BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
CANSELORI
UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1. Nama Penyelidik : DR. FARIDAH ABDUL RASHID ✓

   Nama Penyelidik-Penyelidik Lain (Jika berkaitan) : PROF. MADYA (Dr.) MAZIDAH AHMAD MANSUR
   EN. HASENAN NORDIN

2. Pusat Pengajian/Pusat/Unit : SAINS PERUBATAN

3. Tajuk Projek : MENGENALPASTI FENOTIP APOLIPOPROTEIN E DENGAN MENGGUNAKAN TEKNIK FOKUS ISOELEKTRIK BAGI KES-KES HIPERLIPIDEMIA DI KELANTAN

4. (a) **Penemuan Projek/Abstrak**

(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

Sampel plasma pesakit (n=250) disaring untuk kandungan kolesterol dan trigliserida oleh analisis enzimatik yang dilakukan menggunakan penganalisa kimia automatik dan kit piawai. Elektroforesis lipoprotein dilakukan ke atas kepingan gel nipis atau tiub gel siap. Plasma yang tidak berkait dipilih (n=24) dan dikaji menggunakan kedua-dua kaedah gel tersebut. Satu subjek berkemungkinan mempunyai fenotip apolipoprotein E-2/2 dikesan menerusi kaedah kepingan gel. Sebanyak 11 subjek lain yang mempunyai corak jaluran lipoprotein yang luar biasa dan berkemungkinan mempunyai fenotip apolipoprotein E yang tak normal dikesan menerusi kaedah tiub gel. VLDL dipisahkan menerusi kaedah mikro yang dibangunkan ke atas pengempar ultra. Fenotip apolipoprotein E dipastikan menerusi pemfokusan isoelektrik (IEF) protein VLDL setelah diluputkan lipid yang dilakukan ke atas gel komersial dan buatan sendiri.

Patients plasma samples (n=250) were screened for cholesterol and triglyceride content by enzymatic analyses performed on an automated chemistry analyzer using standard kits. Lipoprotein electrophoresis was performed on thin slab or pre-casted tube gels. Unrelated plasma were selected (n=24) and studied using both gel methods. One subject with a possible apolipoprotein E-2/2 phenotype was detected by the slab gel method. Another 11 subjects with unusual lipoprotein banding patterns and possible altered apolipoprotein E phenotypes were detected by the tube gel method. VLDL were isolated by a micromethod developed on an ultracentrifuge. Apolipoprotein E phenotype was confirmed by flat bed isoelectric focussing (IEF) of delipidated VLDL proteins on commercial and homemade IEF gels.

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak :

Bahasa Malaysia	Bahasa Inggeris
KOLESTEROL	CHOLESTEROL
TRIGLISERIDA	TRIGLYCERIDE
ELEKTROFORESIS LIPOPROTEIN	LIPOPROTEIN ELECTROPHORESIS
KEPINGAN GEL NIPIS	THIN SLAB GELS
TIUB GEL SIAP	PRE-CASTED TUBE GELS
FENOTIP APOLIPOPROTEIN E	APOLIPOPROTEIN E PHENOTYPE
PEMFOKUSAN ISOELEKTRIK	ISOELECTRIC FOCUSSING (IEF)
PROTEIN VLDL	VLDL PROTEINS
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5. Output Dan Faedah Projek

(a) Penerbitan (termasuk laporan/kertas seminar)

(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

	Jenis	Tajuk	Pengarang	Tahun	Tempat
1.	Abstrak	Application of apolipoprotein E phenotyping in hyperlipidemia	Faridah Abdul Rashid	11-12 Sept 1993	4th Combined Meeting of Malaysian & Singapore Society of Pathologists, USMCK Abstrak-Programme Book Penerbitan-Malaysian J of Pathology
2.	Kertas seminar	Apolipoprotein E	Faridah Abdul Rashid	15-16 Sept 1993	Bengkel Peningkatan Mutu Perkhidmatan Makmal Biokimia Klinikal, USMCK Pembentangan-Seminar Penerbitan-Proceedings
3.	Poster, Abstrak, Kertas penuh	Micromethod for isolating very low density lipoproteins (VLDL, d<1.006 g/ml) from leftover human lipemic plasma (d<1.006 g/ml)	Faridah Abdul Rashid & Zakaria Abu Samah	6-7 Nov 1993	Malaysian Association of Clinical Biochemists (MACB) Conference on Diabetes Mellitus, the Regent, KL Poster-Exhibition Abstrak-Programme Book Kertas penuh-Proceedings

(b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.**

(Jika ada dan jika perlu, sila gunakan kertas berasingan)

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PERKEMBANGAN PRODUK : USAHA PENGHASILAN IEF GEL NIPIS 1MM TEBAL

MENGANDUNGI UREA (THIN IEF GELS, 1MM THICK, CONTAINING UREA) DAN

BERMUTU TINGGI SEDANG DITERUSKAN DENGAN BEBERAPA PENGUBAHSUAIAN KAEDAH

PROSPEK KOMERSIALISASI : SEKIRANYA PENGHASILAN UREA IEF GEL NIPIS

BERJAYA, PROSPEK KOMERSIALISASI ADALAH BESAR. PERSAINGAN HEBAT

ADALAH DARI PHARMACIA /LKB

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(c) **Latihan Gunatenaga Manusia**

i) **Pelajar Siswazah :** TIADA .....

