 178.32±14.2 mg/dl. Triglyceride level was also significantly higher in diabetic male 160.9±60.38 mg/dl as compared to non-diabetic males 131.7±28.57mg/dl. LDL was also significantly higher in diabetic males 143.2±38.9 mg/dl as compared to the value found in non diabetic males 97.6±16.28 mg/dl. HDL and VLDL values 43.23±5.77 mg/dl and 30.2±11.9 mg/dl respectively were not significantly different changed in diabetic males as compared to the value found for HDL and VLDL in non-diabetic males 45.9±7.23 mg/dl, 26.7±4.96 mg/ dl respectively (Table-4).

Group	n	Fasting blood sugar (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Diabetic Males	23	161±65.6	190.1±16.79	160.9±60.38	43.23±5.77	143.2±38.9	30.2±11.9
Non-diabetic males	30	78±6.7	178.32±14.2	131.7±28.57	45.9±7.23	97.6±16.28	26.7±4.96
t-test		6.0438	2.7087	2.1427	1.4949	5.2786	1.3251
p-value		P<0.001	P<0.05	P<0.05	P>0.05	P<0.001	p>0.05
Significance level		HS	Significant	Significant	NS	HS	NS

Table 4. Serum fasting blood sugar level and lipid profile in diabetic males and non diabetic males.

HS= highly significant, NS= not significant

The comparison of fasting blood sugar and lipid profile between diabetic females and non diabetic females are presented in Table-5. It was found that fasting blood sugar in diabetic females was significantly higher 148±69.9 mg/dl as compared to non diabetic females 82±7.6 mg/dl. Cholesterol, triglyceride and LDL levels were significantly higher in diabetic females 181.2±23.5mg/dl, 155.9±53.4mg/dl and 135.5±34.0 mg/dl respectively as compared to that found in non-diabetic females for cholesterol, TG and LDL 178.2±11.2 mg/dl, 120.5±27.9mg/dl, and 94.1±22.4 mg/dl respectively. However HDL were significantly

Nov., 2013

low in diabetic females 49.84.58 mg/dl as compared to non diabetic females 58.7±18.67 mg/dl. VLDL value

was not significantly changed in diabetic females 29.0 ± 9.99 mg/dl as compared to the found in nondiabetic females 28.2 ± 5.09 mg/dl (Table-5).

Group	n	Fasting blood sugar (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Diabetic	17	148±69.9	181.2±23.5	155.9±53.4	49.8±4.58	135.5±34.0	29.0±9.99
females							
Non- diabetic	20	82±7.6	148.2±11.2	120.5±27.9	58.7±18.67	94.1±22.4	28.2±5.09
females							
t-test		3.8795	5.3007	2.4625	2.0602	4.291	0.2988
p-value		P<0.001	P<0.001	P<0.05	P<0.05	P<0.001	p>0.05
Significance		HS	HS	Significant	Significant	HS	NS
level							

Table 5. Serum fasting blood sugar level and lipid profile in diabetic females and non diabetic females.

HS= highly significant, NS= not significant

Discussion

In present study, lipid profile of 40 type-2 diabetes mellitus patients was estimated and compared with 50 age & sex matched non-diabetic ,non-obese and normotensive healthy controls.

The present study showed that, total- cholesterol, triglyceride, and LDL cholesterol were significantly higher and HDL cholesterol was significantly lower in diabetic patients than in control groups. Similar result was reported by *Sapna smith et al; (2008)*. The serum total cholesterol, triglyceride, LDL, VLDL levels increased in male as compared to female. Serum HDL level was reduced significantly in both male and female diabetic groups.

Cohen JR et al; (1979) showed significant increase in the level of serum cholesterol and LDL cholesterol in diabetics when compared with controls. In their study, serum HDL -cholesterol level did not differ significantly in the two groups. *Sharma JH e.al,(1970)* and *Jain EE et. al, (1980) also* observed increase in the levels of total cholesterol, serum triglycerides in diabetic subjects as compared to normal controls. The studies of *Santen A.R et al (1972)* and *Peret T et. al, (1974)* observed mean serum triglyceride levels higher in diabetics as compared to control subject. *Bijlani M et al (1984)* and *Sapna smith et. al,(2008)* found HDL to be significantly lower in diabetics as compared to normal subjects.

Fredrick DJ et.al, (1994) & Michel et. al, (1989), have also reported level in type -2 diabetic there is significant increased level of cholesterol, triglyceride and LDL and decreased level of HDL cholesterol in type-2 diabetic patients as compared to non-diabetic control subjects.

Some studies, have strongly suggested an inverse correlation of HDL-cholesterol level with the development of ischemic heart disease .*Castelli WP* & *Miller NE et. al,(1977)*. Most of the studies have revealed the inverse relationship of HDL- cholesterol with atherosclerosis to be independent of other lipid abnormalities. In the present study too, HDL cholesterol was lower in type-2 diabetics in both sexes, while total cholesterol was increased in both groups. These observations can be at least partly explained by the known inverse correlation of HDL-cholesterol with adiposity and triglyceride levels .

The males have marginally high serum lipid levels as compared to diabetic females. Similar results have been observed in other studies of *Sapna smith et.al*,(2008). Significant difference in lipid profile of male and female diabetics is because sex hormones play unique for lipid metabolism. This study has clearly shown that all lipid fractions (except HDL) are abnormally elevated in diabetics when compared with controls.

