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Volume 6, 2015

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Errata of Health Science Journal 2015 Vol. 6 No. 1 page i and 29
Written: Confirmation of Plasmodium falciparum treatment failure cases by polymerase change reaction genotyping
Corrected: Confirmation of Plasmodium falciparum treatment failure cases by polymerase chain reaction genotyping

Health Science Journal of Indonesia

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Editorial Note

One of the winner of Nobel prize for medicine 2015 was a Chinese scientist, Tu You You. She was famous for her work in developing Artemisin, an antimalarial drug. Artemisin is an extract of *Artemisia annua* which was known as a traditional Chinese medicine (TCM) for malaria since the fourth century. As a modern medicine, the drug was systematically chosen from hundreds of potential TCM in 1960s. Two animal studies in mice and monkeys in 1970s confirmed the drugs potential antimalarial efficacy in 1971. In 1990s, the world health organization (WHO) recommends the use of the drug widely. Later the drug was recommended to be given in combination due to several report of drug resistance. Despite of all its controversies, in 2006, Artemisin in combination therapy (ACT) has become the treatment of choice for malaria worldwide.\(^1,2\)

In Indonesia the use of Artemisin was started in early 2000s, following the recommendation from the WHO.\(^3\) Since that various studies have been conducted by Ministry of Health to look at the efficacy and safety of the drug in either uncomplicated or complicated malaria. Armady et.al studied the efficacy and safety of a single dose Artemisinin-Napthoquine (AN) on *P. vivax* infection, as presented in this edition of Health Science Journal of Indonesia (HSJI). They concluded that Artemisin has a similar efficacy with Dihydroartemisinin-Piperaquine (DHP) for the treatment of *P. vivax* infection in adults. It has also an acceptable safety profile.\(^4\) This result is in line with previous studies of ACT in various population in the world. Further studies and long-term safety monitoring on Artemisin is still needed.

The success story of Artemisin certainly has shine some hope that any herbal medicine can be rediscover as an acceptable treatment in western medicine, as long as it has clinically proven and tested. In this edition of HSJI, results from two pre-clinical (animal) studies of chemical entities that were derived from herbal medicine were presented. Rachmawati et.al described the effects of combined infusion of *sambiloto, salam, kayu manis*, and *temulawak* on decreasing blood levels of glucose, cholesterol, and triglyceride.\(^5\) Their results shows that The infusion administration of the combined medicinal plants (*sambiloto, salam, kayu manis* and *temulawak*) at dose 491.4 mg/200 g bw for 7 days decreased blood glucose, cholesterol and triglyceride levels of hyperglycemic SD rats.

While Yunarto et al examine effect of purified gambir leaves extract to prevent atherosclerosis\(^6\) and showed a significant positive results. Gambir leaves purified extract had the effect of preventing the thickening of the walls and foam cell formation rat aorta.

After the aviation industry, the drug development is known as the second highly regulated field in the world. Since the ill-fate story of Thalidomide users in early 1960s, a drug has to be proven as safe and effective before it use for its intended indication by going into three phases of clinical trials. Prior to that, a pre-clinical studies with various staged using at least two kind of animals is needed. The process took 10 to 15 years to complete and was blamed as one of the reason for a slow growth of drug development. In addition, it requires a lot of resources which make it seems possible only for big pharmaceutical industry to conduct.\(^7\)

The safety data of Artemisin was infact controversial in the beginning, as the drug was tested for its safety only on Dr. Tu and her colleague before it was tested for its efficacy for malaria patients.\(^2\) However, as proven in the later stage of its clinical studies and after more than a decade of its worldwide use, the safety profile of Artemisin seems acceptable.

The results from pre-clinical studies of some Indonesian herbal/traditional medicine seems promising. As the story of Artemisin, any drugs need to enter a long-term of clinical studies before it can be use by population at large. In addition, a continuous drug monitoring after the drug gets into the market is needed. Thus, we expect more Indonesian researchers are inspired and produced a good quality result on new drugs.

Grace Wangge
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Inne Yuliawati, Minarma Siagian, Thamrin Abudi, Bastaman Basuki

Jumlah sektor dan faktor risiko lain yang terkait dengan kelelahan di antara pilot komersial jarak pendek di Indonesia

Health Science Journal of Indonesia 2015;6:69-75

Latar belakang: Kelelahan penerbang sipil, termasuk pada penerbangan jarak dekat, dapat mempengaruhi fungsi kognitif penerbang sehingga membatasi keselamatan penerbangan. Tujuan penelitian ini untuk mengidentifikasi faktor-faktor yang mempengaruhi kelelahan penerbang sipil pada penerbangan jarak dekat di Indonesia.


Hasil: Di antara 785 penerbang yang melaksanakan pengujuan kesehatan, 382 bersedia berpartisipasi, dan 239 penerbang yang memenuhi kriteria. Rata-rata skala kelelahan adalah 4,66 (standar deviasi 1,202). Faktor-faktor dominan yang mempertinggi skala kelelahan adalah jumlah sektor 24 jam terakhir, jam terbang penugasan di luar jadwal, dan kehilangan waktu tidur. Setiap penambahan 1 sektor dalam 24 jam terakhir, jam terbang penugasan di luar jadwal, dan kehilangan waktu tidur meningkatkan 0,371 skala kelelahan [koefisien regresi (β) = 0,371; P = 0,000]. Selanjutnya setiap penambahan 1 jam terbang penugasan di luar jadwal mempertinggi 0,033 skala kelelahan (β = 0,033; P = 0,000). Sedangkan setiap penambahan 1 nilai ESS mempertinggi 0,043 skala kelelahan (β = 0,043; P = 0,008).


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Intan Mariska, Danardi Sosrosumihardjo, Widura Imam Moestopo, Bastaman Basuki

Dukungan purser dan faktor lainnya dengan kecenderungan depresi pada pramugari penerbangan sipil di Indonesia

Health Science Journal of Indonesia 2015;6:76-80

Latar belakang: Depresi dapat terjadi juga di antara pramugari dalam melakukan pekerjaannya. Tujuan penelitian ini membuktikan pengaruh dukungan purser dan faktor lainnya terhadap kecenderungan depresi pada pramugari penerbangan sipil di Indonesia.


Hasil: Di antara 242 pramugari yang memenuhi kriteria inklusi adalah 145 orang. Dukungan purser, dukungan di luar pekerjaan, dan beban kerja mental merupakan faktor dominan yang mempengaruhi kecenderungan depresi. Dengan peningkatan 1 poin dukungan purser akan menurunkan 0,552 poin depresi [koefisien regresi = (β) = -0,552; P = 0,033]. Dengan peningkatan 1 point dukungan di luar pekerjaan akan menurunkan 1,191
poin depresi ($\beta = -1,191; P = 0,000$). Namun sebaliknya, dengan peningkatan 1 poin beban kerja mental akan meningkatkan 0,549 poin depresi ($\beta = 0,549; P = 0,045$).

**Kesimpulan:** Semakin tinggi dukungan purser dan dukungan di luar pekerjaan akan semakin mengurangi kecenderungan depresi, sebaliknya semakin tinggi beban kerja mental akan semakin mempertinggi kecenderungan depresi.

**Kata kunci:** depresi, dukungan purser, beban kerja mental, pramugari

---

**Inne Yuliawati, Minarma Siagian, Thamrin Abudi, Bastaman Basuki**

**Pengaruh beban kerja dan faktor risiko lain terhadap sindrom metabolik di antara pilot komersial jarak pendek di Indonesia**

Health Science Journal of Indonesia 2015;6:81-6

**Latar belakang:** Sindroma metabolik (MS) dapat menyebabkan kecacatan di antara pilot di Indonesia. Tujuan dari penelitian ini adalah untuk mengidentifikasi faktor-faktor risiko MS antara pilot komersial jarak pendek di Indonesia.


**Hasil:** Di antara 521 pramugari sebanyak 393 setuju berpartisipasi, 19 dieksklusi karena menderita gangguan durasi haid sebelum bekerja, sehingga diperoleh 374 subyek untuk analisis, dan 35,8% di antaranya menderita gangguan durasi haid. Stres kerja, jenis penerbangan dan usia merupakan faktor risiko dominan terhadap gangguan durasi haid. Pramugari dengan stres kerja memiliki risiko menderita gangguan durasi haid 58% lebih tinggi [risiko relatif suai (RRa) = 1,58; interval kepercayaan (CI) = 0,96-2,62; P = 0,071]. Pramugari dengan jenis penerbangan jarak jauh dalam tiga bulan terakhir memiliki risiko 69% lebih tinggi mengalami gangguan durasi haid (RRa=1,69; CI=1,17-2,43). Pramugari berumur 30–39 tahun memiliki risiko gangguan durasi haid 50% lebih rendah (RRa = 0,50; 95% CI = 0,22-1,02; P = 0,057).

**Kesimpulan:** Pramugari dengan stres kerja, jenis penerbangan jarak jauh dalam tiga bulan terakhir, dan berusia 19–24 tahun memiliki risiko lebih tinggi gangguan durasi haid.

**Kata kunci:** durasi haid, stres kerja, pramugari, Indonesia

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**Melissa Audry Rampen, Setyawati Budiningsih, Agus Supriyadi, Bastaman Basuki**

**Stres kerja terhadap risiko gangguan durasi menstruasi pada pramugari sipil di Indonesia**

Health Science Journal of Indonesia 2015;6:87-91

**Latar belakang:** Gangguan durasi haid pada pramugari dapat mengganggu performa kerja. Tujuan penelitian adalah untuk mengetahui faktor-faktor risiko yang terhadap gangguan durasi haid pada pramugari.


**Hasil:** Di antara 521 pramugari sebanyak 393 setuju berpartisipasi, 19 dieksklusi karena menderita gangguan durasi haid sebelum bekerja, sehingga diperoleh 374 subyek untuk analisis, dan 35,8% di antaranya menderita gangguan durasi haid. Stres kerja, jenis penerbangan dan usia merupakan faktor risiko dominan terhadap gangguan durasi haid. Pramugari dengan stres kerja memiliki risiko menderita gangguan durasi haid 58% lebih tinggi [risiko relatif suai (RRa) = 1,58; interval kepercayaan (CI) = 0,96-2,62; P = 0,071]. Pramugari dengan jenis penerbangan jarak jauh dalam tiga bulan terakhir memiliki risiko 69% lebih tinggi mengalami gangguan durasi haid (RRa=1,69; CI=1,17-2,43). Pramugari berumur 30–39 tahun memiliki risiko gangguan durasi haid 50% lebih rendah (RRa = 0,50; 95% CI = 0,22-1,02; P = 0,057).

**Kesimpulan:** Pramugari dengan stres kerja, jenis penerbangan jarak jauh dalam tiga bulan terakhir, dan berusia 19–24 tahun memiliki risiko lebih tinggi gangguan durasi haid.

**Kata kunci:** durasi haid, stres kerja, pramugari, Indonesia
Armedy Ronny Hasugian, Hadjar Siswantoro, Michael P. Fay, Emiliana Tjitra

Artemisinin-napthoquine dibandingkan dihydroartemisinin-piperaquine pada subyek dewasa penderita Plasmodium vivax infection

Health Science Journal of Indonesia 2015;6:92-8

Latar belakang: Penelitian ini untuk membandingkan efikasi dan keamanan Artemisinin Napthoquine (AN) dengan dosis tunggal dan obat pilihan ACT lain, dengan Dihydroartemisinin-Piperaquine (DHP) pada pengobatan P.vivax.


Hasil: Total 158 subyek dianalisis. Sejumlah 80 subyek direkrut pada kelompok AN dan 78 dengan DHP. Median Parasite Clearance Estimator (PCE) yaitu 2,32 (kisaran: 1,42 – 7,78; IQR: 1,99 – 2,82) jam pada AN dan 2,05 (kisaran: 1,30 – 8,30; IQR: 1,82 – 2,46) jam pada DHP. Parasite clearance sudah terjadi dalam 64 jam. Adequate clinical parasitological response (ACPR) at day 42 was 100% (95% CI: 95,2-100) pada AN, dan 100% (95%CI: 94,9-100) pada DHP. Kejadian Sampingan ditemukan ringan dan dapat ditoleransi.

Kesimpulan: Artemisinin-napthoquine dan dihydroartemisinin-piperaquine memiliki efikasi dan keamanan yang sebanding untuk pengobatan malaria vivaks dewasa. Walau pun pembersihan parasit AN lebih lama dibandingkan DHP, 100% pembersihan terjadi pada kedua kelompok pengobatan dalam 64 jam.

Kata kunci: malaria, Artemisinin, napthoquine, dihydroartemisinin, vivax

Nuning Rahmawati, Awal Prichatin Kusuma Dewi, Yuli Widiyastuti

Efek gabungan tanaman obat terhadap infus glukosa darah, kolesterol, dan trigliserida di antara tikus Sprague Dawley- hiperglikemia

Health Science Journal of Indonesia 2015;6:99-104

Latar belakang: Kadar glukosa, kolesterol dan trigliserida darah yang tinggi dapat memicu terjadinya berbagai penyakit sehingga diperlukan cara untuk mengontrol ketiga parameter tersebut, salah satunya dengan obat tradisional. Penelitian dilakukan untuk membuktikan efek penurunan kadar glukosa, kolesterol dan trigliserida darah tikus Sprague-Dawley (SD) dari infusa kombinasi sambiloto, salam, kayu manis, dan temulawak.

Metode: Penelitian terhadap 3 kelompok uji (kontrol, perlakuan, dan metformin sebagai kontrol positif) dilakukan pada bulan Juni-Agustus 2014 di laboratorium hewan coba B2P2TOOT Jawa Tengah. Hewan uji adalah tikus galur SD usia... Induksi hiperglikemik tikus uji dengan pemberian high fructose diet (HFD), yaitu campuran fruktosa (36%), kuning telur (20%), dan pellet standar (44%) dalam 0,36 g /200 g Berat Badan (BB) selama 70 hari. Setelah diperoleh tikus gula darat positif, dilanjutkan pemberian infusa formula selama 7 hari. Ketiga parameter uji meliputi kadar glukosa, trigliserida, dan kolesterol total tikus uji hari ke-77 dibandingkan dengan hari ke-70. Pemeriksaan histopatologi organ pankreas dilakukan pada akhir percobaan.

Hasil: Pemberian HFD selama 70 hari menyebabkan peningkatan signifikan kadar glukosa (p=0,0001), kolesterol (p=0,001) dan trigliserida total (p=0,006) darah. Peningkatan berat badan tikus uji dibandingkan tikus kelompok kontrol tidak berbeda bermakna (p=0,792). Pemberian infusa ramuan sambiloto, salam, kayu manis, dan temulawak dosis 491,4 mg/200 g BB menurunkan kadar glukosa, kolesterol, dan trigliserida darah tikus uji berurutan sebesar 37,09% 19,51%, dan 79,29%.

Kesimpulan: Pemberian infusa ramuan sambiloto, salam, kayu manis, dan temulawak dosis 491,4 mg/200 g BB selama 7 hari secara signifikan menurunkan kadar glukosa darah, kolesterol. dan trigliserida tikus hiperglikemik.

Kata kunci: hiperglikemik, tikus, sambiloto
Nanang Yunarto, Nurul Aini

Efek gambir murni ekstrak daun untuk mencegah aterosklerosis pada tikus

Health Science Journal of Indonesia 2015;6:-105-10


Metode: Desain penelitian ini adalah eksperimental laboratorium yang dilakukan di Laboratorium Farmasi dan Laboratorium Hewan, Badan Litbangkes, Kemenkes RI pada tahun 2014. Ekstrak gambir dipurifikasi untuk diperoleh kandungan katekin yang optimal, selanjutnya dilakukan uji aktivitas antioksidan menggunakan pereaksi 2,2-difenil-1- pirkilhidrazil (DPPH) dengan pembanding asam askorbat. Penelitian ini menggunakan 36 ekor tikus putih jantan galur Sprague Dawley berusia 2,5 bulan yang dibagi secara acak ke dalam enam kelompok yaitu kelompok normal, kontrol negatif (akuades), kontrol positif (atorvastatin 2 mg/200 g bb), ekstrak dosis I (20 mg/200 g bb), dosis II (40 mg/200 g bb) dan dosis III (80 mg/200 g bb). Tikus diinduksi dengan makanan yang mengandung lemak tinggi dan perlakuan pengobatan sesuai kelompoknya selama 60 hari, kecuali kontrol normal.

Hasil: Kadar katekin dalam ekstrak gambir terpurifikasi diperoleh sebesar 92,69%. Dari uji aktivitas antioksidan diperoleh IC50 11,76 μg/mL. Hasil pengukuran aktivitas antiaterosklerosis menunjukkan bahwa jika dibandingkan dengan kontrol negatif, ketiga dosis ekstrak gambir terpurifikasi mampu mencegah terjadinya aterosklerosis dengan menghambat penebalan dinding aorta dan pembentukan sel busa (p<0,05). Aktivitas antiaterosklerosis meningkat dengan bertambahnya dosis.

Kesimpulan: Ekstrak gambir terpurifikasi mempunyai efek mencegah penebalan dinding dan pembentukan sel busa aorta tikus.

Kata kunci: gambir, katekin, antiaterosklerosis

Cicih Opitasari

Pendidikan ibu, prematuritas dan risiko asfiksia bayi baru lahir di rumah sakit terpilih di Jakarta

Health Science Journal of Indonesia 2015;6:111-5


Hasil: Pada analisis ini diperoleh 2777 sampel dari 4191 yang memiliki data lengkap. Proporsi asfiksia pada bayi baru lahir adalah 6,5%. Jika dibandingkan wanita berpendidikan rendah, wanita berpendidikan rendah memiliki risiko melahirkan bayi asfiksia 4,3 kali lebih tinggi [rasio odds suaian (ORa) = 4,31; P=0,000], sedangkan wanita berpendidikan menengah dibandingkan dengan yang tinggi berisiko 3,3 kali lebih tinggi melahirkan bayi dengan asfiksia (ORa=3,31; P=0,000). Selanjutnya, jika dibandingkan bayi cukup bulan, bayi prematur memiliki risiko 3,1 kali lebih tinggi mengalami asfiksia (ORa=3,07; P=0,000), sedangkan bayi postmatur 63% (namun tidak signifikan) lebih tinggi berisiko mengalami asfiksia (P=0.118).

Kesimpulan: Wanita yang memiliki tingkat pendidikan rendah dan menengah serta bayi prematur memiliki risiko lebih tinggi mempunyai bayi baru lahir yang asfiksia. (Health Science Journal of Indonesia 2015;6:-)

Kata kunci: pendidikan, prematur, asfiksia bayi baru lahir
Listrik dan faktor lain yang terhadap kemungkinan dengan Puskesmas mempunyai kerusakan vaksin campak rusak

Health Science Journal of Indonesia 2015;6:116-20

Latar belakang: Puskesmas yang memiliki vaksin campak rusak dipengaruhi oleh beberapa faktor risiko. Oleh karena itu perlu diidentifikasi beberapa faktor risiko dominan yang mempengaruhinya.

Metode: Analisis menggunakan sebagian data Riset Fasilitas Kesehatan (Rifaskes) tahun 2011. Rifaskes tersebut dilakukan di seluruh Puskesmas di 33 provinsi di Indonesia. Selanjutnya, pada analisis ini hanya menggunakan data Puskesmas di provinsi yang memiliki cakupan imunisasi campak di atas angka prevalensi nasional (81,6%), dan Puskesmas yang memiliki angka prevalensi campak di atas angka prevalensi nasional (1,18%). Analisis data statistik dilakukan dengan menggunakan analisis regresi logistik untuk menentukan beberapa faktor risiko dominan yang mempengaruhi Puskesmas yang memiliki vaksin campak rusak.

Hasil: Sebanyak 7(Riau, Jakarta, Nusa Tenggara Barat, Nusa Tenggara Timur, Sulawesi Tengah, Sulawesi Selatan, Gorontalo) sebanyak 1259 Puskesmas memenuhi kriteria inklusi. Puskesmas yang berlokasi di perdesaan dibandingkan dengan perkotaan berisiko 3,4 kali lipat yang merupakan Puskesmas yang memiliki vaksin campak rusak [rasio odds suaiain (ORa) = 3,37; 95% interval kepercayaan (CI) = 1,34-8,26]. Selanjutnya, Puskesmas dengan ketersediaan listrik PLN kurang dari 24 jam dibandingkan dengan tersedia selama 24 jam mempunyai risiko 2,1 kali lipat merupakan Puskesmas yang memiliki vaksin campak rusak (ORa = 2,10; 95% CI = 1,02-4,33).

Kesimpulan: Puskesmas yang mempunyai kerusakan vaksin campak yang rusak lebih banyak terjadi di Puskesmas di perdesaan dan yang tidak tersedia listrik PLN maupun ketersediaan listrik sehari-hari kurang dari 24 jam merupakan Puskesmas yang memiliki vaksin campak rusak.

Kata kunci: vaksin campak rusak, Puskesmas

Tahap infeksi HIV dan risiko infeksi oportunistik Tuberkulosis

Health Science Journal of Indonesia 2015;6:121-5

Latar belakang: Infeksi oportunistik (IO) oleh tuberkulosis adalah penyebab kematian tertinggi orang yang terinfeksi HIV di seluruh dunia, terutama pada infeksi HIV yang tidak diobati. Tujuan penelitian ini adalah untuk mengetahui faktor risiko infeksi oportunistik TB pada orang dengan HIV.


Hasil: Di antara 490 pasien HIV, 238 (48,6%) pasien melaporkan TB sebagai IO. Semakin muda kelompok subyek dengan HIV semakin besar persentase IO TB. Penderita HIV laki-laki mempunyai risiko 24% lebih tinggi terinfeksi TB dibandingkan perempuan [risiko relatif suaiain (RRa) = 1,24; P = 0,023]. Sedangkan subyek dengan tingkat stadium 1 sampai dengan 3 dibandingkan subyek dengan tingkat stadium akhir berisiko 52% terinfeksi TB (RRa = 1,55; P = 0,000).

Kesimpulan: Infeksi HIV stadium 4 dan penderita laki-laki lebih berisiko terkena IO TB. (Health Science Journal of Indonesia 2015;6:-)

Kata kunci: Tuberkulosis, HIV, infeksi oportunistik, Indonesia
Tegar A. P. Siregar2 T. Mirawati Sudiro, Whinie Lestari, Beti Ernawati Dewi

Beberapa konservasi epitop non struktural 3 protein virus dengue serotipe 4

Health Science Journal of Indonesia 2015;6:126-31

Latar belakang: Protein Non Struktural 3 (NS3) virus dengue menginduksi respon antibodi netralisasi dan respon sel T CD4+ dan CD8+, serta berperan dalam replikasi virus. Protein NS3 memiliki epitop-epitop sel T dan B yang terdapat perbedaan kelestarian pada berbagai strain virus dengue serotipe 4 (DENV-4). Penelitian ini bertujuan untuk mengetahui kelestarian epitop sel T dan B pada protein NS3 DENV-4 strain-strain dunia dan keempat serotipe virus dengue strain Indonesia.


Hasil: Delapan epitop sel T dan 2 epitop sel B dari protein NS3 DENV-4 081 ternyata identik dan keempat epitop sel T dan B pada protein NS3 DENV-4 081 ternyata identik dan lestari pada protein NS3 dari 124 strain DENV-4 dunia dan keempat epitop NS3 dari keempat serotipe DENV strain Indonesia.

Kesimpulan: Kelestarian yang luas dari epitop sel T dan B pada strain dunia sebesar 72% kekestan dan keempat epitop sel T dan B pada strain Indonesia kekestan 75%.

Kata kunci: virus dengue, protein NS3, epitop sel T, epitop sel B

Roselinda, Vivi Setiawaty

Tipe pekerja seks komersial dan risiko sifilis

Health Science Journal of Indonesia 2015;6:132-6


Metode: Data dari Survei WPS menggunakan kuesioner terstruktur di 7 kota (Kupang, Samarinda, Pontianak, Yogyakarta, Timika, Makassar dan Tangerang) di Indonesia tahun 2007, desain potong lintang dan responden dipilih secara cluster random sampling dari WPS langsung dan tidak langsung yang memenuhi kriteria definisi operasional. Diagnosa Sifilis ditegakkan dengan pemeriksaan laboratorium Rapid Plasma Reagen (RPR) dan Treponema pallidum Haemaglutination Assay (TPHA).