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ii) **Pelajar Prasiswazah :** TIADA .....

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iii) **Lain-lain :** .....

SAINTIS - SEORANG (SAMBILAN)

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TEKNOLOGIS TERLATIH - SEORANG (SAMBILAN, 6 BULAN)

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6. **Peralatan Yang Telah Dibeli :**

i. ANTON PAAR DIGITAL DENSITY METER DM35 (MR5,750.00)

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ii. METTLER PJ3000 BALANCE (MR4,360.00)

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UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

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Presentation Code:	P23

APPLICATION OF APOLIPOPROTEIN E PHENOTYPING IN HYPERLIPIDEMIA

Faridah Abdul Rashid

Department of Chemical Pathology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

Approximately 10-20% of total VLDL protein is apolipoprotein E. Apolipoprotein E is an arginine-rich single polypeptide containing 299 amino acids, with a molecular weight between 34 - 39 000. Its synthesis in the liver is modulated by the apolipoprotein E gene on chromosome 19. Serum apolipoprotein E levels are higher in women than in men, and correlate with serum triglyceride levels. The highest serum concentrations of apolipoprotein E are observed in type III hyperlipoproteinemia. Apolipoprotein E isoforms are determined by 3 allelic genes. The isoforms, designated E-2, E-3 and E-4, are distinguished based on cystine and arginine content. This study determined the apolipoprotein E phenotype in 21 lipemic plasma.

Apoliprotein E (isoelectric points between 5.7-6.2) was focussed on slab polyacrylamide iscelectric focussing gels of pH 4 - 6.5 containing 6 M urea. Apoliprotein E was resolved into 6 isoforms. The distribution of apolipoprotein E phenotypes obtained was E-3/3 (62%), E-4/3 (24%), E-3/2 (9%), and E-4/2 (5%). No homozygous E-2/2 nor E-4/4 phenotypes were found.

MICROMETHOD FOR ISOLATING VLDL FROM HUMAN LIPEMIC PLASMA

Zakaria Abu Samah and Faridah Abdul Rashid

Department of Chemical Pathology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Several methods for isolating low density lipoproteins (VLDL, density < 1.006 g/ml) have been published. However these methods described preparative techniques and required large amounts of plasma which were often not applicable to clinical samples as sometimes the samples received were insufficient. The objective of our study was to develop a micromethod for isolating VLDL from either fresh or leftover human lipemic plasma. The isolated VLDL were intended for compositional analysis and apolipoprotein E phenotyping by isoelectric focussing. Three-ml blood samples were collected from fasting human adult subjects. Plasma obtained was stored in tubes overnight at 4 C. Traces of chylomicrons were removed by aspiration of the top creamy layer. Four ultracentrifuge tubes (capacity > 300 µl) were filled with prestained unadjusted plasma (5 µl Fat Red/200 µl plasma/tube). Ultracentrifugation was in a table-top ultracentrifuge, the Beckman Airfuge at 90,000 rpm for 3 hours at 25 C to float VLDL. Lipoprotein banding pattern was noted in each tube following ultracentrifugation. VLDL were aspirated (15 µl) in the top most fraction (F1) from each tube into a fresh Eppendorf tube. Altogether 60 µl of VLDL were recovered for each plasma sample. VLDL purity was checked by lipoprotein electrophoresis on Beckman Paragon Lipo gels. Following electrophoresis and staining with Sudan Black (7% W/W), the stained gels were scanned in a clinical densitometer, the Helena Ciniscan. VLDL were found to comprise 72-75% of the first top fraction in each tube; the remaining 28-25% consisted mainly of HDL. A successive fraction (F2) that was removed from each tube comprised chiefly of LDL (55%), VLDL + HDL (45%). The bottom most fraction (F3) consisted of LDL (34%) and VLDL + HDL (57%). We have thus shown that the Beckman Airfuge could be utilised to isolate VLDL in the clinical laboratory. However, purity of the isolated VLDL was ambiguous when tested on commercial electrophoresis gels. We therefore suggest that purity of large lipoproteins be checked by 10, 12 or 15% of PAGE (polyacrylamide gel electrophoresis). Commercial gradient gels of 5-15% could be used for PAGE of small lipoproteins (IDL, LDL and HDL). An alternative method for identification of the isolated lipoproteins is to employ the use of a particle size analyser. This will omit the need for PAGE. Lipoprotein sizing is required prior to compositional analysis.