Summary & Conclusion

In the present study ,there was a significant increase in fasting blood sugar, total- cholesterol, HDL, triglyceride and LDL levels in diabetic patients when compared with the healthy control subjects. However, there was no change in VLDL. No significant change was observed in fasting blood sugar, total-cholesterol, HDL, triglyceride and VLDL levels in diabetic male patients when compared with the diabetic female patients, except for HDL levels which were significantly higher in female diabetic patients as compared to male diabetic patients. A significant

References

- Ali A.; Khem A.; Syed (1999). Disturbances of lipoprotein metabolism in diabetes. J Ayub Med Coll Abottabad ;3-5.
- Anebela R.P. and Carlos P.M. (2006). Diabetes and mitochondial function :Role of hyperglycaemia and oxidative stress .Toxicology and Applied Pharmacolog;212:167-78.
- Bijlani M.; Esteghamati A.R.; Esfahanian F. and Ochei J. and Kolhatkar (1984). A Medical Laboratory Sciences Theory and Practice. Tata M. Graw Hill, 188-198.
- Boguslaw L. (2001): Pathophysilogy of oxidative stress in diabetes mellitus. Journal of Diabetes and its Complications ;15:203-10.
- Chattrejee C.C. (1992) : Human physiology(vol I) Role of endocrine in lipid metabolism. (Editoredical allied agency) .546-550,Culcutta- INDIA.
- Chatrejee M .N. and Shinde R. (2005) : Text book of medical laboratory technology. Metabolism of carbhohydrates.(Jaypee Brothers Medicall publisher) Sixtedition. 266-330, Delhi-India.

increase in fasting blood sugar, total- cholesterol, and triglyceride and LDL levels was observed in diabetic male patients when compared with the healthy control males except for VLDL and HDL levels. A significant increase in fasting blood sugar, total- cholesterol, HDL, triglyceride and LDL level was observed in diabetic females when compared with the healthy control females except VLDL level.

From this study, it was evident that diabetes mellitus has a real impact on lipid metabolism. This was substantiated by the fact that all the lipid fractions were elevated in diabetics when compared to the healthy control. Hyperlipidemia and hypertriglyceridemia are quite common abnormalitie in diabetics causing the patients at cardiac risk. Therefore, diabetic patients should be monitored for dyslipidemia and cardiac risk at early stage of disease to avoid further CVD complications in diabetic patients.

- 7. Cohen J.R. and Lesterm M.A. (1979) : Diabetes and cardiovascular disease.Diabetes Care .22: 20.
- Folsom A.R.; Szklo N. and Stevens J. (1997)
 Aprospetive study of coronary heart diseases in realation to fasting insulin , glucose and diabetes, diabetes care 20:935-942.
- 9. George P. and Iuduvik. B. (2003) : lipid and diabetes J.Clin .basic cardial 3: 159-162.
- Godkar P. and Godker D. (2003) : Text book of medical laborarty technology.Ed .2 chemistry of carbhohydrates (Bhalani publishing house s. 176-233, New Delhi-India
- **11. Haffner S.M.** (2004) : American Diabeties Association Dyslipidemia management in adults with diabetes. Diabeyes Care .Jan:27 Suppl 1:S68-71.
- 12. Hong D. and Chris R. (2005) : Endothelial cell dysfunction and the vascular complications associated with type 2 diabetes: assessing the health of the endothelium. Vascular Health Risk Manag.; 1:55-71.
- **13.** Jain E.E.; Gonzalez J.J. and Ranney J.E. (1993) : Lipid level differences and hypertension

effect in blacks and whites with type II diabetes. Ethn.Dis 3: 242-249.

- **14.** Krolewski S.; Czyzyk A. and Janeczko Dzt (1997) : Mortality from cardivascular diseases among diabetic diabetologia . 13:345-350.
- 15. Mozaffarian D.; Martijn B.; Alberto A.; and Mer, J. (2006) : Trans Fatty Acids and cardiovascular diseases. NEJM 354:1601-13.
- Nessar A. (2005) : Advanced glycation end products role in pathology of diabetic complications. Diabetes Research and Clinical Practice 67:3-21.
- Peret T.; Oba K.; Igari Y.; Nakano H. and Metori S. (1974) : A four-year prospective study on the serum elevated lipoprotein concentration on ischemic heart disease and cerebral infarction in patients with type 2 diabetes. Nippon Ronen Igakkai Zasshi, 38(4): 507-513
- Ronald M. and Krauss M. (2004) : Lipids and Lipoproteins in Patients with type 2 Diabetes . Diabetes Care 27:1496-1504.

- Samia J.; Asghar S. and Fayaz (2000) : Relation of high density lipoprotein cholestrerol concentration to type of diabetes and its control. Biomedical. 16:19-24
- 20. Santen A.R. and Heshmat N.R. (1970) : Dyslipidemia in type 2 Diabetes mellitus: More athergnenic lipid profile in women. Acta Med. Iranica,: 111-118.
- 21. Smith Sapna and Lall Alok M. (2008) : Lipid Profile Levels of Diabetics and Non-Diabetics Among Naini Region of Allahabad, IndiaTürk Biyokimya Dergisi [Turkis Journalof Biochemistry–Turk J Biochem] 33 (4) ; 138–141.
- 22. Sharma J.H.; . Konen J.C. and Dignan M.B. (1970) : Racial differences inlipid and lipoprotein levels in diabetes. Metab. 41: 851–855.
- 23. World Health Organization (1994) : Expert committee on prevation and treatment of diabetes mellitus . WHO technical series No 844. Geneva World Health Organization