Hasil: Sebanyak 1750 responden ikut dalam penelitian dan 12,2% terindikasi sifilis. Kota Makassar mempunyai prevalensi yang tertinggi sebesar 55,2%. WPS yang berlokasi di luar pulau Jawa memiliki risiko terinfeksi sifilis 3,16 kali lebih tinggi dibandingkan dengan WPS yang berlokasi di pulau Jawa [risiko relatif suaiuan (RRa) = 3,16 ; P= 0,000]. Tipe WPS tidak langsung memiliki risiko 46% lebih besar untuk terinfeksi sifilis dibandingkan dengan WPS yang langsung [RRa = 1,46; 95%; P = 0,002], sedangkan WPS yang mencari pengobatan ke dokter memiliki risiko 58% lebih besar dibandingkan yang berobat ke sarana kesehatan langsung (RRa = 1,58; P = 0,006).

Kesimpulan: Lokasi WPS yang berada di luar pulau Jawa, tipe WPS tidak langsung memiliki risiko lebih tinggi untuk terinfeksi penyakit sifilis. Wanita pekerja seks yang mencari pengobatan ke dokter menyebabkan penyakit terindikasi lebih tinggi dibandingkan jika berobat ke sarana pelayanan kesehatan lainnya.

Kata kunci: Sifilis, Wanita Pekerja Seks, Indonesia
Inne Yuliawati, Minarma Siagian, Thamrin Abudi, Bastaman Basuki

The number of sectors and other risk factors related to fatigue among short-haul commercial pilots in Indonesia

Health Science Journal of Indonesia 2015;6:69-75

Background: Fatigue could impair cognitive function in pilots which may lead to accidents in short-haul flight. The aim of this study was to identify the risk factors related to fatigue among short-haul commercial pilots in Indonesia.

Methods: A cross-sectional study with purposive sampling was conducted among Boeing 737 series typed-rating pilots taking medical examination at the Civil Aviation Medical Center, Jakarta from May 5-26, 2014. Fatigue was measured with Self-Reporting Questionnaire, Fatigue Severity Scale (FSS). Data were collected using anonymous self-reporting questionnaire on demographics, workload, sleep restriction (Epworth Sleepiness Scale-ESS), personal factors, and managerial support. Linear regression was used to identify dominant risk factors related to fatigue.

Results: During data collection, 785 pilots were taking medical examination, 382 pilots were willing to participate, and 239 pilots met the criteria. The FSS mean was 4.66 ± 1.202. The number of sectors in 24 hours, flight times of unplanned flights in 30 days, and sleep restriction were dominant factors of fatigue. Each additional sector increased FSS by 0.371 points [regression coefficient (β) = 0.371; P = 0.000]. Furthermore, each additional ESS, increased FSS by 0.043 points (β = 0.043; P = 0.008), while each additional unplanned flights increased FSS by 0.033 points (β = 0.033; P = 0.000).

Conclusions: Additional number of sectors in 24 hours, additional unplanned flight times within 30 days, and sleep restriction increased the risk of fatigue among short-haul commercial pilots in Indonesia.

Keyword: Fatigue, number of sectors, pilots, Indonesia

Intan Mariska, Danardi Sosrosumihardjo, Widura Imam Moestopo, Bastaman Basuki

The purser support and tendency of depression among civilian female flight attendants in Indonesia

Health Science Journal of Indonesia 2015;6:76-80

Background: Depression can be occurred among female flight attendants. The purpose of this study was showing a correlation between purser support and other factors with depression among female civilian flight attendants in Indonesia.

Methods: Cross sectional study with purposive sampling in female civilian flight attendants who were taking routine medical check up at Civil Aviation Medical Center Jakarta, May 12th to18th 2014. Those who took antidepressants or benzodiazepines and who drank alcohol more than 3 times in a day were excluded for this study. The depression was measured using Beck Depression Inventory Questionnaire. The purser support, co-workers support, and support outside of work were measured using the NIOSH Generic Job Stress Questionnaire. Risk factors for depression were analyzed using linear regression.

Results: Out of 242 female flight attendants who were taking routine medical check up, 145 persons met the study criteria. Purser support, support outside of work, as well as mental work load were dominant risk factors related to depression among female civilian flight attendants. One point of purser support decreased 0.552 point of depression [regression coefficient (β) = -0.552; p = 0.033]. One point increase of support outside of work decreased 1.191 point of depression [β = -1.191; p = 0.000]. On the other hand, one point of mental workload increased 0.549 points depression (β = 0.549; p = 0.045).

Conclusion: More purser and outside of work supports decreased the risk of depression, however, more mental work load increased depression among civilian flight attendants in Indonesia.

Keywords: depression, purser support, mental workload, female flight attendants.
Inne Yuliawati, Minarma Siagian, Thamrin Abudi, Bastaman Basuki

**The effect of workload and other risk factors of metabolic syndrome among short-haul commercial pilots in Indonesia**

Health Science Journal of Indonesia 2015;6:81-6

**Background:** Metabolic syndrome (MS) could cause sudden incapacitation among pilots in Indonesia. The aim of this study was to identify risk factors of MS among short-haul commercial pilots in Indonesia.

**Methods:** A cross-sectional study with purposive sampling was conducted among commercial pilots taking medical examination at the Civil Aviation Medical Center, Jakarta from July 27-August 30th, 2014. Metabolic syndrome was assessed according to the National Cholesterol Education Program Adult Treatment panel III criteria and criteria. Risk factors were collected using anonymous self-reporting questionnaire. The laboratory data were extracted from medical records. Cox regression was used to identify dominant risk factors of MS.

**Results:** During data collection, 2135 pilots taking medical examination, Total male Asian pilots met the inclusion criteria was 864 pilots. Prevalence of MS was 18.28%. Compared to 20-35 year-old group, subjects aged 56-65 years-old had 88% higher risk for MS [adjusted relative risk (RRa) = 1.88; P = 0.019]. In term of number of sectors in the last 24 hours, compared to 0-3 sectors, subjects who had 6-7 sectors in the last 24 hours had 66% higher risk for MS (RRa = 1.66; P = 0.033), while subjects who had 8 or more sectors in 24 hours had 82% more risk for MS (RRa = 1.82; P = 0.072).

**Conclusions:** The pilot aged 56-65 years-old, who had 6 or more sectors in the last 24 hours, had higher risk for metabolic syndrome among short-haul commercial pilots in Indonesia.

**Keyword:** metabolic syndrome, number of sectors, pilots, Indonesia

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Melissa Audry Rampen,1 Setyawati Budiningsih,1 Agus Supriyadi,2 Bastaman Basuki1

**Job stress and risk of menstrual duration disorder in female civilian flight attendants in Indonesia**

Health Science Journal of Indonesia 2015;6:87-91

**Background:** Menstrual duration disorder may cause impaired work performance. The research objective was to identify risk factors related to menstrual duration disorder in female flight attendants.

**Methods:** A cross-sectional study with convenient sampling was conducted on civilian female flight attendants age 19–50 years who underwent routine medical examination at Civil Aviation Medical Center and Garuda Sentra Medika, Jakarta on May 18-29 2015. Menstrual duration disorder is menstruation more than 8 days and/or shorter than usual period (3-5 days). Stress was identified by using criteria of National Institute for Occupational Safety and Health Generic Job Stress Questionnaire Mental Demands Form Number 11. Relative risk was analyzed using Cox regression.

**Results:** Among 521 female civilian flight attendants, 393 were willing to participate in this study. Nineteen subjects were excluded, leaving 374 subjects for this analysis, and 35.8% of subjects had menstrual duration disorder. Job stress, flight type and age were dominant risk factors for menstrual duration disorder. Subjects with job stress and long haul flight within three months had higher risk for having menstrual duration disorder by 58% [adjusted relative risk (RRa) = 1.58; confidence interval (CI) = 0.96-2.62; P = 0.071] and 69% (RRa = 1.69; CI = 1.17-2.43) respectively. Those between aged 30-39 years had 50% had less risk of having menstrual duration disorder (RRa=0.50; 95% CI = 0.22-1.02; P = 0.057).

**Conclusion:** Female civilian flight attendants with job stress, long haul flight within three months and younger age had higher risk to be menstrual duration disorder.

**Key words:** menstrual duration, job stress, female civilian flight attendant, Indonesia
Artemisinin-napthoquine versus dihydroartemisinin-piperaquine in adult subjects with *Plasmodium vivax* infection

Health Science Journal of Indonesia 2015;6:92-8

**Background:** This study aimed to compare the efficacy and safety of a single dose Artemisinin-Napthoquine (AN), as an alternative drug, to Dihydroartemisinin-Piperaquine (DHP), as a three-day standard regimen on *P. vivax* infection.

**Methods:** This was an open randomized study with subjects recruited from St. Gabriel hospital in Maumere, and Police, Army, and Navy Hospital in Jayapura in 2007 and 2008. This study was part of a previously published study for malarial infections. Efficacy was the absence of clinical and parasitological malaria until day 42, performed as Adequate Clinical and Parasitological Response (ACPR). Safety was performed based on adverse event in any day of follow up which was never reported at day recruitment (d0).

**Results:** In this study 158 subjects of *P. vivax* were analyzed, the 80 subjects was treated with AN and 78 with DHP. The median Parasite Clearance Estimator (PCE) was 2.32 (range: 1.42 – 7.78; Interquartile Ranges (IQR) were 1.99 – 2.82) hours in AN and 2.05 (range: 1.30 – 8.30; IQR: 1.82 – 2.46) hours in DHP group. The parasite clearance was complete by 64 hours. The ACPR was 100% (95% Confidence Interval (CI): 95.2-100) in the AN, and 100% (95% CI: 94.9-100) in the DHP group. Both drugs have similar mild and tolerated adverse events.

**Conclusions:** Both drugs have similar efficacy and safety for the treatment of *P. vivax* infection in adults. Although AN had a longer PCE compared to DHP. In 64 hours, 100% clearance was achieved in both groups.

**Key word:** malaria, Artemisinin, napthoquine, dihydroartemisinin, *P vivax*

The effects of combined medicinal plants infusion on blood glucose, cholesterol, and triglyceride levels in hyperglycemic *Sprague-Dawley* rats

Health Science Journal of Indonesia 2015;6:99-104

**Background:** High levels of blood glucose, cholesterol, and triglyceride tend to increase the incidence of several diseases. This study aimed to prove the effects of combined infusion of *sambiloto*, *salam*, *kayu manis*, and *temulawak* on decreasing blood levels of glucose, cholesterol, and triglyceride in Sprague-Dawley (SD) rats.

**Methods:** The rats were divided into 3 groups (control, treatment, and metformin as positive control). The study was conducted at the animal laboratory of MPTMRDC, Central Java, on June to September 2014. Hyperglicemia was induced by administering high fructose diet (HFD), a mixture of fructose (36%), egg yolk (20%), and standard pellets (44%) in 0.36 g/200 g Body Weight (BW) for 70 days. The combined infusion was given orally to the hyperglycemic rats for 7 consecutive days. The parameters were blood levels of glucose, cholesterol, and triglycerides. The pancreas was examined histopathologically at the end of the study.

**Results:** HFD for 70 days led to significant increase in glucose (p=0.0001), cholesterol (p=0.001), and total triglycerides (p=0.006) levels. The increase of body weight of experiment group had no significant difference with control group (p=0,792). The combined infusion of 491.4 mg/200 g BW significantly reduced blood glucose, cholesterol, and triglyceride levels in rats by 37.09, 19.51, and 79.29 %, respectively.

**Conclusion:** The administration of the combined infusion with a dose of 491.4 mg/200 g BW for 7 consecutive days decreased blood glucose, cholesterol, and triglyceride levels in hyperglycemic rats.

**Keywords:** hyperglycemia, rat, glucose, *sambiloto*
Nanang Yunarto, Nurul Aini

**Effect of purified gambir leaves extract to prevent atherosclerosis in rats**

Health Science Journal of Indonesia 2015;6:105-10

**Background:** Atherosclerosis is a risk factor for coronary heart disease (CHD). Catechin have high antioxidant activity that can prevent atherosclerosis. Gambir (*Uncaria gambir*, Roxb.) leaves extract have high catechin content thereby potentially inhibiting atherosclerosis. This research was aimed to examine effect of purified gambir leaves extract to prevent atherosclerosis in rats.

**Methods:** The experimental laboratory study was conducted in the Laboratory of Pharmacy and Animal Laboratory, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia in 2014. Gambir leaves extract were purified to gain optimum catechin. Afterwards, antioxidant activity was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, with ascorbic acid as positive control. Thirty six white male Sprague Dawley rats aged 2.5 months were randomly divided into six groups, i.e. normal control group, negative control group (aquadest), positive control group (atorvastatin 2 mg/200 g bw), extract dose I (20 mg/200 g bw), dose II (40 mg/200 g bw) and dose III (80 mg/200 g bw). The rats were given high fat diet and treatment according to their group for 60 days, except for normal control group.

**Results:** Catechin content in the purified gambir leaves extract was 92.69%. From antioxidant activity test, IC$_{50}$ was found to be 11.76 µg/mL. Anti-atherosclerotic activity study showed that compared to negative control, all three doses of purified gambir leaves extract were able to prevent atherosclerosis through inhibition of aortic wall thickening and foam cell formation due to high fat diet (p<0.05). Anti-atherosclerotic activity increased with increasing dose.

**Conclusion:** Gambir leaves purified extract had the effect of preventing the thickening of the walls and foam cell formation rat aorta

**Keywords:** gambir, catechin, antiatherosclerosis

Cicih Opitasari

**Maternal education, prematurity and the risk of birth asphyxia in selected hospitals in Jakarta**

Health Science Journal of Indonesia 2015;6:111-5

**Background:** Birth asphyxia can causes hypoxic ischemic organ damage in neonates. According to advanced Indonesian Basic Health Research 2007, asphyxia was the second highest cause of infant death after infection (13.8%). This study aimed to identify several risk factors associated with birth asphyxia.

**Methods:** This study was carried out in one government and one private hospitals in Jakarta. All medical records of pregnant women who gave birth between January 1 to December 31 of 2011 were included. Birth asphyxia was defined as an Apgar score less then seven at one minute after birth. Logistic regression was used to analyze the risk factors related to birth asphyxia.

**Results:** As many as 2777 samples out of 4191 were included for this analysis. The proportion of asphyxia in newborn babies was 6.5%. Compared with those who had high educated mothers, those who had low education level had 4.3-fold higher risk to have an asphyxiated baby [adjusted odds ratio (ORa) = 4.31; P=0.000]. Meanwhile middle educated mothers had 3.3-fold higher risk to have birth asphyxia (ORa=3.31; P=0.000). In terms of gestasional age at birth, compared with those who had full term birth, those who had preterm birth had 3.1-fold higher risk to have birth asphyxia (ORa=3.07; P=0.000). Meanwhile, although not significant, those who had postterm birth had 63% more risk (P=0.118) to have birth asphyxia.

**Conclusion:** The mother who had lower and middle education levels as well as preterm babies had higher risk for having birth asphyxia baby.

**Keyword:** maternal education, prematurity, birth asphyxia
Anggita Bunga Anggraini, Nyoman Fitri

Electricity and other factor related to Public Health Center in Indonesia had measles vaccine damage

Health Science Journal of Indonesia 2015;6:116-20

Background: The Public Health Center (PHC) had broken measles vaccine was influenced by a number of risk factors. Therefore, it was necessary to identify some dominant risk factors that related to PHC had measles damage vaccines.

Methods: The analysis used a part of the data of Research Health Facilities (Rifaskes) in 2011. The Rifaskes was conducted in all health centers in all (33) provinces in Indonesia. Furthermore, this analysis uses data only health center in the province who have measles immunization coverage the national prevalence rate (81.6%) or more, and health centers that have measles prevalence rate above the national prevalence rate (1.18%) or more. Statistical data analysis performed using logistic regression analysis to determine some of the risk factors related to the health center had has measles vaccine damaged.

Results: A number of 7 provinces (Riau, Jakarta, West Nusa Tenggara, East Nusa Tenggara, Central Sulawesi, South Sulawesi, Gorontalo) of 1259 PHC met the inclusion criteria. Health centers located in rural areas compared with urban areas had 3.4-fold had measles damage vaccines [adjusted odds ratio (ORa) = 3.37; 95% confidence interval (CI) = 1.34 - 8.26]. Furthermore, the health center with the availability of the electricity for less than 24 hours compared with available 24 hours had 2.1-fold the risk of a health center that had measles damage vaccines (ORa = 2.10; 95% CI = 1.02 - 4.33).

Conclusion: Health center in rural areas, or did not have have commercial electric power, or did not have not have the availability of day-to-day electricity less than 24 hours had more risk to have measles damage vaccines.

Keywords: measles, public health center, vaccine

Roselinda, Vivi Setiawaty

The stages of HIV infection and the risk of opportunistic Tuberculosis infection

Health Science Journal of Indonesia 2015;6:121-5

Introduction: Opportunistic infection (OI) by tuberculosis is the most common cause of death for people infected with HIV worldwide, mainly in persons with untreated HIV infection. The aim of the study was to determine the risk factors associated with TB as an OI of HIV-infected patients.

Methods: The study conducted in Voluntary Counseling Testing (VCT) clinics in several hospitals of seven provinces (North Sumatera, West Sumatera, Riau Islands, South Sulawesi, North Sulawesi, Maluku, and Papua). This was a cross sectional study and the respondents selected by purposive sampling. There were 490 HIV patients included in this study. Data were collected using a specific questionnaire. Statistical analyses were done using STATA 9.0 version.

Results: Among 490 HIV patients, there were 238 (48.6%) patients with TB as IO. The percentage of TB as OI in the younger HIV-infected group was higher than the older group. Among the HIV-infected persons, males had a 24 % higher risk of becoming infected with TB than females [adjusted relative risk (RRa)=1.24; P = 0.023]. Stage 4 HIV-infected persons had 52% higher risk of TB as OI compared to stages 1-3 (RRa = 1.55; P = 0.000).

Conclusion: Stage 4 of HIV cases and male patients were more at risk for developing TB IO.

Keywords: Tuberculosis, HIV, opportunistic infection, Indonesia
Tegar A. P. Siregar, T. Mirawati Sudiro, Whinie Lestari, Beti Ernawati Dewi  

**Some epitopes conservation in non structural 3 protein dengue virus serotype 4**

Health Science Journal of Indonesia 2015;6:126-31

**Background:** Non Structural 3 (NS3) protein of dengue virus (DENV) is known to induce antibody, CD4+ and CD8+ T cell responses, as well as playing role in viral replication. NS3 protein has T and B cell epitopes, which has conservation difference between DENV-4 strains. This study aimed to identify conservation of T and B cell epitope in NS3 protein among DENV-4 strains and four serotypes DENV of Indonesia strains.

**Methods:** Research was held at the Department of Microbiology, Faculty of Medicine, Universitas Indonesia, June 2013 to April 2014. NS3 amino acid sequence of DENV-4 081 strain was obtained after NS3 gene of DENV-4 081 PCR products were sequenced. T and B cell epitopes of NS3 protein of DENV-4 081 strain were analysed and compared to NS3 proteins of 124 DENV-4 strains around the world and four serotypes of Indonesia strains.

**Results:** Eight positions of T cell epitopes and two positions of B cell epitopes of NS3 DENV-4 081 were identical and conserved to NS3 protein of 124 DENV-4 strains around the world. B cell epitope of NS3 DENV-4 081 protein at aa 537-544 was found identical and conserved to four serotypes DENV of Indonesia strains.

**Conclusion:** This wide conservation of T and B epitopes in almost all DENV-4 strains around the world and all serotypes of Indonesia strains.

**Keywords:** dengue virus, NS3 protein, T cell epitope, B cell epitope

Roselinda, Vivi Setiawaty

**Type of female sex worker and other risk factors of syphilis**

Health Science Journal of Indonesia 2015;6:132-6

**Background:** Syphilis is one of the chronic sexual transmission diseases which is caused by Treponema pallidum bacteria that can cause disability in patients and babies born. This analysis aims at looking at the relationship type and work duration as Female Sex Workers (FSW) and the syphilis cases within 7 cities in Indonesia.

**Methods:** The data was taken from Survey on FSW using a structured questionnaire in 7 cities (Kupang, Samarinda, Pontianak, Yogyakarta, Timika, Makassar and Tangerang) in Indonesia in 2007, the cross-sectional design and respondents are selected by cluster random sampling directly and indirectly towards the WPS who fulfill the operational definition criteria. Syphilis diagnosis was confirmed by laboratory tests Rapid Plasma Reagents (RPR) and Treponema pallidum Haemaglutination Assay (TPHA).

**Results:** There were 1750 respondents who participated in the study and about 12.2% were infected with the syphilis. Makassar has the highest prevalence about 55.2%. The WPS who are located outside of Java have the syphilis infection risk about 3.16 times higher than the WPS located in Java [Adjusted Relative Risk (RRa) = 3.16; P = 0.000]. The indirect WPS had 1.46 times risk for syphilis infection compared to direct WPS (RRa = 1.46; P = 0.002), whereas the FSW who seek treatment from doctor have a risk about 58% more risk compared to the direct health facilities treatment (RRa = 1.58; P = 0.006).

**Conclusion:** The location of FSW which is outside of Java, the FSW does not directly have a higher risk of being infected with syphilis. Female sex workers who seek the doctor treatment are able to be indicated earlier rather than they are who seek treatment to other health care facilities.

**Keyword:** Syphilis, Female Sex Worker, Indonesia
The number of sectors and other risk factors related to fatigue among short-haul commercial pilots in Indonesia

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Abstract

Background: Fatigue could impair cognitive function in pilots which may lead to accidents in short-haul flight. The aim of this study was to identify the risk factors related to fatigue among short-haul commercial pilots in Indonesia.

Methods: A cross-sectional study with purposive sampling was conducted among Boeing 737 series typed-rating pilots taking medical examination at the Civil Aviation Medical Center, Jakarta from May 5-26, 2014. Fatigue was measured with Self-Reporting Questionnaire, Fatigue Severity Scale (FSS). Data were collected using anonymous self-reporting questionnaire on demographics, workload, sleep restriction (Epworth Sleepiness Scale-ESS), personal factors, and managerial support. Linear regression was used to identify dominant risk factors related to fatigue.

Results: During data collection, 785 pilots were taking medical examination, 382 pilots were willing to participate, and 239 pilots met the criteria. The FSS mean was 4.66 ± 1.202. The number of sectors in 24 hours, flight times of unplanned flights in 30 days, and sleep restriction were dominant factors of fatigue. Each additional sector increased FSS by 0.371 points [regression coefficient (β) = 0.371; P = 0.000]. Furthermore, each additional ESS, increased FSS by 0.043 points (β = 0.043; P = 0.008), while each additional unplanned flights increased FSS by 0.033 points (β = 0.033; P = 0.000).

Conclusions: Additional number of sectors in 24 hours, additional unplanned flight times within 30 days, and sleep restriction increased the risk of fatigue among short-haul commercial pilots in Indonesia. (Health Science Journal of Indonesia 2015;6:69-75)

Keyword: Fatigue, number of sectors, pilots, Indonesia
Fatigue could impair cognitive function in pilots, including judgment, memory, concentration, selective attention and decision making. These factors have been known as contributory factors to aircraft accidents and incidents. The National Aeronautics and Space Administration (NASA) Aviation Safety Reporting System indicated that 21% of reported aviation incidents were fatigue related.

The causes of pilot fatigue are primarily related to sleep loss for both long-haul and short-haul flights. Night flights and jet lag are the most important factors that generated fatigue in long-haul flights. While other studies revealed that fatigue in short-haul operation was caused by the number of sectors, more than one unplanned flights in a month, cumulative duty time in the preceding week, and sleep restriction. Repeated mild sleep restriction without sufficient opportunity for recuperation, could cause cumulative fatigue and lead to great risk for aviation safety.