Reference: Bronzert T.J. and Brewer Jr., H.B. New micromethod for measuring cholesterol in plasma lipoprotein fractions. Clin Chem 1977. 23: 2089-2098.

PRELIMINARY INVESTIGATIONS ON THE USE OF KETOM LEAVES (*MITRAGYNA SPECIOSA*) FOR THE TREATMENT OF DRUG ADDICTION IN MALAYSIA

Yahya Ahmad, Badrul Amini Rashid, Zabedah M. Yunus, Jalilah Hassan dan Zakiah Ismail

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A survey on the use of *Mitragyna speciosa* was carried out in several villages in the Northern States of Malaysia. About 65 respondents were interviewed during the exercise. The results

Apolipoprotein E

Faridah Abdul Rashid, Dept. of Chemical Pathology, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

Triglyceride-rich lipoproteins (chylomicrons, VLDL) are metabolized intravascularly by lipoprotein lipase forming lipoprotein remnants (chylomicron remnants, VLDL remnants). Apolipoprotein E is important in lipoprotein remnant-receptor interactions and subsequent removal of these remnants. Lipoprotein remnants are taken up by two types of hepatic receptors, the LDL (B,E) receptor and the chylomicron remnant (E) receptor. The LDL (B,E) receptor is responsible for binding and removal of LDL from the circulation. The chylomicron remnant (E) receptor is responsible for the conversion of IDL to LDL and uptake of chylomicron remnants. In the receptor interaction of apolipoprotein E, the isoforms E-3 and E-4 are recognized as normal components of various lipoprotein remnants and thus these remnants are catabolized normally. On the other hand, the isoform E-2 is recognized as abnormal and the triglyceride-rich lipoprotein remnants which have apoE-2 in their surface coat are not catabolized normally, and thus partially accumulate in the plasma as β-VLDL. Apolipoprotein E (isoelectric points between 5.7 - 6.2) can be resolved by isoelectric focussing in 6 M urea into a number of isoforms (determined by three allelic genes), designated apolipoprotein E-2, E-3 and E-4. These isoforms are distinguished on the basis of cysteine and arginine content. Heterozygous and homozygous apolipoprotein E phenotypes relate to the different types and severity of hyperlipidemia that a person suffers from. This has important implications with respect to atherosclerosis and risk of heart disease. Heterozygous apolipoprotein E phenotypes are E-4/3, E-4/2 and E-3/2, and homozygous apolipoprotein E phenotypes are E-2/2, E-3/3 and E-4/4.

Apolipoprotein E phenotypes were determined using lipemic plasma. VLDL were isolated from the lipemic plasma. Isoelectric focussing (IEF) was performed on aliquots of the VLDL. Apolipoprotein E were resolved into 5 or 6 visible bands by IEF. The apolipoprotein E phenotype was determined from one or two most predominantly

visible of these 5 or 6 bands. The apolipoprotein E phenotype was confirmed by scanning and quantitation by automated integration of 'areas under the curve'. The predominant apolipoprotein E band quantitated gave a homozygous apolipoprotein E phenotype. The two most predominant apolipoprotein E bands quantitated gave a heterozygous apolipoprotein E phenotype. The distribution of apolipoprotein E phenotypes obtained in one study (n=21) was: E-3/3 (61.9%), E-4/3 (23.8%), E-3/2 (9.5%) and E-4/2 (4.8%). No homozygous E-2/2 nor E-4/4 phenotypes were found in this study.

From a medical standpoint, as apolipoprotein E phenotype is genetically determined and thus inherited. a potential epidemiological value of apolipoprotein E phenotyping is in early detection of apolipoprotein E2 homozygosity There is an association between apolipoprotein E2 homozygosity and type III hyperlipoproteinemia. Some 1% of the population are apolipoprotein E2 homozygotes whereas type III is much rarer, occurring in 1 in 10 000. With cosanguinity prevalent in hyperlipidemia in Kelantan, screening for abnormal apolipoprotein E phenotypes among adults with hyperlipidemia should be extended to include their children and first-degree relatives. Early detection of abnormal homozygous apolipoprotein E-2/2 phenotype should be followed with providing advice and genetic counselling to affected individuals.

Another application of apolipoprotein E phenotyping is in the management of patients with type III hyperlipoproteinemia. Some 75-95% of patients with type III hyperlipoproteinemia will be E2 homozygotes. Type III hyperlipoproteinemia is detected by lipoprotein electrophoresis on agarose gel. Apolipoprotein E phenotyping may be helpful since it helps to identify the biochemical basis of the hyperlipidemia. Once the apolipoprotein E phenotype is determined, management of patients with type III hyperlipidemia can then proceed based on known underlying biochemical defect and the associated defective metabolism of lipoproteins. Appropriate therapy can then be tailored and given to patients who must then be regularly monitored for progress.