Indonesia is an archipelago nation and short-haul domestic flights between cities or small islands play an important role. High domestic passenger rate and intense competition among airlines in Indonesia pushed airline management to a culture of short-haul pilots flying more sectors, flying unplanned flights during rest periods and cumulatively flying close to legal maximum of flight and duty time limitations, as was clarified by 30% of subjects during preliminary study. This condition may adversely affect the safety of short-haul flights in Indonesia.

The aim of this study was to identify the dominant risk factors related to fatigue among short-haul commercial pilots in Indonesia.

METHODS

This cross-sectional among the population of active pilots working for commercial airlines was conducted under Civil Aviation Safety Regulation (CASR) part 121 in Indonesia. Subjects were selected by purposive sampling from commercial pilots taking medical examinations at the Civil Aviation Medical Center in Jakarta from May 5 – 26, 2014.

The inclusion criteria were active commercial pilots holding an Airline Transport Pilot License (ATPL) or Commercial Pilot License (CPL) type license, operating in short-haul flights of less than 2 hours flight time per sector, and having a Boeing 737 series type rating. The exclusion criteria were subjects taking hypnotics or stimulants. Subjects who were willing to participate signed an informed consent letter. They were asked to fill a self-reporting questionnaire regarding demographics, workload, sleep restriction, managerial support, personal factors at home, behavior, and overall fatigue experience in the last week.

Fatigue was measured using Fatigue Severity Scale (FSS) that consisted of 9 questions relating to fatigue and its impact on functioning and behavioral aspects in the past week. The FSS questions were: (1) my motivation is lower when I am fatigued; (2) exercise brings on my fatigue; (3) I am easily fatigued; (4) fatigue interferes with my physical functioning; (5) fatigue causes frequent problem for me; (6) my fatigue prevents sustained physical functioning; (7) fatigue interferes with carrying out certain duties and responsibilities; (8) fatigue is among my most disabling symptoms; and (9) fatigue interferes with my work, family, or social life. Subjects answered the questions on a scale of “1” indicating agrees to and “7” indicating disagrees to. Mean FSS score was used as a continuous measure of fatigue scale.

While the number of sectors was any flight which has a take-off and landing at different airports which are not less than 50 nautical miles apart in the last 24 consecutive hours.

The risk factors were frequency and flight time of unplanned flights, flight duty time in the last 24 hours, sleep restriction, managerial support, personal factors at home, and health behaviors. Flight time was divided into the last 24 hours and into 7 days.

Unplanned flight was considered as flight duty, performed by the pilot, which was not in their schedule or during their day-off, in the last 30 consecutive days. This variable was divided into frequency and flight time of unplanned flight.

Flight time was considered as total elapsed time from the moment the aircraft first moved under its own power for takeoff, until the time it comes to rest at the end of the flight. This variable was divided into flight time in the last 24 hours and into 7 days.

Flight duty time was total elapsed period from the time a pilot report for duty to the time he completed all official duties in the last 24 hours.

Sleep restriction was measured by the Epworth Sleepiness Scale (ESS) that has 8 questions relating to how likely the subjects were to doze off or fall asleep in the following situations: (1) sitting and reading; (2) watching television; (3) sitting inactive.
in a public place (e.g. theatre, meeting); (4) as a passenger in a car for an hour without break; (5) lying down in the afternoon when circumstances permit; (6) sitting and talking to someone; (7) sitting quietly after a lunch without alcohol; (8) driving a car, while stopping for a few minutes in traffic. Subjects answered the questions on a scale of 0 = would never doze; 1 = slight chance; 2 = moderate chance; 3 = high chance. The value of each scale was summed and used as a continuous measure of ESS.9

Managerial support was measured using the Whitehall organizational justice that has 6 question relating to the subjects’ situation at the company they worked for, which were: (1) do you get sufficient information from line management (your superior)?; (2) do you get consistent information from line management (your superior)?; (3) how often do you get help and support from your colleagues?; (4) how often are your colleagues willing to listen to your work related problems?; (5) how often do you get help and support from your immediate superior?; (6) how often is your immediate supervisor willing to listen to your problems? Subjects answered the questions on a scale of 1 = never; 2 = seldom; 3 = sometimes; 4 = often. Managerial support was scaled as ordinal measure.4

Personal factors at home were measured by the Home Stress Checklist that has 4 questions relating to the stress situation that the subjects experienced at home every day. The question and choices of answer were: (1) how is your role at home? as the main source of family income/ as a father or mother/ as a husband/wife/ as a financial support to other family members/ as a payer or pay various bill/ as a gardener/ as home decorator/ as a household repairmen (answer can be more than one); (2) which are the physical factors at household/neighborhood that you feel can annoy you? neighborhood noise/ the narrow house/ homes that’s not well organized/ leaks and physical damage in other household/ dense neighborhood/ neighborhoods prone to flooding/ unsafe neighborhood (answer can be more than one); (3) how do you describe your tension at home: low (a bit of dispute, can be openly discussed) or moderate (there is some tension, but can be tolerable) or severe/high; (4) when you are at home, how often do you have the opportunity to yourself and do activity that’s relaxing? The answers were either every day, rarely, or several times a week. For questions number 1 and 2, “low” was if the subject answered 0 – 2, “moderate” if the subject answered 3-5, and “high” if the subject answered ≥6. For question number 3, “low” was if the subject stated their tension at home as a bit of dispute, can be openly discussed; “moderate” if the subject stated there is some tension but was tolerable, and “severe/high” if the subject felt the tension at home was severe/high. Personal factor at home was in ordinal scale.10

Health behavior was considered as the frequency of drinking alcohol per week, frequency of drinking coffee per day, duration of smoking (months), number of cigarettes per day, and frequency of sport activities per week.11,12

Ethical clearance was granted from the Research Ethical Commission of Faculty of Medicine Universitas Indonesia and data collection was approved by the Head of the Civil Aviation Medical Center.

Linear regression was used to identify dominant risk factors related to fatigue11, and computed using Stata released 9.

RESULTS

In the 16-day data collection period, there were 785 pilots taking medical examination and 382 were willing to participate. None of the subjects were excluded because of taking stimulants or hypnotics. Pilots who were excluded were 81 long-haul flight pilots, 43 Airbus 320 type-rated pilots, and 19 Avions de Transport Regional (ATR) type-rated pilots. Finally, there were 239 pilots with Boeing 737 series type-rated short-haul commercial pilots.

Table 1 showed that no subject reached the maximum (9) for FSS. The average FSS was mid-scale (4.66) between the least and the maximum and the average number of sectors was 3.58 in the last 24 hours. There were subjects with a sleep period of 4 hours, but the average sleep period was 6.83 hours, while average ESS was 8. Flight time in the last 7 days, sleep and wakefulness periods slightly fluctuated (coefficient of variations was less than 20%), while frequency and flight time of unplanned flights fluctuated widely. Moreover, the highest variation among subjects was for the duration of smoking at 98.6%.

Table 2 showed that age, physical factors at home, opportunity to do relaxing activities, and role at home did not have any effect on fatigue. The number of sectors in the last 24 hours, frequency and unplanned flight time in the last 30 days, flight time in the last 24 consecutive hours and 7 consecutive days, flight duty time in the last 24 hours, ESS, and tension at home were more likely to increase the risk of FSS.
Table 1. Several demographics, workload, sleep history, and personal habits in short-haul commercial Indonesian pilots (n=239)

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue Severity Scale (FSS)</td>
<td>1</td>
<td>7</td>
<td>4.66</td>
<td>1.202</td>
<td>25.79</td>
</tr>
<tr>
<td>Age</td>
<td>20</td>
<td>63</td>
<td>35.66</td>
<td>9.392</td>
<td>26.34</td>
</tr>
<tr>
<td>Number of sectors in the last 24 hours</td>
<td>2</td>
<td>8</td>
<td>3.58</td>
<td>1.332</td>
<td>37.21</td>
</tr>
<tr>
<td>Frequency of unplanned flights</td>
<td>1</td>
<td>16</td>
<td>2.89</td>
<td>2.605</td>
<td>90.14</td>
</tr>
<tr>
<td>Flight time of unplanned flights</td>
<td>1</td>
<td>60</td>
<td>13.68</td>
<td>11.695</td>
<td>85.49</td>
</tr>
<tr>
<td>Flight time in the last 7 days</td>
<td>20</td>
<td>35</td>
<td>24.82</td>
<td>3.530</td>
<td>14.22</td>
</tr>
<tr>
<td>Flight time in the last 24 hours</td>
<td>3</td>
<td>12</td>
<td>5.49</td>
<td>1.561</td>
<td>28.43</td>
</tr>
<tr>
<td>Flight duty time the last 24 hours</td>
<td>4</td>
<td>15</td>
<td>9.16</td>
<td>2.489</td>
<td>27.17</td>
</tr>
<tr>
<td>Sleep period in the last 24 hours</td>
<td>4</td>
<td>10</td>
<td>6.83</td>
<td>1.183</td>
<td>17.32</td>
</tr>
<tr>
<td>Wakefulness period in the last 24 hours</td>
<td>10</td>
<td>20</td>
<td>15.21</td>
<td>1.965</td>
<td>12.92</td>
</tr>
<tr>
<td>Epworth Sleepiness Scale (ESS)</td>
<td>2</td>
<td>20</td>
<td>7.89</td>
<td>4.212</td>
<td>53.38</td>
</tr>
<tr>
<td>Role in family</td>
<td>1</td>
<td>3</td>
<td>1.57</td>
<td>0.623</td>
<td>39.68</td>
</tr>
<tr>
<td>Physical factor at home/ neighborhood</td>
<td>1</td>
<td>3</td>
<td>1.26</td>
<td>0.485</td>
<td>38.49</td>
</tr>
<tr>
<td>Tension at home</td>
<td>1</td>
<td>3</td>
<td>1.39</td>
<td>0.498</td>
<td>35.83</td>
</tr>
<tr>
<td>Opportunity of relaxing activities</td>
<td>1</td>
<td>3</td>
<td>1.80</td>
<td>0.856</td>
<td>47.56</td>
</tr>
<tr>
<td>Duration of smoking (months)</td>
<td>2</td>
<td>240</td>
<td>48.17</td>
<td>47.497</td>
<td>98.60</td>
</tr>
<tr>
<td>Number of cigarettes per day</td>
<td>1</td>
<td>16</td>
<td>7.47</td>
<td>3.902</td>
<td>52.24</td>
</tr>
<tr>
<td>Frequency of drinking alcohol per week</td>
<td>1</td>
<td>6</td>
<td>1.84</td>
<td>1.280</td>
<td>69.57</td>
</tr>
<tr>
<td>Frequency of drinking coffee per day</td>
<td>1</td>
<td>6</td>
<td>1.53</td>
<td>0.864</td>
<td>56.47</td>
</tr>
<tr>
<td>Frequency of sport activities per week</td>
<td>1</td>
<td>9</td>
<td>2.35</td>
<td>1.837</td>
<td>56.47</td>
</tr>
</tbody>
</table>

Table 2. Demographics, workload, sleep restriction and risk of fatigue  in short-haul commercial Indonesian pilots (n=239)

<table>
<thead>
<tr>
<th></th>
<th>Crude regression coefficient</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.009</td>
<td>-0.007;0.027</td>
<td>0.270</td>
</tr>
<tr>
<td>Constant</td>
<td>4.330</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of unplanned flights in the last 30 days</td>
<td>0.139</td>
<td>0.068;0.211</td>
<td>0.000</td>
</tr>
<tr>
<td>Constant</td>
<td>4.512</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight time in last 7 days</td>
<td>0.053</td>
<td>0.010;0.096</td>
<td>0.015</td>
</tr>
<tr>
<td>Constant</td>
<td>3.328</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight time in last 24 hours</td>
<td>0.175</td>
<td>0.079;0.271</td>
<td>0.000</td>
</tr>
<tr>
<td>Constant</td>
<td>3.692</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight duty time in last 24 hours</td>
<td>0.080</td>
<td>0.019;0.141</td>
<td>0.010</td>
</tr>
<tr>
<td>Constant</td>
<td>3.919</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep restriction</td>
<td>0.037</td>
<td>0.001;0.074</td>
<td>0.041</td>
</tr>
<tr>
<td>Constant</td>
<td>4.358</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical factors at home</td>
<td>0.002</td>
<td>-0.314;0.320</td>
<td>0.986</td>
</tr>
<tr>
<td>Constant</td>
<td>4.653</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tension at home/ neighborhood</td>
<td>0.224</td>
<td>-0.083;0.532</td>
<td>0.152</td>
</tr>
<tr>
<td>Constant</td>
<td>4.344</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opportunity to do relaxing activity</td>
<td>0.018</td>
<td>-0.161;0.198</td>
<td>0.841</td>
</tr>
<tr>
<td>Constant</td>
<td>4.623</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Role at home</td>
<td>-0.139</td>
<td>-0.385;0.106</td>
<td>0.266</td>
</tr>
<tr>
<td>Constant</td>
<td>4.876</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. The dominant factors related to fatigue in short-haul commercial Indonesian pilots (n=239)

<table>
<thead>
<tr>
<th></th>
<th>Adjusted regression coefficient</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sectors in 24 hours</td>
<td>0.371</td>
<td>0.270;0.472</td>
<td>0.000</td>
</tr>
<tr>
<td>Epworth Sleepiness Scale</td>
<td>0.043</td>
<td>0.011;0.075</td>
<td>0.008</td>
</tr>
<tr>
<td>Unplanned flight time</td>
<td>0.033</td>
<td>0.017;0.048</td>
<td>0.000</td>
</tr>
<tr>
<td>Constant</td>
<td>2.832</td>
<td>2.361;3.303</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3 showed that each additional sector in the last 24 hours increased FSS. One additional number of sector in the last 24 hours increased FSS by 0.371 points. Moreover, one additional ESS point increased FSS by 0.043 points. Lastly, one additional unplanned flight increased FSS by 0.033 points.

DISCUSSION

In interpreting this study, firstly the small number of subjects willing to participate must be considered. The low participation was probably because pilots felt uncomfortable if the exceeded flight time was discovered by the DGCA and affect their certification process. Secondly, fatigue was measured in subjective concept (FSS). Nevertheless, FSS is a widely used self-reporting questionnaire relating to subjects’ fatigue and its impact on functioning and behavioral aspects in the past week. FSS has good reliability and validity with Cronbach $\alpha = 0.85$.5,14

Furthermore, there were some factors effecting fatigue which were not measured in this study, for example the circadian rhythm, vibration, noise, and automation.15,16 Short-haul pilots often had unplanned flights, early starts and late finishes, which can result in sleep restriction and disrupt circadian rhythm. This effect was minimized by using ESS to subjectively determine the subjects’ sleepiness scale. ESS has been used in many research, recommended by the Fatigue Risk Management System (FRMS) of the International Civil Aviation Organization (ICAO) and has good reliability and validity with Cronbach $\alpha = 0.77$-0.88.9 In addition, this study was only directed to Boeing 737 type-rated short haul commercial pilots and therefore the effect of vibration and automation were expected to be similar among subjects.

The average FSS (4.66±1.202) was almost the same as the British Airline Pilots Association (BALPA) pilots in United Kingdom (UK), Germany, French and Italy which was 4.7±1.0.5

The number of sectors was the highest factor to significantly increase fatigue, which increased FSS by 0.371 for every additional sector in the last 24 hours. These results were consistent with a study by Powell in New Zealand that stated each additional sector was equivalent to an increase of 0.38 on the 7-point Samm-Perelli fatigue scale.3 Bourgeois-Bougrine in France also found that each additional sector was equivalent to an increase of 0.48 on the visual analog scale of fatigue.3 Take-offs and landings are critical phase of flight that requires high concentration and intense conversation between pilots and Air Traffic Controller (ATC).2,4 Thus several repeated sectors in a short haul operation can cause cumulative fatigue that will impair a pilot’s cognitive function leading to accidents or incidents.17

Other dominant factors of fatigue were unplanned flight time and sleep restriction. Each additional unplanned flight time in the last 30 days and sleep restriction increased FSS. This result was consistent with the studies in UK and BALPA pilots.2,5 By flying unplanned flights, pilots had less rest period, which cumulatively lead to sleep restriction and increase the risk for fatigue.

The results of multivariate analysis showed that the frequency of unplanned flights, flight time in the last 24 hours and 7 days, and flight duty time in the last 24 hours were not dominant factors of fatigue. These results were not consistent with the study by Bourgeois-Bougrine in France which revealed that each additional flight time in 4 days was significantly correlated with higher visual analog scale of fatigue.3 Powell in New Zealand also revealed that each additional flight duty time was significantly correlated with higher Samm-Perelli fatigue scale.4 The explanation for this dissimilarity was probably the different characteristic and measurement of fatigue, and although some subjects may fly the same flight time or flight duty time, each subjects flew different numbers of sectors. That is probably why the number of sector was the highest factor to significantly increase fatigue.

Personal factors at home and managerial support were not dominant factors of fatigue. These results were not consistent with Deros in Malaysia which revealed that fatigue in Malaysian pilots was due to trouble sleeping because of personal worries.18 While Steptoe revealed that subjects with low perception of organizational justice were less likely
to have lowered fatigue scale. These differences were explained by Yuliana who studied the same population in 2013. It was stated that pilots were highly intellectual and rational people who had the ability to use coping strategy such as comfort in religion that will decrease distress level as much as 51%. But this requires further study to investigate the effects of distress and coping strategy on fatigue.

This study revealed that fatigue was a multi-factorial physiologic condition that was caused by an imbalance between workload and recovery sleep. Fatigue could impair a pilot’s cognitive function to safely operate an aircraft. Therefore, it is important for pilots to get enough rest-period, understand their fatigue level before considering a flight duty especially unplanned flights, assemble a good Crew Resource Management (CRM), and notably manage safety culture and safety report for every flight duty (pilots must be free to express their concern and share their thoughts of every flight safety related conditions).

As a member ICAO, Indonesia strives to implement FRMS to our regulations. This study was conducted as the first step of FRMS implementation, to gather data and information of fatigue in short-haul operation. Further studies are suggested to establish the presence of fatigue in different operations and organize a cooperative arrangement between The Ministry of Transportation Republic of Indonesia, every operating airline in Indonesia, as well as airport operator to work in harmony for adopting every step of FRMS as amended by the ICAO.

In conclusion, additional number of sectors in 24 hours, additional unplanned flight times within 30 days, and sleep restriction increased the risk of fatigue among short-haul commercial pilots in Indonesia.

Acknowledgment

The authors sincerely thank The Director General of Civil Aviation, Ministry of Transportation for facilitating this study, and to all commercial pilots who willingly took time to participate and contribute invaluable in this study.

REFERENCES


Purser support and the tendency of depression among civil female flight attendants in Indonesia

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Abstract

Background: Depression can occur in female flight attendants. The purpose of this study was to find the correlation between purser support and other factors with depression among female civil flight attendants in Indonesia.

Methods: This cross-sectional study with purposive sampling was carried out on female civilian flight attendants undergoing routine medical check up at the Civil Aviation Medical Center Jakarta from May 12 to 18, 2014. Those taking antidepressants or benzodiazepines, or drink alcohol more than 3 times a day, were excluded for this study. Depression was measured using the Beck Depression Inventory Questionnaire. Purser support, co-workers support, support outside workplace, and workload were measured using the NIOSH Generic Job Stress Questionnaire. Risk factors for depression were analyzed using linear regression.

Results: Out of 242 female flight attendants undergoing routine medical check up, 145 persons met the study criteria. Purser support, support outside work, as well as mental workload were the dominant risk factors related to depression among female civilian flight attendants. One point increase of purser support, decreased depression by 0.552 point [regression coefficient (β) = -0.552; p = 0.033]. One point increase of support outside work, decreased depression by 1.191 points [β = -1.191; p = 0.000]. On the other hand, one point increase of mental workload, increased depression by 0.549 point (β = 0.549; p = 0.045).

Conclusion: Increased purser and outside of work supports decreased the risk of depression, however, more mental workload increased depression among civilian flight attendants in Indonesia. (Health Science Journal of Indonesia 2015;6:76-80)

Keywords: depression, purser support, mental workload, female flight attendants.
A person with depression will show loss of energy and interest, guilt, hard to focus, changing the way they do their activity, loss of cognitive ability, speech, and vegetative functions, more over, they think about suicide. Depression can occurred among female flight attendants. This is a serious issue because it would affect their performance and presence at work.

Flight attendants are responsible for passenger safety, making sure all passengers are comfortable and safe, serving the need of passengers, and also as a bridge of communication between cabin and cockpit. All these tasks must be reported to the purser as a supervisor. A purser is the head of senior and junior cabin crews who controls and reports all of the activities and events that occurred during flight to captain pilot as a flight leader.

The Department of Environmental Health of the United States compared health conditions of female flight attendant to the general population. This study showed that the tendency of depression in female flight attendants was 3.8% higher than the general population. The National Institute for Occupational Safety and Health (NIOSH) of the United States showed that support from purser and outside of work, and also mental workload have a significant correlation with the tendency of depression.

The aim of this study was to identify the correlation between purser support and other factors and the tendency of depression among female flight attendants in Indonesia.

METHODS

This was a cross sectional study with purposive sampling in female flight attendants undergoing routine medical examinations at the Civil Aviation Medical Center, Jakarta, from May 12-18, 2014.

The inclusion criteria were active working female flight attendants, aged 18-50 years old, and agreed to participated this study. The exclusion criteria were those working as purser or cabin 1, taking antidepressants or benzodiazepine, or drink alcohol more than three times in a day.

The subjects who agreed to participate in this study were asked to sign an informed form after given an explanation about this study.

The data collected included several demographic characteristics, habits (physical activity and smoking, and job (working hours in a day and mental workload). The tendency of depression was measured using the Beck Depression Inventory Questionnaire, which had good validity and reliability (Cronbach’s alpha 0.88). This questionnaire consisted of 21 questions about depression symptoms which occurred during the last two weeks such as sadness, self-blame, suicidal tendencies, crying, sensitive, feeling not physically attractive, sleep disturbances, feeling tired, weight loss, somatic preoccupation and loss of libido.

The subjects were asked to answer each statement on the questionnaire by giving a mark in the box that perfectly fit with their condition during the last 2 weeks. Each box represents score from 0-3, which means 0 for the lowest answer and score 3 for the highest answer. The score for each statement was added to achieve total score. The minimum was 0 and the maximum was 63. Higher scores mean the subject had a higher tendency for depression. Psychosocial factors (purser support, support outside of work, and coworker support) were measured using the NIOSH generic job stress questionnaire. The questions were (1) How much do each of these people go their way to do things to make your work life easier for you?; (2) How much can each of these be relied on when things get tough at work?; (3) How easy is it to talk with each following people?; and (4) How much of the following are willing to listen to your personal problem? The subjects were asked to choose an answer that perfectly fit their condition during the last 1 month, by selecting an answer 1 = do not have any such person, 2 = not at all, 3 = a little, 4 = somewhat, 5 = very much. Psychosocial support was scored by adding each answer from each statement to achieve a minimum score 4 and a maximum score 20. A lower score means the subject has higher support.

This study was analyzed by linear regression and the calculation with Stata software released 9.

Ethical approval was obtained from the Health Research Ethical Committee of the Faculty of Medicine, Universitas Indonesia. This study was conducted with permission from the Chief of the Civil Aviation Medical Center, Jakarta.

RESULTS

Out of 242 female flight attendants doing routine medical check up, 159 persons agreed to participate the study, however, 14 respondents were excluded.
from this study. The 14 excluded consisted of 8 purser/cabin 1, 2 persons drank alcohol more than three times in a day, 1 person consumed benzodiazepin, and 3 persons did not completely fill the questionnaire. Leaving 145 persons meeting the study criteria.

Table 1 shows that the youngest subject was 18 years old and the oldest subject was 44 years old. The average age was 24 years old, the average working hours in a week was 8 hours, and the average job tenure was less than 5 years.

Variables with homogenous distribution based on variance coefficients <20% were age, purser support, support outside of work, and co-workers support, whereas variables with heterogenous distribution were depression, job tenure, working hours, and mental workload.

Table 2 shows age, job tenure, purser support, support outside of work, and co-workers support were more likely to decrease the tendency of depression. Whereas, job tenure and mental work load were more likely to increase the tendency of depression.

Table 3 shows the model selected. Purser support, support outside of work, as well as mental work load were dominant risk factors related to depression among female civilian flight attendants. One point of increased purser support will decrease depression by 0.552 point [regression coefficient (β) = -0.552; p = 0.033]. One point of increased support outside of work will decrease depression by 1.191 points [β = -1.191; p = 0.000]. On the other hand, one point of increased mental workload increased depression by 0.549 point (β = 0.549; p = 0.045).

Table 1. Several demographic characteristics, job and psychosocial factors (n=145)

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression (units)</td>
<td>0</td>
<td>34</td>
<td>7.88</td>
<td>7.987</td>
<td>101.30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>18</td>
<td>44</td>
<td>24.10</td>
<td>4.706</td>
<td>19.52</td>
</tr>
<tr>
<td>Working hours</td>
<td>3</td>
<td>18</td>
<td>8.59</td>
<td>2.602</td>
<td>30.29</td>
</tr>
<tr>
<td>Job tenure (units)</td>
<td>1</td>
<td>25</td>
<td>4.67</td>
<td>3.832</td>
<td>82.05</td>
</tr>
<tr>
<td>Mental workload</td>
<td>5</td>
<td>17</td>
<td>8.61</td>
<td>2.367</td>
<td>27.49</td>
</tr>
<tr>
<td>Purser support (units)</td>
<td>8</td>
<td>20</td>
<td>14.41</td>
<td>2.810</td>
<td>19.50</td>
</tr>
<tr>
<td>Co-worker support (units)</td>
<td>8</td>
<td>20</td>
<td>15.37</td>
<td>2.461</td>
<td>16.01</td>
</tr>
<tr>
<td>Support outside work (units)</td>
<td>9</td>
<td>20</td>
<td>16.54</td>
<td>2.821</td>
<td>17.05</td>
</tr>
<tr>
<td>Physical activity</td>
<td>0</td>
<td>3</td>
<td>1.06</td>
<td>0.872</td>
<td>82.26</td>
</tr>
</tbody>
</table>

Remarks: SD = standard of deviation; CV = coefficient of variation

Table 2 Several demographic factors, job, habits, and psychosocial factors tendency of depression  (n=145)

<table>
<thead>
<tr>
<th></th>
<th>Crude regression coefficient</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.164</td>
<td>-0.443;0.114</td>
<td>0.247</td>
</tr>
<tr>
<td>Constant</td>
<td>11.837</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Job tenure</td>
<td>-0.217</td>
<td>-0.559;0.125</td>
<td>0.213</td>
</tr>
<tr>
<td>Constant</td>
<td>8.889</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purser support</td>
<td>-0.625</td>
<td>-1.084;0.1676</td>
<td>0.008</td>
</tr>
<tr>
<td>Constant</td>
<td>16.893</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td>-0.209</td>
<td>-1.778;1.360</td>
<td>0.793</td>
</tr>
<tr>
<td>Constant</td>
<td>8.162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Support outside work</td>
<td>-1.227</td>
<td>-1.649;0.806</td>
<td>0.000</td>
</tr>
<tr>
<td>Constant</td>
<td>28.192</td>
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<td></td>
</tr>
<tr>
<td>Flight time</td>
<td>-0.193</td>
<td>-2.812;2.425</td>
<td>0.884</td>
</tr>
<tr>
<td>constant</td>
<td>8.186</td>
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<td></td>
</tr>
<tr>
<td>Co-worker support</td>
<td>-0.698</td>
<td>-1.221;0.173</td>
<td>0.009</td>
</tr>
<tr>
<td>Constant</td>
<td>18.600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>0.164</td>
<td>-1.349;1.677</td>
<td>0.830</td>
</tr>
<tr>
<td>Constant</td>
<td>7.702</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.725</td>
<td>-1.158;2.608</td>
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</tr>
<tr>
<td>Constant</td>
<td>7.630</td>
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<td></td>
</tr>
<tr>
<td>Working hours</td>
<td>0.401</td>
<td>-0.101;0.904</td>
<td>0.117</td>
</tr>
<tr>
<td>Constant</td>
<td>4.428</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental workload</td>
<td>1.081</td>
<td>0.552;1.609</td>
<td>0.000</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.439</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Dominant factors related to tendency of depression (n=145)

<table>
<thead>
<tr>
<th></th>
<th>Adjusted regression coefficients</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purser support</td>
<td>-0.552</td>
<td>-1.059;0.045</td>
<td>0.033</td>
</tr>
<tr>
<td>Outside work support</td>
<td>-1.191</td>
<td>-1.723;0.659</td>
<td>0.000</td>
</tr>
<tr>
<td>Co-worker support</td>
<td>0.542</td>
<td>-0.135;1.086</td>
<td>0.116</td>
</tr>
<tr>
<td>Mental workload</td>
<td>0.549</td>
<td>0.012;1.091</td>
<td>0.045</td>
</tr>
<tr>
<td>Constant</td>
<td>22.478</td>
<td>10.853;34.103</td>
<td>0.000</td>
</tr>
</tbody>
</table>

DISCUSSION

The limitation that should be noted in this study was that the subjects were purposively selected among female flight attendants in Indonesia who were taking routine medical examinations at the Civil Aviation Medical Center, Jakarta.

In this study, purser support had a significant correlation with the tendency of depression. It decreased the tendency of depression among female flight attendants in Indonesia. Female flight attendants with higher purser support had a lower tendency of depression. This was supported by findings of the MacDonald et al study. The study consisted of 73 female flight attendants employed by two commercial airlines in the United States of America. The results showed that purser support was negatively correlated with the tendency of depression, with p ≤ 0.01.

Another research in Amsterdam revealed that purser support had a good impact for female flight attendants with emotional problems in their work. The higher the social support they received, the less stress factors they would experience. Therefore, the tendency of depression would decrease.

Ruguilies et al analyzed the impact of psychosocial work characteristics on the tendency of depression among 4133 (49% women) of the workforce in Denmark between 1995 and 2000. They found that female workers who had low supervisor support had a higher chance to experience depression [relative risk (RR) = 2.03; 95% confidence interval (CI) = 1.20-3.43].

The present study also revealed that there was a significant correlation between support outside of work and the tendency of depression. The higher the support from outside of work, the less depression the experienced. This was supported by The National Institute for Occupational Safety and Health (NIOSH) in the United States which showed that support outside of work was negatively correlated with the tendency of depression, with p ≤ 0.01.

Furthermore, the present study showed that there was a significant correlation between mental work load and the tendency of depression. The higher mental work load they received, the higher the depression experienced. A previous study also had similar result showing mental work load was positively correlated with the tendency of depression.

In this study, job tenure and depression was not significantly related. This may be due to adaptation by the female flight attendants to their work environment. Job tenure increased working experience, knowledge, and working skills of the female flight attendants making them more skilled at overcoming difficulties. This was also supported with the results of another study which stated that the higher the job tenure, the lower the tendency of depression experienced. A prior study also found that job stressors will decrease when they have worked for a longer period of time. The greater the working experience, knowledge, and responsibilities the better the adaption to the job stressors.

In conclusion, increased purser and outside of work supports decreased the risk of depression, however, more mental work load increased depression among civilian flight attendants in Indonesia.

Acknowledgment

The authors would like to thank all subjects who voluntarily participated in the study. We would also like to thank the Chief Aviation Medical Center for his permission to conduct this study at the Civil Aviation Medical Center.

REFERENCE

3. Ballard TJ, Corradi L, Mazzanti C, et al. Integrating qualitative methods into occupational health research:
The effect of workload and other risk factors of metabolic syndrome among short-haul commercial pilots in Indonesia

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Abstract

Background: Metabolic syndrome (MS) could cause sudden incapacitation among pilots in Indonesia. The aim of this study was to identify risk factors of MS among short-haul commercial pilots in Indonesia.

Methods: A cross-sectional study with purposive sampling was conducted among commercial pilots taking medical examination at the Civil Aviation Medical Center, Jakarta from July 27-August 30th, 2014. Metabolic syndrome was assessed according to the National Cholesterol Education Program Adult Treatment panel III criteria and criteria. Risk factors were collected using anonymous self-reporting questionnaire. The laboratory data were extracted from medical records. Cox regression was used to identify dominant risk factors of MS.

Results: During data collection, 2135 pilots taking medical examination. Total male Asian pilots met the inclusion criteria was 864 pilots. Prevalence of MS was 18.28%. Compared to 20-35 year-old group, subjects aged 56-65 years-old had 88% higher risk for MS [adjusted relative risk (RRa) = 1.88; P = 0.019]. In term of number of sectors in the last 24 hours, compared to 0-3 sectors, subjects who had 6-7 sectors in the last 24 hours had 66% higher risk for MS (RRa = 1.66; P = 0.033), while subjects who had 8 or more sectors in 24 hours had 82% more risk for MS (RRa = 1.82; P = 0.072).

Conclusions: The pilot aged 56-65 years-old, who had 6 or more sectors in the last 24 hours, had higher risk for metabolic syndrome among short-haul commercial pilots in Indonesia. (Health Science Journal of Indonesia 2015;6:81-6)

Keyword: metabolic syndrome, number of sectors, pilots, Indonesia
Metabolic syndrome is associated with development of cardiovascular disease, stroke, and diabetes mellitus.\textsuperscript{1,2} Those conditions could cause an onboard sudden incapacitation among commercial pilots thus increase the risk of accidents in Indonesia. Moreover, metabolic syndrome has been associated and characterized with elevated cortisol level which also have been shown increase acutely in response to work demands, sleep deprivation and fatigue.\textsuperscript{1,2}

Sleep deprivation and fatigue are major concern among commercial pilots that lead to great risk for aviation safety.\textsuperscript{3} The causes of pilot fatigue are primarily related to sleep loss for both long-haul and short-haul flights. Night flights and jet lag are the most important factors that generated fatigue in long-haul flights.\textsuperscript{4} While other studies revealed that fatigue in short-haul operation was caused by the number of sectors,\textsuperscript{4,5} more than one unplanned flights in a month,\textsuperscript{3,6} cumulative flight time in the preceding week,\textsuperscript{3} and sleep restriction.\textsuperscript{7}

Indonesia is an archipelago nation and short-haul domestic flights between cities or small islands play an important role. High domestic passenger rate and tense competition among airlines in Indonesia\textsuperscript{8} pushed airline management to a culture of short-haul pilots flying more sectors, flying unplanned flights during rest periods and cumulatively flying close to legal maximum of flight and duty time limitations, as was clarified by 30\% of subjects during preliminary study. This condition may adversely affect the safety of short-haul flights in Indonesia.

The aim of this study was to identify the dominant risk factors related to metabolic syndrome among short-haul commercial pilots in Indonesia.

**METHODS**

This cross-sectional study was conducted among the population of active pilots working for commercial airlines in Indonesia. Subjects were selected by purposive sampling from commercials pilots taking medical examinations at the Civil Aviation Medical Center in Jakarta from May 5 – 26, 2014.

The inclusion criteria were male Asian commercials pilots who actively having flight duty in the last 7 days prior to data collection and holding an Airline Transport Pilot License (ATPL) or Commercial Pilot License (CPL) type license, and operating in short-haul flights of less than 2 hours flight time per sector. The exclusion criteria were subjects has been diagnosed with diabetes mellitus or taking hypnotics or stimulants.

Subjects willing to participate signed an informed consent letter, then were asked to fill in a self-reporting questionnaire regarding demographics, smoking habits, type rating, and pilots’ workload including flight time in the last 30 and 7 days; flight time in the last 24 hours; number of sectors in the last 24 hours; frequency of unplanned flight and flight time during unplanned flights in the last 30 days, and fatigue using Fatigue Severity Scale (FSS).

Metabolic syndrome was assessed using the National Cholesterol Education Program (NCEP) Adult Treatment Panel-III (ATP-III) criteria as having three or more of the following: fasting blood glucose of at least 110 mg/dl, blood pressure at least 130/85 mmHg, waist circumference of greater than 90 cm for Asian men or 102 for Caucasian men, triglycerides greater than 150 mg/dl, and HDL cholesterol of less than 40 mg/dl in men. Data were collected from medical record.\textsuperscript{1,2}

The risk factors were age, smoking habits, type license, type rating, and pilots’ workload including flight time in the last 30 and 7 days; flight time in the last 24 hours; number of sectors in the last 24 hours; frequency of unplanned flight and flight time during unplanned flights in the last 30 days, and fatigue using Fatigue Severity Scale (FSS).

The number of sectors was defined as any flight which has a take-off and landing at different airports which are not less than 50 nautical miles apart in the last 24 consecutive hours.\textsuperscript{9} Flight time was considered as total elapsed time from the moment the aircraft first moved under its own power for takeoff, until the time it comes to rest at the end of the flight. This variable was divided into flight time in the last 24 hours, in the last 7 days and 30 days.\textsuperscript{9}

Unplanned flight was considered as flight duty, performed by the pilot, which was not in their schedule or during their day-off, in the last 30 consecutive days. This variable was divided into frequency and flight time of unplanned flight.

Fatigue was measured using Fatigue Severity Scale (FSS) that consisted of 9 questions relating to fatigue and its impact on functioning and behavioral aspects in the past week. The FSS questions were: (1) my motivation is lower when I am fatigued; (2) exercise brings on my fatigue; (3) I am easily fatigued;...
(4) fatigue interferes with my physical functioning; (5) fatigue causes frequent problem for me; (6) my fatigue prevents sustained physical functioning; (7) fatigue interferes with carrying out certain duties and responsibilities; (8) fatigue is among my most disabling symptoms; and (9) fatigue interferes with my work, family, or social life. Subjects answered the questions on a scale of “1” indicating agrees to and “7” indicating disagrees to. This variable was divided into clinical fatigue if mean FSS score was greater than 5.10

Ethical clearance was granted from the Research Ethical Commission of Faculty of Medicine Universitas Indonesia and data collection was approved by the Head of the Civil Aviation Medical Center.

Cox regression was used to identify dominant risk factors related to metabolic syndrome,11 variable sub-group were categorized using Receiver Operating Characteristic (ROC) and computed using Stata released 9.

RESULTS

In the 25-day data collection period, there were 2135 pilots taking medical examination and 1545 pilots willing to participate. There were pilots who did not meet the inclusion criteria including 461 pilots flying intermediate and long haul flights,190 pilots not actively flying in the past 7 days because of taking annual leave, having no schedule for flight duty or being a ground instructor and 3 female pilots. There were 891 pilots met the inclusion criteria of had short-haul flight duty in the last 7 days but 27 pilots were excluded because has been diagnosed with diabetes mellitus. None of the subjects excluded for taking hypnotics or stimulant medication. Finally, the subject in this study was 864 commercial pilots.

Table 1 showed that the prevalence of metabolic syndrome among subject were 18.28%. The highest prevalence of metabolic syndrome criteria was prehypertension at 62.28%, followed by HDL cholesterol level less than 40 mg/dl at 36.3% and waist circumference greater than 90 cm at 34.71%. More than half (56.0%) of the subjects had clinical fatigue.

Table 2 showed that the subjects who had and did not have metabolic syndrome were similarly distributed with respect to type rating, clinical fatigue, flight time in the last 7 days, frequency of unplanned flights, as well as flight time of unplanned flights.

<table>
<thead>
<tr>
<th>Category of BMI</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>10</td>
<td>1.16</td>
</tr>
<tr>
<td>Normal</td>
<td>372</td>
<td>43.11</td>
</tr>
<tr>
<td>Overweight</td>
<td>371</td>
<td>42.94</td>
</tr>
<tr>
<td>Obese</td>
<td>111</td>
<td>12.85</td>
</tr>
<tr>
<td>Waist Circumference &gt; 90 cm</td>
<td>300</td>
<td>34.71</td>
</tr>
<tr>
<td>Triglycerides &gt; 150 mg/dl</td>
<td>116</td>
<td>13.43</td>
</tr>
<tr>
<td>Fasting blood glucose &gt; 110 mg/dl</td>
<td>132</td>
<td>15.28</td>
</tr>
<tr>
<td>Prehypertension &gt; 130/85 mmHg</td>
<td>538</td>
<td>62.27</td>
</tr>
<tr>
<td>HDL &lt; 40 mg/dl</td>
<td>314</td>
<td>36.34</td>
</tr>
<tr>
<td>Clinical fatigue</td>
<td>484</td>
<td>56.02</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>484</td>
<td>56.02</td>
</tr>
</tbody>
</table>

Compared with pilot who had Boeing rating group, the pilot who had Avions de transport regional (ATR) rating group were more likely had higher risk to be metabolic syndrome. On the side, compared with pilot who had flight time in the last 30 days for 15-69 hours, those who had 70-89 hours were less likely had 21% to be metabolic syndrome.

Table 3 showed that age group, flight time in the last 24 hours, and number of sectors in the last 24 hours three dominants factors related were related to metabolic syndrome.

Furthermore, compared to 20-35 year-old group, subjects aged 56-65 years-old had 88% higher risk for metabolic syndrome [adjusted relative risk (RRa) = 1.88; P = 0.019]. In term of number of sectors in the last 24 hours, compared to 0-3 sectors, subjects who had 6-7 sectors in the last 24 hours had 66% higher risk for metabolic syndrome (RRa = 1.66; P = 0.033), while subjects who had 8 or more sectors in 24 hours had 82% more risk for metabolic syndrome (RRa = 1.82; P = 0.072).

DISCUSSION

In interpreting this study, it should be considered that there are unmodifiable factor such as age, ethnic, gender, metabolic genes, and it takes time to develop.1,2 This effect has been minimize by selecting male and Asian, subjects. There are also other factor related to hypercortisolism resulted from chronic fatigue that were not measured in this study such circadian rhythm, psychological factor that lead
Table 2. Several demographies and workload characteristics and risk of metabolic syndrome

<table>
<thead>
<tr>
<th></th>
<th>Metabolic syndrome</th>
<th>Crude Relative Risk</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No (n=706)</td>
<td>Yes (n=158)</td>
<td></td>
<td></td>
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<tr>
<td>Type of license</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Commercial pilot License</td>
<td>412</td>
<td>83.2</td>
<td>83</td>
<td>16.8</td>
</tr>
<tr>
<td>Airline transport pilot License</td>
<td>294</td>
<td>79.7</td>
<td>75</td>
<td>20.3</td>
</tr>
<tr>
<td>Type rating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boeing</td>
<td>397</td>
<td>82.4</td>
<td>85</td>
<td>17.7</td>
</tr>
<tr>
<td>Airbus</td>
<td>105</td>
<td>86.1</td>
<td>17</td>
<td>13.9</td>
</tr>
<tr>
<td>Avions de transport regional</td>
<td>44</td>
<td>74.6</td>
<td>15</td>
<td>25.4</td>
</tr>
<tr>
<td>Others</td>
<td>160</td>
<td>79.6</td>
<td>41</td>
<td>20.4</td>
</tr>
<tr>
<td>Smoking habits</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>562</td>
<td>81.0</td>
<td>132</td>
<td>19.0</td>
</tr>
<tr>
<td>1-7 cigarettes/day</td>
<td>34</td>
<td>75.6</td>
<td>11</td>
<td>24.4</td>
</tr>
<tr>
<td>8-15 cigarettes/day</td>
<td>39</td>
<td>90.7</td>
<td>4</td>
<td>9.3</td>
</tr>
<tr>
<td>15-21 cigarettes/day</td>
<td>51</td>
<td>82.3</td>
<td>11</td>
<td>17.7</td>
</tr>
<tr>
<td>22 or more cigarettes/day</td>
<td>20</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clinical fatigue</td>
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<td></td>
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<td></td>
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<tr>
<td>No</td>
<td>312</td>
<td>82.1</td>
<td>68</td>
<td>17.9</td>
</tr>
<tr>
<td>Yes</td>
<td>394</td>
<td>81.4</td>
<td>90</td>
<td>18.6</td>
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<td>Flight time in the last 30 days</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-69 hours</td>
<td>334</td>
<td>79.7</td>
<td>85</td>
<td>20.3</td>
</tr>
<tr>
<td>70-89 hours</td>
<td>244</td>
<td>85.0</td>
<td>43</td>
<td>15.0</td>
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<tr>
<td>90-109 hours</td>
<td>104</td>
<td>83.9</td>
<td>20</td>
<td>16.1</td>
</tr>
<tr>
<td>110 or more hours</td>
<td>24</td>
<td>70.6</td>
<td>10</td>
<td>29.4</td>
</tr>
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<td>Flight time in the last 7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-19 hours</td>
<td>343</td>
<td>80.5</td>
<td>83</td>
<td>19.5</td>
</tr>
<tr>
<td>20-24 hours</td>
<td>183</td>
<td>82.1</td>
<td>40</td>
<td>17.9</td>
</tr>
<tr>
<td>25-29 hours</td>
<td>137</td>
<td>84.0</td>
<td>26</td>
<td>16.0</td>
</tr>
<tr>
<td>30 or more 30 hours</td>
<td>43</td>
<td>82.7</td>
<td>9</td>
<td>17.3</td>
</tr>
<tr>
<td>Frequency of unplanned flights in the last 30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>528</td>
<td>\</td>
<td>121</td>
<td>18.6</td>
</tr>
<tr>
<td>Once</td>
<td>100</td>
<td>83.3</td>
<td>20</td>
<td>16.7</td>
</tr>
<tr>
<td>2 times</td>
<td>57</td>
<td>80.3</td>
<td>14</td>
<td>19.7</td>
</tr>
<tr>
<td>3 times</td>
<td>13</td>
<td>86.7</td>
<td>2</td>
<td>13.3</td>
</tr>
<tr>
<td>4 times</td>
<td>8</td>
<td>88.9</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>Flight time of unplanned flights in the last 30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>558</td>
<td>81.4</td>
<td>128</td>
<td>18.6</td>
</tr>
<tr>
<td>1-5 hours</td>
<td>97</td>
<td>83.6</td>
<td>19</td>
<td>16.4</td>
</tr>
<tr>
<td>6-10 hours</td>
<td>23</td>
<td>71.9</td>
<td>9</td>
<td>28.1</td>
</tr>
<tr>
<td>11-16 hours</td>
<td>17</td>
<td>89.5</td>
<td>2</td>
<td>10.5</td>
</tr>
</tbody>
</table>

* n/a = not applicable

...to chronic stress and sleep deprivation.

Age was the highest dominant factor to significantly increase the risk of metabolic syndrome which subjects age 56-65 year-old had 88% higher probability to metabolic syndrome compared to subject age less than 35 years old. This result was not consistent with the research performed in the Naval Submarines Medical Research Laboratory among 199 submarines involving in stressful working condition. This study revealed that submariner age 30-44 year-old had 25% probability of metabolic syndrome. This difference between these study probably because of the number and the community of subjects were different although they might had similar work stress related condition. Other than that, age is one of an unmodifiable risk factors of metabolic syndrome.

Other dominant factor was number of sectors in the last 24 hours, which subjects flying 6-7 sectors in the
Table 3. Several dominant factors and risk of metabolic syndrome

<table>
<thead>
<tr>
<th>Metabolic syndrome</th>
<th>No (n=706)</th>
<th>Yes (n=158)</th>
<th>Adjusted relative risk</th>
<th>95 % confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-35 year-old</td>
<td>281</td>
<td>84.9</td>
<td>50</td>
<td>15.1</td>
<td>1.00</td>
</tr>
<tr>
<td>36-45 year-old</td>
<td>291</td>
<td>80.6</td>
<td>70</td>
<td>19.4</td>
<td>1.28</td>
</tr>
<tr>
<td>46-55 year-old</td>
<td>105</td>
<td>80.2</td>
<td>26</td>
<td>19.8</td>
<td>1.31</td>
</tr>
<tr>
<td>56-65 year-old</td>
<td>29</td>
<td>70.7</td>
<td>12</td>
<td>29.3</td>
<td>1.88</td>
</tr>
<tr>
<td><strong>Flight time in the last 24 hours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5 hours</td>
<td>376</td>
<td>78.7</td>
<td>102</td>
<td>21.3</td>
<td>1.00</td>
</tr>
<tr>
<td>6-8 hours</td>
<td>303</td>
<td>85.1</td>
<td>53</td>
<td>14.9</td>
<td>0.67</td>
</tr>
<tr>
<td>9 or more hours</td>
<td>27</td>
<td>90.0</td>
<td>3</td>
<td>10.0</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Number of sectors in the last 24 hours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3 sectors</td>
<td>333</td>
<td>83.9</td>
<td>64</td>
<td>16.1</td>
<td>1.00</td>
</tr>
<tr>
<td>4-5 sectors</td>
<td>299</td>
<td>81.3</td>
<td>69</td>
<td>18.7</td>
<td>1.20</td>
</tr>
<tr>
<td>6-7 sectors</td>
<td>57</td>
<td>76.0</td>
<td>18</td>
<td>24.0</td>
<td>1.66</td>
</tr>
<tr>
<td>8 or more sectors</td>
<td>17</td>
<td>70.8</td>
<td>7</td>
<td>29.2</td>
<td>1.82</td>
</tr>
</tbody>
</table>

last 24 hours had an increase of 66% probability for metabolic syndrome compared to subjects flying less than 3 sectors in the last 24 hours. This study showed that number of sectors which consisted of multiple take-offs and landings as critical phase of flight induced an acute-stress response in the hypothalamo-pituitary-adrenocortical axis. This response resulting in elevated cortisol level that accumulatively associated with fatigue as one of primary causes of hypercortisolism and has been linked to metabolic syndrome.

Short-haul commercial pilots flying 6-8 hours in the last 24 hours had 23% lower probability to metabolic syndrome compared to pilots flying less than 5 hours in the last 24 hours. This result probably because of healthy worker effect among pilots flying more than 9 hours in the last 24 hours.

In addition, cardio-metabolic disorder associated with chronically elevated cortisol such as central obesity, insulin resistance, glucose intolerance, dyslipidemia, and stress-mediated hypertension are also drastically improved with regular exercise.

This study revealed that short-haul pilots who are prone to early starts and late finishes can result in sleep restriction and disrupt circadian rhythm thus lead to sleep deprivation and cumulative fatigue. This condition ultimately resulting in elevated cortisol level and has been linked to metabolic syndrome. Certain measures, however can be taken to help reduce the vulnerability to hypercortisolism including regular exercise, efficient rest periods that are sufficient in length, and mental relaxation techniques.

As a member ICAO, Indonesia strives to implement Fatigue Research Management System (FRMS) to our regulations. This study was conducted as first step of FRMS implementation, to gather data and information on risk factors of fatigue, mainly workload related factors and the effect of workload on metabolic syndrome among short-haul commercial pilots in Indonesia. Further studies are suggested to establish the presence of metabolic syndrome and fatigue in different operations and organize a cooperative arrangement between The Ministry of Transportation Republic of Indonesia, every operating airline in Indonesia, as well as airport operator to work in harmony for adopting every step of FRMS as amended by the ICAO.

In conclusion, the pilot aged 56-65 years-old, who had 6 or more sectors in the last 24 hours, had higher risk for metabolic syndrome among short-haul commercial pilots in Indonesia.

Acknowledgment

The authors sincerely thank The Director General of Civil Aviation, Ministry of Transportation for facilitating this study, and to all commercial pilots who willingly took time to participate and contribute invaluably in this study.

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2. Chandola T, Brunner E, Marmot M. Chronic stress at
Job stress and risk of menstrual duration disorder in female civilian flight attendants in Indonesia

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Abstrak

Latar belakang: Gangguan durasi haid pada pramugari dapat mengganggu performa kerja. Tujuan penelitian adalah untuk mengetahui faktor-faktor risiko yang terhadap gangguan durasi haid pada pramugari.


Hasil: Di antara 521 pramugari sebanyak 393 setuju berpartisipasi, 19 diekskluasi karena menderita gangguan durasi haid sebelum bekerja, sehingga diperoleh 374 subyek untuk analisis, dan 35,8% di antaranya menderita gangguan durasi haid. Stres kerja, jenis penerbangan dan usia merupakan faktor risiko dominan terhadap gangguan durasi haid. Pramugari dengan stres kerja memiliki risiko menderita gangguan durasi haid 58% lebih tinggi [risiko relatif suaijan (RRa) = 1,58; interval kepercayaan (CI) = 0,96-2,62; P = 0,071]. Pramugari dengan jenis penerbangan jarak jauh dalam tiga bulan terakhir memiliki risiko 69% lebih tinggi mengalami gangguan durasi haid (RRa=1,69; CI=1,17-2,43). Pramugari berumur 30–39 tahun memiliki risiko gangguan durasi haid 50% lebih rendah (RRa = 0,50; 95% CI = 0,22-1,02; P = 0,057).

Kesimpulan: Pramugari dengan stres kerja, jenis penerbangan jarak jauh dalam tiga bulan terakhir, dan berusia 19–24 tahun memiliki risiko lebih tinggi gangguan durasi haid. (Health Science Journal of Indonesia 2015;6:87-91)

Kata kunci: durasi haid, stres kerja, pramugari, Indonesia

Abstract

Background: Menstrual duration disorder may cause impaired work performance. The research objective was to identify risk factors related to menstrual duration disorder in female flight attendants.

Methods: A cross-sectional study with convenient sampling was conducted on civilian female flight attendants aged 19–50 years who underwent routine medical examination at Civil Aviation Medical Center and Garuda Sentra Medika, Jakarta on May 18-29 2015. Menstrual duration disorder is menstruation more than 8 days and/or shorter than usual period (3-5 days). Stress was identified by using criteria of National Institute for Occupational Safety and Health Generic Job Stress Questionnaire Mental Demands Form Number 11. Relative risk was analyzed using Cox regression.

Results: Among 521 female civilian flight attendants, 393 were willing to participate in this study. Nineteen subjects were excluded, leaving 374 subjects for this analysis, and 35.8% of subjects had menstrual duration disorder. Job stress, flight type and age were dominant risk factors for menstrual duration disorder. Subjects with job stress and long haul flight within three months had higher risk for having menstrual duration disorder by 58% [adjusted relative risk (RRa) = 1.58; confidence interval (CI) = 0.96-2.62; P = 0.071] and 69% (RRa = 1.69; CI = 1.17-2.43) respectively. Those between aged 30–39 years had 50% had less risk of having menstrual duration disorder (RRa=0.50; 95% CI = 0.22-1.02; P = 0.057).

Conclusion: Female civilian flight attendants with job stress, long haul flight within three months and younger age had higher risk to be menstrual duration disorder. (Health Science Journal of Indonesia 2015;6:87-91)

Key words: menstrual duration, job stress, female civilian flight attendant, Indonesia
Female flight attendant is one of the profession that requires optimal health in order to maintain the safety of flight. Female flight attendants often encounters health problems due to the environment. The problem, among other others, on reproductive disorders such as menstrual duration disorder. This problem may encounter the performance the female flight attendants in doing their job, which could compromise the flight safety.

Previous studies showed that several risk factors (age, habits, job stress, occupational, flight hours) related to menstrual duration disorder among flight attendants. Female flight attendants have higher risk for having menstrual disorder compared to other non-flight attendant females and female ground crews. Furthermore, female flight attendants have more job stress than non-flight attendants. The job stress, among others, influences menstrual duration disorder in female civilian flight attendants.

In Indonesia, the study of several risk factors related to menstrual duration disorder is still rare. The study aimed to identify job stress and several other risk factors related to menstrual duration disorder among female flight attendants.

METHODS

The subjects of this purposively cross-sectional study consisted of female civilian flight attendants age 19–50 years who conducted routine medical examinations in Indonesian Aviation Medical Center in Jakarta and Garuda Sentra Medika in May 18-29, 2015.

Menstrual duration disorder defined as duration of menstruation more than 8 days and/or shorter than usual period (3-5 days).

Job stress was measured using the National Institute for Occupational Safety and Health (NIOSH) Generic Job Stress Questionnaire Mental Demands Form Number 11. The questionnaire has good validity and reliability (interval consistency - Cronbach’s alpha 0.61).

The questionnaire consisted of 5 questions: (1) My job requires a great deal of concentration; (2) My job requires me to remember many different things; (3) I must keep my mind on my work at all times; (4) I can take it easy and still get my work done.; (5) I can let my mind wander and still do the work.

The answer in the form of a Likert scale with four choices: strongly agree, slightly agree, slightly disagree dan strongly disagree. Each answer was given a score of 1-4 by the researchers according to the female flight attendant answers.

Number 1 for the lowest score and number 4 for the highest score.

The score assessment inverted to question numbers 1-3, and then carried out a summation of the whole score. The lowest score at 5 and a highest of 20. The higher the score, the more likely a flight attendant job stress.

For this analysis we used the cut-off point for job stress that identified based on Receiver Operating Characteristics Curve (ROC) analysis. The results are: 0 =without job stress (total score 12 or less; 1 = = with job stress (total score 13 or more)

The demographic, occupation, habit characteristics, job stress and menstrual duration disorder data were collected based on interview using a questionnaire for this study. Height and weight data was obtained from the medical records.

For this study, occupational risk factors were categorized: the most frequent flight type since last week, last three months, and last year (short haul was 2 hours or less, intermediate haul was 2 – 6 hours, and long haul was 6 hours or more); rating type (Boeing, Airbus, ATR and others); total flight hours within three months (21-225 hours and 226–500 hours).

Age was categorized into 4 categories: 19 – 24 years, 25 – 29 years, 30 – 39 years and 40 – 50 years. Smoking was categorized into 3 categories: never smoke, was smoking and currently smoking.

Body mass index (BMI), for this study BMI based on WHO categorization, and we re-categorized into 2 categories: normal and overweight/obese. For Asia race: Normal = 18.5 – 22.9 kg/m2; overweight = 23.0 – 24.9 kg/m2 or more. While for Caucasian race: Normal = 18.5 – 24.9 kg/m2; overweight = 25.0 kg/m2 or more.

Flight type categorized as 1 = short haul; 2 = intermediate haul; and 3 = long haul. Short haul if one flight sector less than 2 hours; intermediate haul when the flight for one sector is 2 to 6 hours; long haul for the flight in one sector more than 6 hours.

The analysis in this study used Cox regression with constant time. Data were analyzed using STATA version 9.

Ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Jakarta. This study was conducted upon approval the Chief of Civil
Aviation Medical Center, Directorate General of Civil Aviation, Jakarta and Chief of Garuda Sentra Medika.

RESULTS

Among the 521 female civilian flight attendants who underwent routine medical examination, 393 subjects agreed to join the study. We excluded 19 subjects who ever had menstrual duration disorder before being employed. Therefore, leaving 374 subjects included for this analysis. None of the included subjects was obese.

Table 1 showed that 35.8% (134/374) subjects had menstrual duration disorder. Subjects with or without menstrual duration disorder were similarly distributed in terms of body mass index and smoking habit, total flight hours within three months, rating type and flight type since last week and last year.

Table 2, our final model, showed that three dominant risk factors (job stress, flight type since last three months and age) related to menstrual duration disorder. Subjects who had job stress had 58% higher risk for having menstrual duration disorder [adjusted relative risk (RRa) = 1.58; P = 0.071].

In term of long haul flight within three months, those who long haul flight within three months compared to short haul flight had 69% higher risk for having menstrual duration disorder (RRa=1.69; P = 0.004).

Furthermore, subjects aged 30-39 years had 50% less risk of having menstrual duration disorder (RRa = 0.50; P = 0.057).

Table 1. Smoking habit, body mass index, occupation, flight and risk of menstrual duration disorder

<table>
<thead>
<tr>
<th>Smoking habit</th>
<th>Menstrual duration disorder</th>
<th>Crude relative risk</th>
<th>95% confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 240)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disorder (n = 134)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>197</td>
<td>63.5</td>
<td>113</td>
<td>36.5</td>
</tr>
<tr>
<td>Ever</td>
<td>21</td>
<td>77.8</td>
<td>6</td>
<td>22.2</td>
</tr>
<tr>
<td>Current</td>
<td>22</td>
<td>59.5</td>
<td>15</td>
<td>40.5</td>
</tr>
<tr>
<td>Body mass index</td>
<td>Normal</td>
<td>181</td>
<td>63.1</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>Overweight</td>
<td>59</td>
<td>67.8</td>
<td>28</td>
</tr>
<tr>
<td>Flight hours within three months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-225 hours</td>
<td>180</td>
<td>63.6</td>
<td>103</td>
<td>36.4</td>
</tr>
<tr>
<td>226-500 hours</td>
<td>60</td>
<td>65.9</td>
<td>31</td>
<td>34.1</td>
</tr>
<tr>
<td>Flight type since last week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short haul</td>
<td>127</td>
<td>65.1</td>
<td>68</td>
<td>34.9</td>
</tr>
<tr>
<td>Intermediate haul</td>
<td>88</td>
<td>65.7</td>
<td>46</td>
<td>34.3</td>
</tr>
<tr>
<td>Long haul</td>
<td>25</td>
<td>55.6</td>
<td>20</td>
<td>44.4</td>
</tr>
<tr>
<td>Flight type since last year</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short haul</td>
<td>89</td>
<td>65.4</td>
<td>47</td>
<td>34.6</td>
</tr>
<tr>
<td>Intermediate haul</td>
<td>117</td>
<td>65.0</td>
<td>63</td>
<td>35.0</td>
</tr>
<tr>
<td>Long haul</td>
<td>32</td>
<td>57.1</td>
<td>24</td>
<td>42.9</td>
</tr>
<tr>
<td>Rating type tewater Avions de transport</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oeing</td>
<td>191</td>
<td>63.2</td>
<td>111</td>
<td>36.8</td>
</tr>
<tr>
<td>irbus</td>
<td>40</td>
<td>70.2</td>
<td>17</td>
<td>29.8</td>
</tr>
<tr>
<td>Avions de transport</td>
<td>7</td>
<td>58.3</td>
<td>5</td>
<td>41.7</td>
</tr>
<tr>
<td>egional thers</td>
<td>2</td>
<td>66.7</td>
<td>1</td>
<td>33.3</td>
</tr>
</tbody>
</table>
Furthermore, levels of cortisol (adrenocorticotropin releasing hormone) stimulate the secretion of CRH (corticotropin releasing hormone) and will suppress GnRH (gonadotropin releasing hormone) in the hypothalamus that releases (adrenocorticotropic hormone (ACTH) which stimulates the production of glucocorticoid hormones. Glucocorticoid hormones (cortisol, adrenaline and androgen) inhibits GnRH, follicle stimulating hormone (FSH)–luteinizing hormone (LH) and ovary, causing follicular atresia, thus lowering the estrogen level and will result in menstrual disorder.

In this study, we noted that female civilian flight attendants with job stress had higher risk for having menstrual duration disorder. This result is similar with the previous study of menstrual duration disorder in female civilian flight attendants in Indonesia. In this study, we also noted that female civilian flight attendants with long haul flight within three months compared to short haul flight had 69% statistically significant higher risk of having menstrual duration disorder. This result was different with a previous study that reported that female flight attendants who had 8 hours or more compared to those who had less than 8 hours flight had more risk (but not statistically significant) had menstrual disorder. The difference might be due to the grouping of hours, which is more than 6 hours in this study, and limited to the last three months.

Menstrual disorder related to sleep cycle may be disturbed by jet lag caused by long haul flight or shift lag in the working environment. This will cause hypothalamo-pituitary-ovarian axis dysfunction, which are also related to stress and the secretion of LH. Sleep directly inhibits LH secretion, thus lowering the LH level on the early follicular phase and affects the menstrual pattern.

Our study noted that compared with the female civilian flight attendants aged 19–24 years, female civilian flight attendants aged 30–39 years had 50% less risk for having menstrual duration disorder.

The adaptation process within working environment between 30–39 years subjects might be better than the 19–24 years old. In addition, aging process that starts at 40 years affects the ovary’s ability to react to the stimulation of gonadotropin hormones.

### DISCUSSION

This study had several limitations, among others, this study was conducted among limited and with purposive selected civilian flight attendants. The selected samples did not represent the general civilian flight attendants. Furthermore, there were some misinterpretation by the subjects while answering the questions listed in the questionnaire. Even though, we explained the questionnaire before the interview.

<table>
<thead>
<tr>
<th>Job stress</th>
<th>Menstrual duration disorder</th>
<th>Adjusted relative risk</th>
<th>95% confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 240)</td>
<td>Disorder (n = 134)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without job stress</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>With job stress</td>
<td>198</td>
<td>62.1</td>
<td>121</td>
<td>37.9</td>
</tr>
<tr>
<td>Flight type since last three months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short haul</td>
<td>105</td>
<td>68.6</td>
<td>48</td>
<td>31.4</td>
</tr>
<tr>
<td>Intermediate haul</td>
<td>113</td>
<td>64.6</td>
<td>62</td>
<td>35.4</td>
</tr>
<tr>
<td>Long haul</td>
<td>22</td>
<td>47.8</td>
<td>24</td>
<td>52.2</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 – 24 years</td>
<td>161</td>
<td>63.6</td>
<td>92</td>
<td>36.4</td>
</tr>
<tr>
<td>25 – 29 years</td>
<td>48</td>
<td>59.3</td>
<td>33</td>
<td>40.7</td>
</tr>
<tr>
<td>30 – 39 years</td>
<td>23</td>
<td>82.1</td>
<td>5</td>
<td>17.9</td>
</tr>
<tr>
<td>40 – 50 years</td>
<td>8</td>
<td>66.7</td>
<td>4</td>
<td>33.3</td>
</tr>
</tbody>
</table>

*Adjusted each other among risk factors listed on this table*

<table>
<thead>
<tr>
<th>Note</th>
</tr>
</thead>
</table>
In conclusion, female civilian flight attendants with job stress, long haul flight within three months and compared with the female civilian flight attendants aged 19–24 years, those who had age 30–39 years had 50% less risk for having menstrual duration disorder.

Acknowledgement
We would like to express our gratitude to Mr. Medianto and Dr. Ichwan Zulhidzaan who allowed us to conduct us to conduct this study at Civil Aviation Medical Center, Directorate General of Civil Aviation, Jakarta, and Garuda Sentra Medika.

REFERENCES
Artemisinin-napthoquine versus dihydroartemisinin-piperaquine in adult subjects with Plasmodium vivax infection

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Abstract

Background: This study was to compare the efficacy and safety between Artemisinin-Napthoquine (AN) as a single dose as well as an alternative drug, Dihydroartemisinin-Piperaquine (DHP) as a three-day standard regimen on P. vivax infection.

Methods: This was an open randomized study performed during the period of April 2007- March 2008 in three Armed Forces Hospitals in Jayapura, Papua Province, and one private hospital in Maumere, East Nusa Tenggara Province. This study was a part from previously published study for any malaria infection. Efficacy was the absence of clinical and parasitological malaria until day 42, performed as Adequate Clinical and Parasitological Response (ACPR). Safety was performed based on adverse event in any day of follow up which never reported at day recruitment (d0).

Results: This study analyses 158 P. vivax cases. A total 80 subjects were treated with AN and 78 with DHP. The median Parasite Clearance Estimator (PCE) was 2.32 (range: 1.42 – 7.78; Interquartile Range (IQR): 1.99 – 2.82) hours in AN and 2.05 (range: 1.30 – 8.30; IQR: 1.82 – 2.46) hours in DHP group. The parasite clearance was complete by 64 hours. The ACPR was 100% (95% Confidence Interval (CI): 95.2-100) in the AN, and 100% (95% CI: 94.9-100) in the DHP group. Both drugs have similar mild and tolerated adverse events.

Conclusions: Both drugs have similar efficacy and safety for the treatment of P. vivax in adults. Although AN has took longer PCE compared to DHP, 100% clearance was achieved in both groups in 64 hours. (Health Science Journal of Indonesia 2015;6:92-8)

Key word: malaria, Artemisinin, napthoquine, dihydroartemisinin, vivax
Malaria, the old disease caused by *Plasmodium* species has been difficult to control until now. *P. falciparum* and *P. vivax* infection (55%; 45%) comprise the main malaria infections in Indonesia. Although *P. vivax* is usually associated with uncomplicated malaria, some cases may become severe. Malaria is a major health issue in the eastern part of the Indonesia. Annual Parasite Incidence (API) in Papua and East Nusa Tenggara was reported as 177 and 81 cases per 100000 people in 2007.

Dihydroartemisinine-Piperaquine (DHP) which has been widely used since 2009 has become the new standard regimen of antimalarial therapy in Indonesia and has demonstrated better efficacy and safety against *P. vivax* in comparison with AAQ. DHP is a fixed once-daily dose taken for three days. The drug is well-tolerated and has better compliance than AAQ in Indonesia. Drug compliance is a complex issue for antimalarial regimens and can be associated with early treatment failure (e.g. Chloroquine). Although safe and efficacious treatment regimens of only three days are available compliance remains an issue. An alternative drug that could be taken in a single dose and directly observed could improve compliance and prevent treatment failure and relapse. Additionally relapses an important issue and difficult to distinguish from reinfection or recrudescence in *P. vivax* infection.

Artemisinin-Naphthoquine (AN) is a alternative drug that can be given in a single dose and is reported to have better efficacy and safety for *P. falciparum*. Artemesunate-Amodiaquine (AAQ) was the first ACT approved in Indonesia since 2004 but the efficacy for *P. vivax* is 52%. AN was developed in China in the 1990s and is known to be more efficacious than artesinin or piperaquine monotherapy. The API decreased was shown in Papua and East Nusa Tenggara in 2011 after Artemisinin Combination Therapy (ACT) was adopted as a standard regimen.

In this report, we compared the efficacy and safety profile of DHP and AN in treating *P. vivax* malaria patients in Indonesian hospitals. We compared the clinical symptoms from day 0 to 42 days and the recurrence of parasitemia from day 7 until day 42.

**METHODS**

**Study Design**

This was an open-label multi-center randomized trial. phase 3 comparative study to determine efficacy and safety of a single dose AN vs DHP as a three-day standard regimen in adults with uncomplicated *P. vivax*. We evaluated 158 subjects infected with *P. vivax* alone. Inclusion criteria were previously reported. Subjects infected by *P. vivax* were required to have circulating asexual parasite levels ≥ 250 parasite/µl.

**Study Setting**

The study was performed during the period of April 2007- March 2008 in three Armed Forces Hospitals in Jayapura, Papua Province and one private hospital in Maumere, Sikka District, East Nusa Tenggara Province. All of the subject were uncomplicated *P. vivax* cases but all of them were hospitalized because AN was a new drug therefore the subjects received DHP had the same treatment as AN. In Papua province, chloroquine resistance, cross-resistant amodiaquine, and low AAQ efficacy and safety for *P. vivax* had been previously reported.

**Study Drugs**

The AN was manufactured by Kunming Pharmaceutical Corporations Arco™. One tablet contains 250 mg of Artemisinin and 100 mg of Naphthoquine (equivalent with 156.6 mg of Phosphate Naphthoquine). The drug was compared with DHP. Duo-Cotexin™ produced by Beijing Holley Cotex Pharmaceutical Co.Ltd; one tablet contains 40 mg and 320 mg of dihydroartemisinin piperazine. Four tablets of Artemisinine-Naphthoquine (Arco™) were administered to the AN subjects by investigator on recruitment day (D0) only. Dihydroartemisin-piperaquine (Duo-Cotexin™) was given to DHP subjects by investigator in one dose a day for three days; one dose was 3 tablets for people with body weight of 35-60 kg or 4 tablets for body weight of 60 kg. Primaquine with 0.5 mg per kg body weight was given to all subject when recurrence occurred or on the last day of the study (Day 42). All drug treatments on all subjects were directly observed.

**Study Procedure**

As detailed previously, parasite asexual and gametocyte count was measured on day 0 at hour 0 (h0) then measured at eight-hour intervals for the first three days (at 8, 16, 24, 32, 40, 48, 56, 64, and 72 hours). Measuring the parasite was conducted on follow up schedule at day 7, 14, 21, 28, 35 and 42. The blood blot slide was taken and read by trained microscopist. The asexual parasite count was calculated based on total parasite per 200 µl leucocyte multiplied by 5000 µl leucocyte.
the gametocyte was calculated per 2000 ul leucocyte multiplied by 5000 µl leucocyte. All subjects were hospitalized until day 3 to observe adverse events even though the parasitemia was cleared.

History taking, clinical examination and adverse events were all performed on the initial recruitment day 0 (d0), and on follow-up visits (d1, d2, d3, d7, d14, d21, d28, d35). and until the last day (d42) or days of recurrence.

**Study outcomes and Data Analysis**

Efficacy was defined according to the WHO 2009 as a Adequate Clinical and Parasitology Response (ACPR) protocol at D42, which is evaluated based on intention to treat (ITT) and Per Protocol (PP) analysis. ACPR is defined as the absence of clinical and parasitological malaria until day 42. The subject who unable to meet ACPR was considered either Early Treatment Failure (ETF), Late Clinical Failure (LCF) or Late Parasitological Failure (LPF). Subjects lost to follow up, withdrawal of consent and protocol violations were considered censored events until day 42 according WHO guideline 2009. ITT is defined as analysis on all the subjects who were recruited and take at least one dose of the study drug; while PP is defined as analysis on all subject who finished the study regimen. The protocol violation was defined as all the subject who unable to meet inclusion and exclusion criteria including the new infection with different species.

Safety was performed based on a occurrence of adverse events that happened until d42 during AN and DHP treatment. Any clinical symptom in anyad of follow-up which never reported at day recruitment(d0) was considered as an adverse event.

Other parameters measured such as proportion of clinical symptom, parasite clearance time and gametocyte clearances were analyzed by chi-square test and t-test or mann-whitney test. Proportion of clinical symptom was defined as any clinical symptom at day of recruitment according to standard clinical malaria manifestation. Proportion of asexual parasitemia and gametocytes were calculated for each eight-hour interval until hour 72 (d72), gametocytes would be counted especially each seven-days interval until day 42(d42). Proportion of parasite clearance was measured for regression models of the log transformed parasite counts were fitted in order to estimate parasite clearance using the Parasite Clearance Estimator Tool (PCE) developed by the World Wide Antimalarial Resistance Network (WWARN).

The study received ethical approval by the Ethics Commission of the National Institute of Health Research and Development, Ministry of Health, Indonesia with no. LB.03.02/2/449/2007. And The Bureau of Food and Drug Control Republic of Indonesia with no. PO.01.01.3.1.1682

**RESULTS**

A total of 158 subjects were included in the study with 80 subjects treated with AN and 78 with DHP (figure 1). In the AN group two cases (2.5%) were analysed as protocol violations, one due to the ingestion of other antimalarial drugs on the d28, and the other because of failure classified as Late Parasitological Failure (LPF) with a different species (*Plasmodium falciparum*) on d32. In DHP group two cases (2.6%) were analysed as withdrawn consent on d0 and d4 and two cases (2.6%) as protocol violations with LPF of different species (*Plasmodium falciparum*) on day 35. Additionally, three cases in the AN group and four cases in the DHP group were lost to follow-up (LTFU). Primaquine was given to all subject.

The baseline characteristics of the two study groups were similar such as sex and fever (table 1). The clinical symptom characteristics at day of admission were similar (table 2). History of vomiting was reported in more than 20% of the subjects in AN group compared to DHP (22.5% vs 6.4%. p = 0.008).

The proportion of parasitemia at eight-hour intervals from 0 until 72 hours can be found in figure 1. The median parasite clearance estimator (PCE) was 2.32 (range: 1.42 – 7.78 ; IQR: 1.99 – 2.82) hours with median PC50 2.98 (range: 0.11 – 11.59) hours and median PC90 8.41 (range: 4.22 – 24.32) hours after AN treatment. While the median PCE after DP treatment was 2.05 (range: 1.30 – 8.30 ; IQR: 1.82 – 2.46) hours with median PC50 2.00 (range: 0.07 – 14.88) hours and median PC90 6.22 (range 3.23 -22.58) hours. Gametocyte clearance was not evidence until 72 hours after treatment for both of drugs (figure 2). All subjects obtained gametocyte clearance on day 21 in the AN group and on day 7 in the DHP group.

The APCR of both drugs on d42 based on ITT and PP analysis was 100% (95% CI: 95.2 –100) for AN and 100%, 95% CI: 94.9 – 100 for DHP. There was no ETF, LCF or LPF with *P. vivax* following recurence cases in both treatment arm. Confidence intervals measures for APCR were by exact binomial method for PP analysis or beta product confidence procedure for ITT analysis and were equivalent because there was 100% response.
Table 1. Several baseline characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Artemisin+ Naphthoquine</th>
<th>DihydroArtemisin+ Piperquine</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subject</td>
<td>(n=80)</td>
<td>(n=78)</td>
<td></td>
</tr>
<tr>
<td>Males (%)</td>
<td>91.3</td>
<td>82.1</td>
<td>0.142</td>
</tr>
<tr>
<td>History of Fever (%)</td>
<td>97.5</td>
<td>98.7</td>
<td>1.000</td>
</tr>
<tr>
<td>Fever ≥ 37.5 °C (%)</td>
<td>56.3</td>
<td>55.1</td>
<td>1.000</td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean (95%CI) parasitemia par/ul</td>
<td>4769 (3751 – 6002)</td>
<td>4628 (3640 – 6002)</td>
<td>0.874</td>
</tr>
<tr>
<td>Geometric mean (95%CI) gametocytemiai par/ul</td>
<td>40 (30 – 54)</td>
<td>33</td>
<td>0.303</td>
</tr>
<tr>
<td>Gametocytemia (%)</td>
<td>88.8</td>
<td>92.3</td>
<td>0.623</td>
</tr>
<tr>
<td>Median (range) haemoglobin in g/dl</td>
<td>13 (7.3 – 23.1)</td>
<td>13 (7.5 – 16.4)</td>
<td>0.523</td>
</tr>
<tr>
<td>Anemia group (Hb&lt;9 g/dl) (%)</td>
<td>13.8</td>
<td>17.9</td>
<td>0.518</td>
</tr>
<tr>
<td>Median (range) age in years</td>
<td>23 (16 – 62)</td>
<td>23 (16 – 64)</td>
<td>0.490</td>
</tr>
<tr>
<td>Median (range) body weight in Kg</td>
<td>60 (39 – 81)</td>
<td>58.5 (41 -75)</td>
<td>0.153</td>
</tr>
</tbody>
</table>

Table 2. Several clinical manifestation characteristics of subjects

<table>
<thead>
<tr>
<th>Clinical Symptom</th>
<th>Artemisin+ Naphthoquine</th>
<th>Artemisin+ Piperquine</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>3.8</td>
<td>0.0</td>
<td>0.245</td>
</tr>
<tr>
<td>Palpitations</td>
<td>7.5</td>
<td>3.8</td>
<td>0.517</td>
</tr>
<tr>
<td>Cough</td>
<td>7.5</td>
<td>10.3</td>
<td>0.742</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>10.0</td>
<td>10.3</td>
<td>1.000</td>
</tr>
<tr>
<td>Anorexia</td>
<td>12.5</td>
<td>14.1</td>
<td>0.950</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>13.8</td>
<td>11.5</td>
<td>0.858</td>
</tr>
<tr>
<td>Unwell</td>
<td>21.3</td>
<td>25.6</td>
<td>0.643</td>
</tr>
<tr>
<td>Sweating</td>
<td>21.3</td>
<td>29.5</td>
<td>0.314</td>
</tr>
<tr>
<td>Vomiting</td>
<td>22.5</td>
<td>6.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Myalgia</td>
<td>23.8</td>
<td>37.2</td>
<td>0.096</td>
</tr>
<tr>
<td>Fatigue</td>
<td>23.8</td>
<td>19.2</td>
<td>0.619</td>
</tr>
<tr>
<td>Dizzy</td>
<td>41.3</td>
<td>34.6</td>
<td>0.487</td>
</tr>
<tr>
<td>Rigors/chills</td>
<td>52.5</td>
<td>50.0</td>
<td>0.877</td>
</tr>
<tr>
<td>Headache</td>
<td>53.8</td>
<td>51.3</td>
<td>0.880</td>
</tr>
<tr>
<td>Nausea</td>
<td>58.8</td>
<td>51.3</td>
<td>0.433</td>
</tr>
</tbody>
</table>

A few adverse events were reported during this study. The most commonly reported adverse events in the AN group were nausea 6.3%(5/80), abdominal pain 5%(4/80), fatigue 3.8%(3/80), cough 3.8%(3/80), dizzy 7.5%(6/80), sleep disturbance 6.3%(5/80) and rigors 5%(4/80). The most common adverse events reported in the DHP group were fatigue 9.1%(7/77), cough 2.6%(2/77), dizzy 3.9%(3/77), vomiting 7.8%(6/77) and abdominal pain 2.6%(2/77).

DISCUSSION

Our study shows AN and DHP had similar efficacy with 100 percent ACPR in all subjects with P.vivax at day 42. This demonstrated that both drugs have essentially fulfilled the WHO criteria in that the percentage of efficacy is higher than 95%. Both drugs also show similar safety profile.

This study is consistent with other AN and DHP studies and confirmed that AN as a single drug can be a potential alternative for treating infection with P. vivax.11-15 In our study the recurrence of P. Vivax was not found. Gametocytemia was found in more than 80 percent of patients at baseline which is characteristic of the sexual stage of P. vivax infection.21 Both drugs can eliminate the gametocyte but gametocytemia was found in a few cases after 72 hours of treatment so the transmission could potentially continue. Prolonged gametocytemia in P. falciparum and P. vivax treatment could be one of many signs of recurrent parasitemia although in this study we observed no recurrence.21,22 The submicroscopic parasitemia study might be required in the future to detect the gametocytopenia level at the end of the treatment study. Because of this the primaquine was needed in the early treatment with ACTs. Primaquine can diminish asexual stage for all of Plasmodium species in humans.

The median PCE, PC50 and PC90 of DHP were faster than AN. The data conformed another study of an artesinin derivative.5,11,12-23 Artesinin derivative was a fast acting drug including dihydroartemisinin and artesinin. However dihydroartemisinin showed higher activity and had parasite clearance time faster compare artesinin.23,24 The parasitemia was clear at 64 hours for all subjects in both treatment. This shows that AN as a single-dose and DHP as a three-dose daily drugs have a similar anti-parasitic potential. Artesinin derivatives can eliminate the parasitemia immediately, but if administered alone as
monotherapy, it must be given for 5 – 7 days because of rapid drug elimination. This disadvantage exists even with a small parasitemia count, but it can be overcome by co-administration of Naphthoquine, which results in adequate parasite clearance time and protection from early recurrence. Moreover, artemisinin resistance for P. vivax has not been reported yet in Indonesia.

The adverse events were categorized as mild in this study for both treatments. This is consistent with the other studies. All adverse events were related to the drugs. Both drugs were tolerated well, despite high numbers of cases in the AN group reporting vomiting at baseline. This shows that both drugs did not give any severe effect.

The limitations of our study were a small sample size and inclusion of only adults. This study was a part of main study for all malaria cases, and was giving us information related the efficacy and safety of AN for treatment P. vivax infection. The inclusion of only adults was related with AN as a new drug, so the children study for AN was needed to use this drug as an alternative of malaria treatment widely. The study had more male subjects than females because we conducted the study at four hospitals and three were armed forces hospitals, which primarily serve men. Even though, there was no different cases between men and women for malaria vivax infection.
In conclusion, both drugs had similar efficacy and safety profiles for the treatment of vivax malaria in adult subjects in hospitals in Indonesia and fulfills WHO criteria. AN took longer to clear the parasitemia than DHP but parasite clearance was complete in all patients by 64 hours. Further evaluation studies are still needed at Primary Health Care centers, especially for efficacy and safety for children. This study supports the recommendation of AN as an alternative drug for *P. vivax* malaria.

**Acknowledgment**

We thank the Police Department Hospital, Army Hospital, and Navy Hospital in Jayapura, and St. Gabriel Hospital in Maumere. We are also grateful to the NIHRD team. We also thank Dr. Nancy Touchette (National Institutes of Health, USA), Prof Inge Sutanto (Faculty of Medicine, Universitas Indonesia), and Dr. Aprilianto Eddy Wiria, PhD (Departement of Parasitology Leiden University Medical Centre) for reviewing this manuscript.

**REFERENCES**

The effects of combined medicinal plants infusion on blood glucose, cholesterol, and triglyceride levels in hyperglycemic Sprague-Dawley rats

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Abstrak

Latar belakang: Kadar glukosa, kolesterol dan trigliserid darah yang tinggi dapat memicu terjadinya berbagai penyakit sehingga diperlukan cara untuk mengontrol ketiga parameter tersebut, salah satunya dengan obat tradisional. Penelitian dilakukan untuk membuktikan efek penurunan kadar glukosa, kolesterol dan trigliserid darah tikaus Sprague-Dawley (SD) dari infusa kombinasi sambiloto, salam, kayu manis, dan temulawak.

Metode: Penelitian terhadap 3 kelompok uji (kontrol, perlakuan, dan metformin sebagai kontrol positif) dilakukan pada bulan Juni-Augustus 2014 di laboratorium hewan coba B2P2TOOT Jawa Tengah Central Java. Hewan uji adalah tikus galur SD usia 70 hari. Induksi hiperglikemik tikus uji dengan pemberian high fructose diet (HFD), yaitu campuran fruktosa (36%), kuning telur (20%), dan pellet standar (44%) dalam 0,36 g /200 g Berat Badan (BB) selama 70 hari. Setelah diperoleh tikus hiperglikemik pada hari ke-70, dilanjutkan pemberian infusa formula selama 7 hari. Ketiga parameter uji meliputi kadar glukosa, trigliserida, dan kolesterol total tikus uji hari ke-77 dibandingkan dengan hari ke-70. Pemeriksaan histopatologi organ pankreas dilakukan pada akhir percobaan.

Hasil: Pemberian HFD selama 70 hari menyebabkan peningkatan signifikan kadar glukosa (p=0,0001), kolesterol (p=0,001) dan trigliserid total (p=0,006) darah. Peningkatan berat badan tikus uji dibandingkan tikuks kelompok kontrol tidak berbeda bermakna (p=0,792). Pemberian infusa ramuan sambiloto, salam, kayu manis, dan temulawak dosis 491,4 mg/200 g BB menurunkan kadar glukosa, kolesterol, dan trigliserida darah tikus uji berurutan sebesar 37,09% 19,51%, dan 79,29%.

Kesimpulan: Pemberian infusa ramuan sambiloto, salam, kayu manis, dan temulawak dosis 491,4 mg/200 g BB selama 7 hari secara signifikan menurunkan kadar glukosa darah, kolesterol, dan trigliserida tikus hiperglikemik. (Health Science Journal of Indonesia 2015;6:99-104)

Kata kunci: hiperglikemik, tikus, glukosa, sambiloto

Abstract

Background: High levels of blood glucose, cholesterol, and triglyceride tend to increase the incidence of several diseases. This study aimed to prove the effects of combined infusion of sambiloto, salam, kayu manis, and temulawak on decreasing blood levels of glucose, cholesterol, and triglyceride in Sprague-Dawley (SD) rats.

Methods: The rats were divided into 3 groups (control, treatment, and metformin as positive control). The study was conducted at the animal laboratory of MPTMRDC, Central Java, on June to September 2014. Hyperglycemia was induced by administering high fructose diet (HFD), a mixture of fructose (36%), egg yolk (20%), and standard pellets (44%) in 0.36 g/200 g Body Weight (BW) for 70 days. The combined infusion was given orally to the hyperglycemic rats for 7 consecutive days. The parameters were blood levels of glucose, cholesterol, and triglycerides. The pancreas was examined histopathologically at the end of the study.

Results: HFD for 70 days led to significant increase in glucose (p=0.0001), cholesterol (p=0.001), and total triglycerides (p=0.006) levels. The increase of body weight of experiment group had no significant difference with control group (p=0.792). The combined infusion of 491.4 mg/200 g BW significantly reduced blood glucose, cholesterol, and triglyceride levels in rats by 37.09, 19.51, and 79.29 %, respectively.

Conclusion: The administration of the combined infusion with a dose of 491.4 mg/200 g BW for 7 consecutive days decreased blood glucose levels, cholesterol, and triglyceride levels in hyperglycemic rats. (Health Science Journal of Indonesia 2015;6:99-104)

Keywords: hyperglycemia, rat, glucose, sambiloto
Diabetes mellitus (DM) is a metabolic disorder characterized by high level of blood glucose, the presence of glucose in urine, along with symptoms of polyphagia, polydipsia, polyuria, blurred vision, weight loss, itching, tiredness, and sleepiness. WHO data showed DM as the 6th leading cause of death in the world. Approximately 1.3 million people die from DM, with 4% dying before the age of 70 years. The prevalence of DM in productive aged urban Indonesians is 4.6%, consisting of 1.1% diagnosed DM and 3.5% undiagnosed DM. DM can be due to various factors, including insulin resistance, a condition in which the body's muscle, fat, and liver cells do not use insulin effectively, and other environmental factors. If not handled properly, DM will cause complications both acute or chronic. The long term use of medicine can cause negative effects especially on kidney function.

There are many medicinal plants which has been proven empirically as having antihyperglycemic properties, such as salam (Syzygium polyanthum) and sambiloto (Andrographis paniculata Ness). As reported by Nurwati (2009), S. polyanthum could lower blood glucose levels indicated by a significant difference between control and treatment group (dose of 4 g/100 g; p = 0.000), characterized by a decrease of malondialdehyde (MDA) levels of alloxan-induced diabetes in male white rats. Ethanolic extract of S. polyanthum leaves at concentrations of 30 and 70% exhibited hypoglycemic effects in glucose loaded rabbits. With 35% leaves infusion, a decrease in glucose levels was reported in glucose loaded rabbits when compared to standard glibenclamide.

Ethanolic extract of Cinnamomum zeylanicum cortex with a dose of 100 mg/kg BW/day for 90 consecutive days has been reported to increase the weight of reproductive organs, with increased motility and sperm count in Wistar rats. C. zeylanicum with doses of 100, 200 and 400 mg/kg BW did not cause death or behavioral changes (nerves, seizures, ataxia) in healthy Wistar rats. Administration of 2 ml cinnamon with a dose of 20 mg/day to HFD-rats caused a significant decrease in fasting blood glucose, insulin, and HbA1c. Ethanol extract of C. xanthorrhiza Roxb with doses of 300, 2000, and 5000 mg/kg BW gave no side effects or toxic effects, and did not cause death in Swiss female mice.

The use of four medicinal plants in the formula was to provide a better synergistic effect compared to the administration of only one herb. Reference? Many have studied the toxicity and anti-hyperglycemic activity of each plant, but data on the efficacy in combination form is not available yet. Therefore, a study needs to be conducted to investigate the effects of combined infusion of sambiloto, salam, kayu manis, and temulawak on blood glucose, cholesterol, and triglyceride levels in Sprague Dawley (SD) rats.

METHODS

This was an experimental study using healthy, white, male and female SD rats (Rattus norvegicus), aged 2-3 months. The rats were obtained from the Experimental Animal Care Unit of Universitas Gadjah Mada, Indonesia. The rats were divided into 3 groups, treatment, negative control, and positive control groups. Each group consisted of 6 white rats, males and females. The doses of A. paniculata and S. polyanthum were based on previous preclinical trials. The additional components, C. Xanthorrhiza and C. Zeylanicum, were based on empirical doses, which were converted to rat doses (multiplied by an extrapolation factor of 0.0182).

Before the study began, the rats were acclimatized for 7 days, housed in the experimental pharmacology laboratory of Medicinal Plant and Traditional Medicine Research and Development Center (MPTMRDC), given drink and fed with standard pellets ad libitum. For inducing hyperglycemia in the rats, the treatment and positive control groups were administered HFD, a mixture of fructose (36%), egg yolk (20%), and standard pellet (44%) for 70 consecutive days. Negative control group was given standard pellets only.

Measurement of blood glucose levels as well as total cholesterol and triglycerid levels was conducted respectively on days 0, 20, 40, 60, and 70. On day 71, the hyperglycemic treatment group was given orally administered combined infusion formula, consisting of S. polyanthum, A. Paniculata(dose?or %), C. Zeylanicum(dose?or %), and C. Xanthorrhiza(dose?or %), at a dose of 491.4 mg/200 g BW for 7 consecutive days. The positive control group was given metformin (45 mg/kg BW) for the next 7 days. On day 77, blood was taken via retro orbital venous plexus and centrifuged. The levels of blood glucose, total cholesterol, and triglyceride were determined. At the end of study, the rats were sacrificed and the pancreas isolated. Histopathological preparations were made and examined microscopically, comparing the treatment group to the control group. The results was analyzed.
using paired and unpaired t-test. This study received ethical approval from the Ethical Committee of National Institute Health Research and Development Center.

RESULTS

The administration of HFD for 70 consecutive days led to significantly increased glucose level as well as total cholesterol and triglyceride levels in the rats. The control group showed no significant changes in the levels of glucose, total cholesterol and triglyceride (p>0.05) as shown in Table 1.

The administration of combined medicinal plants infusion, which consisted of S. polyanthum, A. paniculata, C. zeylanicum, and C. xanthorrhiza at a dose of 491.4 mg/200 g BW significantly decreased the glucose level of treatment SD rat group (p<0.05). The total cholesterol and triglyceride levels of treatment group were also reduced. The administration of metformin at a dose of 45 mg/kg BW decreased both glucose and total cholesterol levels (Table 2).

The rat body weights (grams) were periodically measured once a week. The change of body weight changes of both treatment and metformin groups showed no significant difference compared to the change of body weight changes of the control group as shown in Table 3.

The average number of abnormal cells in pancreas in the control, treatment, and metformin groups were 23.0; 25.5 and 45.5 from 100 cells respectively (Figure 1). It can be concluded that the administration of the infusion formula at a dose of 491.4 mg/200 g BW for 70 consecutive days shows no significant different between the abnormal cell number of the control and the treatment groups (P = 0.387). Therefore, the administration of the infusion formula was safe for the pancreas of SD rats.

Table 1. Glucose, total cholesterol, and triglyceride levels caused by hyperglycemic induction after 70 days HFD administration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day/p value</th>
<th>Control</th>
<th>Infusion</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0</td>
<td>116.83±10.028</td>
<td>100.33±5.279</td>
<td>116.5±13.096</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>128.83±4.119</td>
<td>143.33±11.827</td>
<td>142.67±11.518</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.058</td>
<td>0.000</td>
<td>0.016</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0</td>
<td>52.17±8.727</td>
<td>48.50±11.589</td>
<td>53.33±7.421</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>50.00±8.438</td>
<td>101.67±8.756</td>
<td>91.83±14.063</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.671</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0</td>
<td>120.83±35.216</td>
<td>119.67±63.481</td>
<td>146.17±18.203</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>98.83±17.600</td>
<td>460.33±163.968</td>
<td>293.00±182.238</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.279</td>
<td>0.006</td>
<td>0.102</td>
</tr>
</tbody>
</table>

Table 2. Effects of combined infusion administration on glucose, total cholesterol and triglyceride levels in experimental induced hyperglycemic rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day/p value</th>
<th>Control</th>
<th>Infusion</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>70</td>
<td>128.83±4.119</td>
<td>143.33±11.827</td>
<td>142.67±11.518</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>118.83±13.934</td>
<td>90.17±7.985</td>
<td>91.50±12.029</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.151</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>70</td>
<td>50.00±8.438</td>
<td>101.67±8.756</td>
<td>91.83±14.063</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>57.67±8.869</td>
<td>81.83±10.088</td>
<td>68.17±9.432</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.306</td>
<td>0.003</td>
<td>0.021</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>70</td>
<td>98.83±17.600</td>
<td>460.33±163.968</td>
<td>293.00±182.238</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>84.17±23.727</td>
<td>95.33±10.948</td>
<td>100.17±25.097</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.043</td>
<td>0.003</td>
<td>0.051</td>
</tr>
</tbody>
</table>
Table 3. Body of weight of rats in grams

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (grams) in Week</th>
<th>W_{12} - W_{9}</th>
<th>p (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>197.7</td>
<td>215.0</td>
<td>226.8</td>
</tr>
<tr>
<td>Treatment</td>
<td>191.0</td>
<td>210.6</td>
<td>214.3</td>
</tr>
<tr>
<td>Metformin (grams)</td>
<td>186.9</td>
<td>204.3</td>
<td>210.9</td>
</tr>
</tbody>
</table>

Figure 1. Pancreatic histopathology of control (a), metformin (b) and treatment (c) groups

Note:  
- abnormal cell (in pancreas and surrounding tissues)
- normal cell

DISCUSSION

This was an experimental pharmacological study using white Sprague-Dawley (SD) rats (*Rattus norvegicus*). The limitation of this study was bias caused by the use of laboratory animals in which glucose levels, specified as a parameter, could be influenced by several factors, such as stress and other environmental factors.

Hyperglycemia in rats was induced by administering a high fructose diet (HFD), a mixture of 36% fructose, 20% egg yolk and 44% standard pellets, for 70 days. Measurement of rat blood glucose levels was conducted on days 0, 20, 40, 50, 70, and 77???(explained). The results (table 1) showed that the administration of HFD cause significant changes in blood glucose levels of rats on day 70. It has been postulated that increasing consumption of fructose may be a contributory factor in the development of obesity and the accompanying metabolic abnormalities. Most studies supporting these hypotheses, suggested that consumption of high amounts of fructose may stimulate lipogenesis and thus alter lipid metabolism and increase body weight. Several studies regarding the relationship between HFD with elevated levels of plasma glucose, insulin, and triglycerides have been made. Twelve week administration of 10% w/v fructose solution improved blood glucose and triglyceride levels in
male Wistar rats. In another study, supplementation of 10% w/v HFD for 4 weeks induced insulin resistance on SD male rats. In this study, HFD also caused a significant increase in cholesterol and total triglyceride. Previously reported that the administration of 10% w/v fructose solution for 12 weeks increased triglycerides in male Wistar rats. Fructose can be absorbed rapidly and is metabolized in liver.

The administration of combined infusion was found to have a positive antihyperglycemic effect by decreasing glucose level in the rats. This could be caused by a specific compound contained in A. paniculata and S. polyanthum. While C. Xanthorrhiza and C. zeylanicum were used as additional supportive effect. A. paniculata contains andrographolid which can increase the use of muscle glucose in streptozotocin induced diabetic rats through stimulation of glutation-4 transporter resulting in reduced plasma glucose levels. Andrographolid also increases the utilization of glucose by overexpressing mRNA and glutation-4 protein levels.

There was no significant body weight (week 0 - week12) difference between treatment and control groups. This indicated that the administration of high fructose diet for 70 consecutive days resulted in the same body weight changes in the treatment and control groups. In a previous research, a diet of 8% high fructose (corn syrup) in male SD rats with a 12-hour access to consumption, caused a significant increase in body weight of rats on week 8. While the consumption of the same diet for 6 months led to a significant increase in body weight, fat, and triglyceride levels compared to the control group. The increase of body weight was a result of daily HFD consumption.

In order to determine inflammatory effects on the cell nucleus, observations of abnormal cells were made on every 100 cells, by counting the number of inflammatory mononuclear cells in the islet Langerhans and surrounding cells with a magnification of 400x. At this magnification, the pancreatic cell could be distinguished from surrounding tissues. There was no significant difference of the abnormal cell number between the control and treatment rats groups. This meant that the administration of the combined infusion did not cause negative histopathologically effect (obstruction) on the rat pancreas. Abnormal cells of pancreas can appear as a result of direct toxic effects of certain chemicals, autoimun reaction, or viral infection. There are many levels of insulitis i.e perinsulitis (limphocyte infiltrating surrounding tissues of the pancreas), medium insulitis (more than 50% of the islet of Langerhans is infiltrated by limphocytes), hard insulitis (more than 50% of the islet of Langerhans is infiltrated by limphocytes) and end stage islet in which necrosis is found in the entire of islets of Langerhans.

In conclusion the administration of the combined infusion formula at a dose of 491.4 mg/200 g BW for 7 days decreased blood glucose, cholesterol and triglyceride levels of hyperglycemic rats and was considered safe for the pancreas.

Acknowledgment

A special thanks to Prof. Abdul Rohman for reviewing this article. This study was funded by MPTMRDC, National Institute of Health Research and Development, Ministry of Health Republic of Indonesia.

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Effect of purified gambir leaves extract to prevent atherosclerosis in rats

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Abstract

**Background:** Atherosclerosis is a risk factor for coronary heart disease (CHD). Catechin have high antioxidant activity that can prevent atherosclerosis. Gambir (Uncaria gambir, Roxb.) leaves extract have high catechin content thereby potentially inhibiting atherosclerosis. This research was aimed to examine effect of purified gambir leaves extract to prevent atherosclerosis in rats.

**Methods:** The experimental laboratory study was conducted in Pharmacy Laboratory and Animal Laboratory, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia in 2014. Gambir leaves extract were purified to gain optimum catechin. Afterwards, antioxidant activity was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, with ascorbic acid as positive control. Thirty six white male Sprague Dawley rats aged 2.5 months were randomly divided into six groups, i.e. normal control group, negative control group (aquades), positive control group (atorvastatin 2 mg/200 g bw), extract dose 1 (20 mg/200 g bw), dose II (40 mg/200 g bw) and dose III (80 mg/200 g bw). The rats were given high fat diet and treatment according to their group for 60 days, except for normal control group.

**Results:** Catechin content in the purified gambir leaves extract was 92.69%. From antioxidant activity test, IC$_{50}$ was found to be 11.76 µg/mL. Anti-atherosclerotic activity study shown that compared to negative control, all three doses of purified gambir leaves extract were able to prevent atherosclerosis through inhibition of aortic wall thickening and foam cell formation due to high fat diet (p<0.05). Anti-atherosclerotic activity increased with increasing dose.

**Conclusion:** Gambir leaves purified extract had the effect of preventing the thickening of the walls and foam cell formation rat aorta. (Health Science Journal of Indonesia 2015;6:105-10)

**Keywords:** gambir, catechin, antiatherosclerosis
In the last decade, cardiovascular disease caused by atherosclerosis develop into a major killer in Indonesia. It has become a national health problem, thus we need an effective prevention efforts in overcoming atherosclerosis. Management therapy often used nowadays, especially in the acute phase, is administration of fibrinolytic or management of intervention, followed by drug therapy. However, such management should be supported by preventive measures in order to prevent the incidence of new patients or the emergence of similar attacks in older patients in the future.12

Atherosclerosis is accumulation of fat in the matrix of the tunica intima, followed by the formation of connective tissue in blood vessel walls. High level of total cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the blood will intensify the process of atherosclerosis.3 Synthetic drugs that are usually used to prevent the atherosclerosis are cholesterol-lowering drugs such as simvastatin and atorvastatin. However, these drugs have side effects such as myopathy, hepatotoxicity, peripheral neuropathy, dizziness, diarrhea, allergies and increase diabetes mellitus risk. Today, several research investigate potential medicinal plants that have similar effect with synthetic drugs, but with more tolerable side effects. Catechins are flavonoid derivatives secondary metabolites that have antioxidant and anti-atherosclerotic activity. Catechin compounds have cholesterol-lowering activity through inhibition of pancreas cholesterol esterase, bile acid binding, and reduction of cholesterol solubility in micelles that can delay the absorption of cholesterol. Administration of catechins for six weeks can reduce the average area of atherosclerotic lesions by 32% in rat aorta.4,6

One of the native plants in Indonesia which contains large amount of catechins is Gambir (Uncaria gambir Roxb.). Gambir leaf extract contains catechins as a major component as well as some other components such as catechutannic acid, quercetin, red catechu, gambir fluorescent, fats and waxes.7 The presence of a high content of catechins in Gambir leaves makes it a potential plant to be used as traditional medicine. This study aimed to examine effect of purified gambier leaves extract to prevent atherosclerosis in rats.

METHODS

This study was an experimental laboratory design and was conducted in Pharmacy laboratory and Animal Laboratory, Center for Biomicde and Basic Technology of Health, National Institute of Health Research and Development, Ministry of Health Republic of Indonesia in 2014.

Preparation and purification of extract

The leaves of gambir was procured from Herbal Plant, Lima Puluh Kota District in West Sumatra and it was authenticated by Herbarium Laboratory, Andalas University, Padang. Gambir extract was prepared by boiling the leaves and pressing them. The liquid extract was filtered and then concentrated using vaccum oven at 80°C to get dried extract. Extract purification process is done through following steps. The gambir extract was grindened into powder, suspended in n-hexane and homogenized using sonicator for 10 minutes. The suspension was filtered using filter paper. The residue is then dissolved in ethyl acetate and homogenized using sonicator for 10 minutes. After that, the solution was partitioned by adding distilled water, then shaken in a separator funnel and allowed to stand for 30-60 minutes until two layers were formed (layer of ethyl acetate on top and a layer of distilled water at the bottom). Both layer formed is then separated. Ethyl acetate layer is evaporated using rotary evaporator (Buchi) until viscous extract is gained. Furthermore, viscous extract is evaporated in a fumehood, and then dried using vacuum oven at 40-50 °C until a fixed weight is obtained. Finally, characterization of the purified extract is conducted. This include organoleptic inspection, moisture content, loss on drying, and ash content.8,9

Catechin assay in purified extract

Catechin standard calibration curve was done by plotting six concentrations, i.e. 25; 50; 100; 150; 200 and 300 ppm. Standards and purified extract samples were analyzed utilizing High Performance Liquid Chromatography/HPLC (Waters), with C18 column sized 4.6 × 150 mm, 0.45 mL/min flow rate, 1.0 mL injection volume and UV detection at 280 nm using PDA detector (Waters). The mobile phase gradient is used with the mobile phase A consist of 0.03% acetic acid in a mixture of acetonitrile: water (5:95) and mobile phase B consist of 0.1% trifluoroaset acid in acetonitrile. Mobile phase gradient conditions was 100% A at minutes 0 to 4, 71.5 A and 28.5 B at minutes 4 through 20, and 100% B until minutes 30.10

Antioxidant activity test

The antioxidant activity test was done using DPPH procedure. The sample with concentration of 0.2 mg/mL was dissolved in 100 mL ethanol. The solution was added with 5 mL of 0.1 mmol/L ethanol
solution of DPPH. The solution was then pipetted to spectrophotometer cuvette and incubated at 27°C for 20 minutes. Control blanks were made from ethanol, meanwhile positive control used was ascorbic acid. Absorbance was measured using a UV-VIS spectrophotometer (Hitachi) at 515 nm.\textsuperscript{11}

### Aortic thickness measurement and foam cell observation

The protocol of animal study has received the ethical clearance from Health Research Ethics Committee Faculty of Medicine University of Indonesia number 365/H2.F1/ETIK/2014.

Thirty six male Sprague Dawley strain rats aged 2.5 months old were randomly divided into six groups: normal group, negative group, positive group (atorvastatin), dose I (20 mg/200 g bw), II (40 mg/200 g bw) and dose III (20 mg/200 g bw) groups. Rats were induced with high cholesterol and saturated fat feeds for 60 days, except for normal group. After that, test animals were decapitated.

For histopathological treatment using hematoxylin and eosin stain (HE), 5 cm abdominal aortic tissue were taken. Observation of the aorta thickness was conducted using microscope equipped (Nikon E 2000) with a micrometer at 100 times magnification. The thickness of the aorta were measured on 8 view zone clockwise: 12:00, 13:30, 15:00, 16:30, 18:00, 19:30, 21:00 and 22:30. The thickness of the aorta was then calculated using the following formula:

\[
\text{aortic thickness} = \frac{\text{number of micrometer scale}}{\text{magnification}} \times 1000 \mu\text{m}
\]

Observations of foam cell formation in histopathology preparations of rat aorta is performed using a microscope at 400 times magnification.\textsuperscript{12}

### Data analysis

Data from the animal experiments were expressed as mean ± SD. The statistical significance of differences between the groups were analyzed with one-way ANOVA, followed by LSD post-hoc test analysis using SPSS software 17.0 version, p values of less than 0.05 were considered to indicate significant differences.

### RESULTS

Extract purification process was done twice. Each purification process used 200 g gambir leaves extract. Purified extract obtained respectively were 129.48 g and 128.72 g. The average yield of purified extract obtained is 64.55%. This indicates a more optimal purification process using ethyl acetate as the solvent. The characterization results showed in Table 1 proved that the purified extract met all parameters required in The Indonesian Herbal Pharmacopoeia.\textsuperscript{13}

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Results</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Solid, powder</td>
<td>Solid, powder</td>
</tr>
<tr>
<td>Color</td>
<td>Yellowish brown</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Odor</td>
<td>Specific gambier</td>
<td>Specific gambier</td>
</tr>
<tr>
<td>Taste</td>
<td>Slightly astringent</td>
<td>Slightly astringent</td>
</tr>
<tr>
<td>Yield of extract</td>
<td>64.88 %</td>
<td>≥ 50 %</td>
</tr>
<tr>
<td>Water content</td>
<td>5.44 %</td>
<td>≤ 14 %</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>7.56 %</td>
<td>≤ 14 %</td>
</tr>
<tr>
<td>Total ash content</td>
<td>0.36 %</td>
<td>≤ 0.5 %</td>
</tr>
<tr>
<td>Residual solvent</td>
<td>0 %</td>
<td>≤ 0.5 %</td>
</tr>
<tr>
<td>Level of catechin</td>
<td>92.69 %</td>
<td>≥ 90 %</td>
</tr>
</tbody>
</table>

Antioxidant activity test of purified extract using DPPH method came with IC\textsubscript{50} value of 11.56 mg/mL. This value is smaller than ascorbic acid used as comparator (IC\textsubscript{50} of 24.72 mg/mL). The smaller the IC\textsubscript{50} value means the higher the antioxidant activity. These data show the antioxidant activity of purified extract of gambir leaves is very strong.

### Table 2. Aortic thickness

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aortic thickness (µm ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>96.26 ± 11.40</td>
</tr>
<tr>
<td>Negative</td>
<td>228.72 ± 17.27</td>
</tr>
<tr>
<td>Positive</td>
<td>142.06 ± 10.35 *#</td>
</tr>
<tr>
<td>Dose I</td>
<td>144.62 ± 8.83 *#</td>
</tr>
<tr>
<td>Dose II</td>
<td>127.38 ± 8.16 *#</td>
</tr>
<tr>
<td>Dose III</td>
<td>109.54 ± 9.53 *#</td>
</tr>
</tbody>
</table>

Remarks: n = 6 rats.
Significantly different: # p<0.05 compared with normal group;
*#p<0.05 compared with negative group, one way Anova

Aortic thickness shown that compared to negative control, all three doses of purified gambir leaves extract were able to prevent atherosclerosis through inhibition of aortic wall thickening. (p<0.05).

After being observed in microscope at 400 times x magnification, the formation of foam cells in negative group shown the foam cells are seen in tunica intima and tunica media seemed more than other group.
DISCUSSION

The purified extract gained has a moisture content of 5.44%, which complies to Indonesia Herbal Pharmacopoeia (IHP), i.e. below 14%. The less water content in the material ingredients can reduce the risk of microbial and fungal growth, or damage caused by insects. Loss on drying (LOD) examination aims to determine water and volatile components lost when heated at 105°C. The purified extract was found to have LOD of 7.56%. LOD value that is bigger than water content explains that in addition to water, there are also volatile components. Determination of total ash content was conducted to determine the amount of material remains after burning at 700°C. The total ash content obtained was 0.36%, which also met Herbal Pharmacopoeia requirements i.e. less than 0.5%. Small ash content value indicates that the material is left slightly. The remaining materials include physiological ash, derived from plant tissue itself as well as non-physiological ash which is a residue of foreign material on the surface of plant, such as sand and soil. It can be said that smaller value of ash content means smaller impurity in the purified extract.\textsuperscript{9,13}

Total catechin content in the purified extract derived from HPLC analysis was 92.69%. The catechin content which is more than 90% met the requirement of IHP. These result was consistent with Kurniatri (2015) that purification process can improve the purity and levels of catechin.\textsuperscript{15}

Catechins are flavonoid of polyphenol groups that function as a free radical scavenger to provide the hydrogen atom. The structure that allows the radical scavenging activity of polyphenols is the presence of 3,4-dihydroxyl (catechol structure) in the B ring, which acts as an electron donor and became the target of radical. The 3-OH structure of ring C is also beneficial to the activity of antioxidant polyphenols. Conjugation bond at C2-C3 with 4-keto group plays a role for the electron delocalization of the B ring, which in turn increases free radical scavenging capacity. Besides that, the 3-OH group and 5-OH, in combination with 4-carbonyl function and C2-C3 double bond, also raise antioxidant activity. In the absence of o-dihydroxy structure in the B ring, the hydroxyl substituents on the catechol ring A can be compensated and the ability antiradikal activity of polyphenols is increased.\textsuperscript{15,16}
The third dose of purified extract was able to reduce the aortic thickness of rats under the mechanism of inhibition of LDL oxidation. Catechin as the largest component in the purified extract has antioxidant activity that is able to reduce LDL oxidation which can induce the formation of several cytokines that can activate pathways stimulated by mitogen-activated protein kinase (MAPK) leading to high expression of the enzyme matrix metalloproteinase-9 (MMP 9). Antioxidants also reduce the toxicity of oxidized LDL on endothelial cells, smooth muscle cells and macrophages as well as reduce degradation oksidatif. Cannabis. Catechins increase HDL levels. HDL has a role to transport cholesterol away from the arteries and back to the liver so it may lower the amount of cholesterol in plaque ateroma. The observation of microscopic atherosclerosis can be seen from the formation of foam cells in the aorta. Foam cells was formed of many macrophages and dendritic cells such as membrane-bound lipids in the cytoplasm. Cells containing the lipid looks like a bubble. The cells' formation begin when phagocytic cells ingest Apolipoprotein-B. The observations of foam cell would appear more clearly with microscope at 400× magnification. Purified extract was able to prevent the formation of foam cells on rats aorta. From microscopic observations, it can be seen that higher dose of purified extract can reduce the formation of foam cells better. Hartay's in vitro study showed that catechin phenolic group, either in extract or single-isomer form, can protect LDL from oxidation triggered by free radical. Furthermore, according to Yang and Koo, catechins have an activity to lower the number of lipid peroxidation products and is able to inhibit LDL oxidation in the endothelium. MMP 9 enzyme contribute a role in stabilizing the atherosclerotic plaque so those plaques would not rupture which in turn may flow through bloodstream and cause blood clots. Decreased expression of MMP 9 shows that catechins can be used as an agent to prevent plaque rupture in the foam cells of aorta. These results are consistent with research from Auclair et al which explain that oral administration of catechins for six weeks can reduce the average area of atherosclerotic lesions in the rat aorta.

In conclusion, gambir leaves purified extract had the effect of preventing the thickening of the walls and foam cell formation rat aorta.

Acknowledgment

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REFERENCES


Maternal education, prematurity and the risk of birth asphyxia in selected hospitals in Jakarta

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Abstract

Background: Birth asphyxia can causes hypoxic ischemic organ damage in neonates. According to advanced Indonesian Basic Health Research 2007, asphyxia was the second highest cause of infant death after infection (13.8%). This study aimed to identify several risk factors associated with birth asphyxia.

Methods: This study was carried out in one government and one private hospitals in Jakarta. All medical records of pregnant women who gave birth between January 1 to December 31 of 2011 were included. Birth asphyxia was defined as an Apgar score less than seven at one minute after birth. Logistic regression was used to analyze the risk factors related to birth asphyxia.

Results: As many as 2777 samples out of 4191 were included for this analysis. The proportion of asphyxia in newborn babies was 6.5%. Compared with those who had high educated mothers, those who had low education level had 4.3-fold higher risk to have an asphyxiated baby [adjusted odds ratio (ORa) = 4.31; P=0.000]. Meanwhile middle educated mothers had 3.3-fold higher risk to have birth asphyxia (ORa=3.31; P=0.000). In terms of gestational age at birth, compared with those who had full term birth, those who had preterm birth had 3.1-fold higher risk to have birth asphyxia (ORa=3.07; P=0.000). Meanwhile, although not significant, those who had postterm birth had 63% more risk (P=0.118) to have birth asphyxia.

Conclusion: The mother who had lower and middle education levels as well as preterm babies had higher risk for having birth asphyxia baby. (Health Science Journal of Indonesia 2015;6:111-5)

Keyword: maternal education, prematurity, birth asphyxia
Asphyxia is one of the major causes of neonatal mortality.\textsuperscript{1} Birth asphyxia can cause hypoxic ischemic organ damage in neonates which subsequently leads to severe or fatal outcome.\textsuperscript{2} The impact of birth asphyxia in the newborns includes attention deficits, hyperactivity, epilepsies, mental retardation, cerebral palsy, and the most severe damage is hipoxic ischemic encephalopathy (HIE).\textsuperscript{2,3}

Global estimates of birth asphyxia is 9\% of neonatal deaths in 2008.\textsuperscript{4} Further analysis of the Indonesian Basic Health Research (Riskesdas) 2007, asphyxia was the second major cause of infant death after infection (13.8\%).\textsuperscript{5} The incidence of birth asphyxia in Dr. Soetomo hospital in 2009 was 8\%.\textsuperscript{6}

Several factors that influence the occurrence of birth asphyxia, among others, maternal age, booking status, pre-eclampsia, primigravidity, maternal fever, maternal education, preterm babies, prolong labour, fetal distress, history of stillbirths, breech presentation, low birth weight.\textsuperscript{7,8} Data related to risk factors of birth asphyxia is still limited in Indonesia. This study aimed to identify the risk factors of birth asphyxia in two hospitals in Jakarta.

METHODS

This study was conducted on purposively selected samples in one government and private hospitals in Jakarta. The data were extracted from medical records of delivery during the period of January 1 to December 31, 2011. The women who gave birth less than 28 weeks of gestation were excluded from this analysis.

The data collected were demographic characteristics and clinical risk factors of birth asphyxia. The dependent variable was birth asphyxia while the independent variables were maternal age, maternal education level, occupations, parity, gestational age, premature rupture of membranes (PROM), prolonge labour, fetal distress, malpresentation/position, intra-uterine growth retardation (IUGR), and fetal nuchal cord.

Birth asphyxia was defined as an Apgar score of less than seven at one minute after birth.\textsuperscript{9,10} An Apgar score consists of five components: appearance, pulse, grimace, activity, and respiration, ranging from zero to ten.\textsuperscript{2} The score in this study was determined by professional health workers.

Age was grouped into three categories: 13-20, 21-35 years, 36-46 years. Education level was divided into three categories: low (uneducated up to completed primary school), middle (completed junior high school), and high (completed senior high school or more). Parity was divided into nulliparous (has never given birth), primiparous (one live birth), and multiparous (two or more live births). Premature rupture of membranes (PROM) was grouped into yes and no (yes = diagnosed as PROM by profesional health workers, or likewise).

Prolonged labour was categorized into yes and no (yes = diagnosed as prolong labour by professional health workers, or likewise). Fetal distress was divided into yes and no (yes= diagnosed as fetal distress by professional health workers). Malpresentation/position was divided into yes and no (yes = diagnosed as malpresentation/position by professional health workers). Intra-uterine growth retardation (IUGR) was categorized as yes and no (yes = diagnosed as IUGR by professional health workers). Fetal nuchal cord was grouped into yes and no (yes= diagnosed as fetal nuchal cord by professional health workers). Gestasional age was divided into full term (37-41 weeks of gestation), preterm (delivered at less than 37 weeks of gestation), postterm (42 weeks of gestation or more).\textsuperscript{11,12}

Logistic regression was used to identify several factors related to the risk of birth asphyxia.\textsuperscript{13} The data analysis used STATA version 9.

Ethical approval was obtained from the National Institute for Health Research and Development Ethics Committee, Ministry of Health, Republic of Indonesia.

RESULTS

Out of 4191 samples obtained, as many as 2777 samples were found with complete data. These sample were used for analysis.

Table 1 showed that the proportion of birth asphyxia was 6.5\% (180/2777). Mothers aged 13-20 years compared to 21-35 years were less likely to have birth asphyxia babies. Compared to the respective reference groups, unemployed/houswife, private employees, entrepreneur/traders, laborer, nulliparous, multiparous, premature rupture of membrane, fetal distress, and intra-uterine growth retardation were more likely to have birth asphyxia.

Those who had birth asphyxia and did not have birth asphyxia were similarly distributed in terms of prolonged labour, malpresentation/position, and fetal nuchal cord.
Table 2, the final model, showed that compared with higher educated mothers, those who had low educational level had 4.3-fold higher risk to have babies with birth asphyxia. In addition, middle educated mothers had 3.3-fold higher risk to have babies with birth asphyxia. In terms of gestasional age at birth, those who had preterm births had 3.1-fold higher risk for birth asphyxia compared with those with full term birth. Meanwhile, although not significant those who had postterm births had 63% more risk to have babies with birth asphyxia (P=0.118).

Table 1. Several demographic, clinical characteristic, and risk of birth asphyxia

<table>
<thead>
<tr>
<th>Birth asphyxia</th>
<th>No (n=2597)</th>
<th>Yes (n=180)</th>
<th>Crude odds ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-35</td>
<td>1987</td>
<td>76.5</td>
<td>140</td>
<td>77.8</td>
<td>1.00 Reference</td>
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<tr>
<td>13-20</td>
<td>151</td>
<td>5.8</td>
<td>6</td>
<td>3.3</td>
<td>0.56 0.25-1.29 0.178</td>
</tr>
<tr>
<td>36-46</td>
<td>459</td>
<td>17.7</td>
<td>34</td>
<td>18.9</td>
<td>1.05 0.71-1.55 0.801</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Military/police/civil servants/state</td>
<td>146</td>
<td>99.3</td>
<td>1</td>
<td>0.7</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>Unemployed/housewife</td>
<td>1800</td>
<td>92.8</td>
<td>140</td>
<td>7.2</td>
<td>11.36 1.57-81.76 0.016</td>
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<tr>
<td>Private employees</td>
<td>531</td>
<td>94.8</td>
<td>29</td>
<td>5.2</td>
<td>7.97 1.07-59.02 0.042</td>
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<tr>
<td>Entrepreneur/traders</td>
<td>84</td>
<td>96.6</td>
<td>3</td>
<td>3.4</td>
<td>5.21 0.53-50.92 0.156</td>
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<tr>
<td>Laborer</td>
<td>36</td>
<td>83.7</td>
<td>7</td>
<td>16.3</td>
<td>28.39 3.38-238.11 0.002</td>
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<tr>
<td>Parity</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>884</td>
<td>95.0</td>
<td>47</td>
<td>5.0</td>
<td>1.00 Reference</td>
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<tr>
<td>Nulliparous</td>
<td>1012</td>
<td>92.5</td>
<td>82</td>
<td>7.5</td>
<td>1.52 1.05-2.20 0.026</td>
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<tr>
<td>Multiparous</td>
<td>701</td>
<td>93.2</td>
<td>51</td>
<td>6.8</td>
<td>1.37 0.90-2.05 0.132</td>
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<tr>
<td>Premature rupture of membranes</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>2345</td>
<td>93.8</td>
<td>155</td>
<td>6.2</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>Yes</td>
<td>252</td>
<td>91.0</td>
<td>25</td>
<td>9.0</td>
<td>1.50 0.96-2.34 0.072</td>
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<tr>
<td>Fetal distress</td>
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<tr>
<td>No</td>
<td>2472</td>
<td>94.1</td>
<td>154</td>
<td>5.9</td>
<td>1.00 Reference</td>
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<td>Yes</td>
<td>125</td>
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<td>26</td>
<td>17.2</td>
<td>3.34 2.12-5.25 0.000</td>
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<tr>
<td>Intra-uterine growth retardation</td>
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<td></td>
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<td>No</td>
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<td>37</td>
<td>84.1</td>
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<td>15.9</td>
<td>2.79 1.23-6.37 0.014</td>
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<td>Prolonged labour</td>
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<td></td>
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<td>No</td>
<td>2539</td>
<td>93.5</td>
<td>176</td>
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<tr>
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<td>93.5</td>
<td>4</td>
<td>6.5</td>
<td>0.99 0.36-2.77 0.992</td>
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<td>Malpresentation/position</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2295</td>
<td>93.5</td>
<td>159</td>
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<td>302</td>
<td>93.5</td>
<td>21</td>
<td>6.5</td>
<td>1.00 0.63-1.60 0.998</td>
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<td>Fetal nuchal cord</td>
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<td></td>
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<td>No</td>
<td>2572</td>
<td>93.6</td>
<td>177</td>
<td>6.4</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>Yes</td>
<td>25</td>
<td>89.3</td>
<td>3</td>
<td>10.7</td>
<td>1.74 0.52-5.83 0.367</td>
</tr>
</tbody>
</table>

Table 2. Relationship between maternal education and gestational age, and the risk of birth asphyxia

<table>
<thead>
<tr>
<th>Birth asphyxia</th>
<th>No (n=2597)</th>
<th>Yes (n=180)</th>
<th>Adjusted odds ratio</th>
<th>95% confidence interval</th>
<th>P</th>
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<tbody>
<tr>
<td>Maternal education</td>
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<td></td>
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<tr>
<td>High</td>
<td>733</td>
<td>28.2</td>
<td>17</td>
<td>9.4</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>Middle</td>
<td>1233</td>
<td>47.5</td>
<td>99</td>
<td>55.0</td>
<td>3.31 1.95-5.62 0.000</td>
</tr>
<tr>
<td>Low</td>
<td>631</td>
<td>24.3</td>
<td>64</td>
<td>35.6</td>
<td>4.31 2.46-7.58 0.000</td>
</tr>
<tr>
<td>Gestasional age at birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full term</td>
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<td>94.8</td>
<td>23</td>
<td>5.2</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>Preterm</td>
<td>257</td>
<td>85.1</td>
<td>45</td>
<td>14.9</td>
<td>3.07 2.12-4.48 0.000</td>
</tr>
<tr>
<td>Postterm</td>
<td>97</td>
<td>89.0</td>
<td>12</td>
<td>11.0</td>
<td>1.63 0.88-3.19 0.118</td>
</tr>
</tbody>
</table>

*Adjusted to each other among the variables listed on this table, maternal age, occupations, parity, and fetal distress
DISCUSSIONS

This study have several limitations such as an Apgar score is not a specific indicator, the records of blood gas and acid status were not available for further assessments of hypoxia and acidosis. Furthermore, this study was only conducted at two hospitals in Jakarta, therefore the results could not be applied to the large population.

One of the main causes of neonatal death in preterm babies was asphyxia. This study noted that preterm birth were at 3.1-fold higher risk for birth asphyxia compared to full term births. This result was consistent with a study by Lee in Southern Nepal who revealed preterm birth had 2.28-fold higher risk for birth asphyxia as well as a study by Pitsawong in Thailand, who reported preterm birth had a 2.08-fold increased risk for birth asphyxia. This study was also in line with a study done by Utomo in the Dr. Soetomo hospital in Surabaya who demonstrated that prematurity increased the risk for birth asphyxia by 4.1-fold. Svenvik in Sweden also found preterm births was the most evident risk factor for low Apgar score. Immature lungs can lead preterm babies to respiratory distress and the episode of not breathing at birth was due to the stiffness of the lungs which will result in birth asphyxia.

This study also found lower educated mothers had higher risk to have birth asphyxia compared to high educated mothers. This finding was similar with study performed by Tabassum in Pakistan which showed Maternal literacy decreased the risk for birth asphyxia (OR=0.5). Lee also suggested that increasing maternal education and literacy significantly decreased the risk of birth asphyxia (RR=0.57).

On the other hand, Rachatapantanakorn found birth asphyxia was not related with maternal education. The different methodologies for defining birth asphyxia may influence the findings.

Education affects an individual’s attitude and behaviour such as improved knowledge of health and care seeking. The higher education the more knowledgeable with respect to the utilization of health facilities. In other words, low education levels prevent women from making decisions independently and accessing the best choices for their health. In Indonesia, Riskesdas 2013 reported that lower educated mothers choose to give birth at home. Education for girls and young women save lives by allowing a mother to make better decisions.

In conclusion, the mother who had lower and middle education levels as well as preterm baby had higher risk for having birth asphyxia baby.

Acknowledgment

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REFERENCES

Electricity and risk of public health center had measles vaccine damage in Indonesia

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Abstract

**Background:** The Public Health Center (PHC) had broken measles vaccine was influenced by a number of risk factors. Therefore, it was necessary to identify some dominant risk factors that related to PHC had measles damage vaccines.

**Methods:** The analysis used a part of the data of Research Health Facilities (Rifaskes) in 2011. The Rifaskes was conducted in all health centers in all (33) provinces in Indonesia. Furthermore, this analysis uses data only health center in the province who have measles immunization coverage the national prevalence rate (81.6%) or more, and health centers that have measles prevalence rate above the national prevalence rate (1.18%) or more. Statistical data analysis performed using logistic regression analysis to determine some of the risk factors related to the health center had has measles vaccine damaged.

**Results:** A number of 7 provinces (Riau, Jakarta, West Nusa Tenggara, East Nusa Tenggara, Central Sulawesi, South Sulawesi, Gorontalo) with 1259 PHC met the inclusion criteria. Health centers located in rural areas compared with urban areas had 3.4-fold risk of a PHC that had measles damage vaccines [adjusted odds ratio (ORa) = 3.37; 95% confidence interval (CI) = 1.34 - 8.26]. Furthermore, the health center with the availability of the electricity for less than 24 hours compared with available 24 hours had 2.1-fold risk of PHC that had measles damage vaccines (ORa = 2.10; 95% CI = 1.02 - 4.33).

**Conclusion:** Public health center in rural areas, or did not have not have commercial electric power, or did not have the availability of day-to-day electricity less than 24 hours had more risk of a PHC that had measles damage vaccines. (**Health Science Journal of Indonesia** 2015;6:116-20)

**Keywords:** measles, public health center, vaccine
Measles can be prevented by immunization and has been included in the national immunization program. Indonesian data in 2007 based on diagnosis and health professionals complaints of respondents, showed measles national prevalence was 1.18%, and immunization coverage for measles was 81.6%.[1,2] It means that Indonesia had high measles immunization coverage, on the other hand, the prevalence was still also high.

Indicators of good quality vaccine, including measles vaccine, management is characterized, among others, by maintained temperature of 2-8°C, no damaged vaccine, and has not exceeded the expired date.[3,4]

High immunization coverage does not explain measles prevalence would low, unless the quality of vaccines is guaranteed. Vaccine quality is not only determined by laboratory tests (test potential vaccines), but also highly dependent on the quality of vaccine management in particular at Public Health Center (PHC).

Indonesia is a country with thousands of PHCs which have various conditions that will influenced the quality vaccine, including measles vaccine. Some PHCs had measles vaccine damage. Therefore, it is necessary to identify the risk factors associated with PHCs which had measles vaccine damage.

This analysis aimed to identify dominant risk factors related to PHC which had damage measles vaccines in the provinces with high immunization coverage, however, having high prevalence measles cases.

**METHODS**

This analysis used a part of National Health Facility Research (Rifaskes) PHC data in 2011. The research had ethical approval from Ethic Committee of the National Institute of Health Research and Development (NIHRD) of the Ministry of Health of Indonesia. This cross-sectional Rifaskes PHC study was conducted by NIHRD, in 8737 PHCs located in all (33) provinces in Indonesia.

This design is a part of the overall Rifaskes study in 2011.[5] The overall design among others, are: Data collected comprised of a data facility, human resources (HR), medical equipment, organization and management, health services were running, and the output of essential health services, as well as the functions of the Quality Indicators Essential Health Center in 2010. The data were collected by PHC Rifaskes 2011 using a questionnaire consisting of 16 blocks. Data were collected through interviews, observation, and assessment of secondary data.

Data collectors were researchers of NIHRD, health polytechnic (polytechnic), university/college, professional organizations, or other medical research institutions that met the required criteria, both at the national and provincial / district / city.

The validity of the study carried out by three School of Public Health (FKM) in Indonesia is leading School of Public Health of: the Universitas Indonesia, University of Hasanudin, and Universitas Airlangga. The validation process carried out 1-2 weeks after the enumerators to collect data. The results were good data collection process and the validity of the data collected had average of more than 80%. So it can be concluded that the process of data on Rifaskes 2011 was valid.

Furthermore, this analysis used data among PHCs in the province with measles immunization coverage prevalence rate (81.6%) or more, and health centers that had measles prevalence rate national prevalence rate (1.18%) or more.[2]

For this analysis, a number of 1259 PHCs in 7 provinces (Riau, Jakarta, West Nusa Tenggara, East Nusa Tenggara, Central Sulawesi, South Sulawesi, and Gorontalo) full filled the inclusion criteria.

PHCs which had measles vaccine damage in Rifaskes 2011 were categorized into ‘yes’ and ‘no’. Measles vaccines damage are measles vaccines in damage condition vaccine vial monitor (VVM) indicator C and D) and expired. Public health center location was categorized into 2 categories (urban and rural). Personnel participation in immunization-related training was divided into two categories (complete and incomplete). Completeness of manuals and training materials were divided into two groups (complete and incomplete). Vaccine stock recordswere categorized into 3 categories (available and complete, available and incomplete, unavailable).

Temperature record chart was divided into 3 categories (available and complete, available and incomplete, unavailable). Vaccine storage availability was divided into 2 groups (refrigerator and others). Thermometer availability was divided into 2 groups (available and unavailable). Completeness of vaccine carrier tools consisting of cold boxes/ vaccine carrier/ flask accompanied with cool packs was divided into two categories (complete and incomplete). Electricity availability at public health centerwas divided into 2 categories (24 hours and <24 hours). Statistical data analysis was performed using logistic regression.
RESULTS

Table 1 shows that in general, there was 3.5% PHCs which had damage vaccines in 7 provinces. East Nusa Tenggara had the highest PHCs which had damage measles vaccines among other provinces which had PHCs with damage measles vaccines while Gorontalo had the lowest proportion of damage measles vaccines.

Table 1. Distribution of PHC which had damage vaccines by province

<table>
<thead>
<tr>
<th>Province</th>
<th>PHC had measles vaccine damaged</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n=1215)</td>
<td>Yes (n=44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Riau</td>
<td>142</td>
<td>11.3</td>
<td>5</td>
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<tr>
<td>Jakarta</td>
<td>0.4</td>
<td>285</td>
<td>22.6</td>
<td>2</td>
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<tr>
<td>West Nusa Tenggara</td>
<td></td>
<td>115</td>
<td>9.1</td>
<td>3</td>
<td>0.2</td>
<td></td>
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<tr>
<td>East Nusa Tenggara</td>
<td></td>
<td>173</td>
<td>13.7</td>
<td>17</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Central Sulawesi</td>
<td></td>
<td>100</td>
<td>7.9</td>
<td>12</td>
<td>1</td>
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<td>South Sulawesi</td>
<td></td>
<td>339</td>
<td>26.9</td>
<td>5</td>
<td>0.4</td>
<td></td>
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<tr>
<td>Gorontalo</td>
<td></td>
<td>61</td>
<td>4.8</td>
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<td>0</td>
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<tr>
<td>Total</td>
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<td>1215</td>
<td>96.5</td>
<td>44</td>
<td>3.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows that PHCs which had damage measles vaccines were similarly distributed in term of personnel participation in immunization related-training, completeness of manual and training materials, vaccine stock record, temperature record chart, and completeness of vaccine carrier tools. Compared to PHCs which had refrigerator and thermometer, those which had others vaccine storage and did not have thermometer were more likely to had higher risk of PHCs had damage measles vaccines.

Table 3 shows that rural area of PHCs (P = 0.008) and availability of government electricity less than 24 hours or not available (P = 0.044) were dominant factors related to PHCs which had measles vaccines damage compare with urban area and availability of government electricity for 24 hours.

Table 2. Several characteristics and risk of public health center had measles vaccine damaged

<table>
<thead>
<tr>
<th>Variable</th>
<th>PHC had measles vaccine damaged</th>
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<th></th>
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<td></td>
<td>No (n=1215)</td>
<td>Yes (n=44)</td>
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</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
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<td>Personnel participation in immunization related-training</td>
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<td>Complete</td>
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<td>0.53 - 1.86</td>
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<td>38</td>
<td>86.4</td>
<td>1.00</td>
<td>Reference</td>
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<td>0.24 - 1.92</td>
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<td>0.13 - 2.26</td>
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<td>Available, complete</td>
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<td>65.9</td>
<td>1.00</td>
<td>Reference</td>
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<td>6.8</td>
<td>0.56</td>
<td>0.17 - 1.85</td>
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<td>0.78 - 3.08</td>
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<td>Vaccine storage availability</td>
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<td>Refrigerator</td>
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<td>39</td>
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<td>Reference</td>
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<td>Others</td>
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<td>5</td>
<td>11.4</td>
<td>2.56</td>
<td>0.97 - 6.73</td>
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<td></td>
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<td>Complete</td>
<td>1128</td>
<td>92.8</td>
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<tr>
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<td>87</td>
<td>7.2</td>
<td>4</td>
<td>9.1</td>
<td>1.30</td>
<td>0.45 - 3.71</td>
</tr>
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</table>
In interpreting the results, this analysis had several limitations, in particular, the data came from a national survey conducted by the Ministry of Health. Various attempts had been made to obtain good data: training activities for collecting data such as practice on doing an interview and fill out the questionnaire.

In addition, we did not have any data regarding PHC personnel knowledge, personnel working period, personnel commitment, as well as refrigerator function that related to the vaccine damage.

In some districts in East Nusa Tenggara, the prevalence of measles was was 1.7% or higher. While the numbers of measles immunization coverage in the province amounted to 94.1%, higher than the national average of 81.6% coverage.6

Electrification ratio in East Nusa Tenggara in 2013 still amounted to 48.3%. On the other hand, province of Gorontalo had 64.04%. Differences in the distribution of electricity in parts of Indonesia may effect to electricity supply.7 Availability of electricity for 24 hours is required for the sustainability of vaccine storage in refrigerator. If measles vaccine is not stored at appropriate temperature, 2-8 °C, vaccine will be quickly broken. Measles vaccine damage is characterized by discoloration of the indicator VVM into condition C and D in which vaccine can not be reused.8

Prior study in two PHCs at Depok (West Java) noted that vaccine storage quality was depended on the electricity, and in case of a power outage, electrical continuity can not be assured because PHCs did not have a generator.9

Another study conducted in a private provider offices in Georgia (USA) showed that the major risk factors related to vaccine storage were lack of thermometer in freezer and lack of thermometer in refrigerator.10

While a study in Semarang (Central Java) showed that the influence factors related to vaccine damage was unavailability of guidelines, personnel lack of knowledge, thermometer unavailability, and inappropriate transported vaccines.11

A previous study in Malaysia showed that intervention to health care such as training, providing resources and material education, and giving additional incentives gave significant improvement in vaccine handling, such as correct vaccine placement, temperature maintenance in 2-8°C, and temperature monitoring.12

In addition, incomplete vaccination also associated with public accessibility to health facilities. Failure in appropriate vaccine storage and handling can reduce vaccine potency, inadequate immune response, and a weak defense against disease. It also can reduce public confidence in the vaccine and vaccination providers when it was happened again and cause losses cost if the vaccine can not be used.13

In conclusion, measles vaccine damages were more occurred in PHC located in rural area and the electricity availability less than 24 hours or not available. Therefore, it is suggested that government should provide electricity for 24 hours in PHC.

REFERENCES


The stages of HIV infection and the risk of opportunistic Tuberculosis infection

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Abstract

Background: Opportunistic infection (OI) by tuberculosis is the most common cause of death for people infected with HIV worldwide, mainly in persons with untreated HIV infection. The aim of the study was to determine the risk factors associated with TB as an OI of HIV-infected patients.

Methods: The study conducted in Voluntary Counseling Testing (VCT) clinics in several hospitals of seven provinces (North Sumatera, West Sumatera, Riau Islands, South Sulawesi, North Sulawesi, Maluku, and Papua). This was a cross sectional study and the respondents selected by purposive sampling. There were 490 HIV patients included in this study. Data were collected using a specific questionnaire. Statistical analyses were done using STATA 9.0 version.

Results: Among 490 HIV patients, there were 238 (48.6%) patients with TB as IO. The precentage of TB as OI in the younger HIV-infected group was higher than the older group. Among the HIV-infected persons, males had a 24 % higher risk of becoming infected with TB than females [adjusted relative risk (RRa)=1.24; P=0.023]. Stage 4 HIV-infected persons had 52% risk of TB as OI compared to stages 1-3 (RRa=1.55; P=0.000).

Conclusion: Patients with stage 4 HIV infection and male were more at risk for developing opportunistic tuberculosis infection. (Health Science Journal of Indonesia 2015;6:121-5)

Keywords: Tuberculosis, HIV, opportunistic infection, Indonesia
Indonesia has the 4th highest number of patients with tuberculosis in the world. Tuberculosis or TB is an infection caused by *Mycobacterium tuberculosis* and has been recognized a long time ago, especially in developing countries such as Indonesia. At the beginning of infection, tuberculosis may cause no symptoms. People infected with HIV are more likely than others to become sick with TB. HIV infection will significantly increase the risk of progression from latent to active TB disease. This risk can be expected to be different if infection occurs in an HIV-negative person, where more than 90% will not develop into active TB disease. Active TB will occur more rapidly in HIV cases with approximately 50% probability to develop into active TB disease. Detection of TB in people with HIV disease is more difficult.1

The World Health Organization reported in 2013, that around 9 million people developed TB and 1.5 million will die. Among the 1.5 million deaths from TB, 360,000 had HIV. The report also stated that 320,000 of the 1.3 million people with tuberculosis who died were people with HIV/AIDS.2 TB negatively impacts the natural history of HIV infection. The opportunistic infection (OI) by tuberculosis is the most common cause of death for people infected with HIV worldwide, mainly in persons with untreated HIV disease. Once people get infected with HIV, the immune system will weaken and lead to the development of TB rapidly.3,4

Several studies found that the prevalence of HIV among new TB cases in several provinces was varied. In 2006, the prevalence of HIV among new TB cases was 2% in Yogyakarta province. In 2008, the prevalence in East Java, Bali, and Papua were 0.8%, 3.8%, and 14% respectively. In Indonesia, TB infection was a challenge for AIDS control since TB is the most infectious disease that infects people with HIV/AIDS (31.8%). In 2013, WHO estimated the number TB patients with HIV positive in Indonesia was 7.5%, this number has increased from 3.3% in 2012.5

Most of the data on TB related to HIV came from HIV control programs, therefore it was important to analyze the risk factors of TB infection among HIV patients. The aim of this study was to determine the risk factors of TB as OI among HIV patients in seven provinces in Indonesia in 2007.

**METHODS**

This study is a part of HIV/AIDS cross-sectional study in seven provinces in Indonesia in 2011. The subjects consisted of HIV/AIDS cases visited by the Voluntary Counseling Testing (VCT) clinics in purposive selected from seven provinces in Indonesia (North Sumatera, West Sumatera, Riau islands, South Sulawesi, North Sulawesi, Maluku, and Papua). In each province, 1 or 2 hospitals were selected purposively.

The subjects consisted of confirmed positive HIV/AIDS using 3 rapid test by different methods or 2 rapid tests by different methods and 1 timed test by ELISA method. Severe cases were excluded.

The subjects were also selected by purposive sampling. There were 490 HIV-AIDS infected patients included in this study. Interviewers and data collection lasted 1 week and carried out by the team from Center for Biomedical and Basic Technology of Health, National Institute of Health Research and Development, Ministry of Health Republic of Indonesia.

The data were obtained by interview using a structured questionnaire. Data were analyzed to obtain the percentage of the variables studied.

This analysis used a part of HIV/AIDS cross sectional study in seven provinces in Indonesia in 2011. The subjects consisted of HIV/AIDS cases visited Volunteer Care and Treatment (VCT) clinics in purposive selected seven provinces (North Sumatera, West Sumatera, Riau islands, South Sulawesi, North Sulawesi, Maluku, and Papua). For each province was selected 1 or 2 hospitals purposively.

The subjects consisted of confirmed positive HIV/AIDS using 3 rapid test by different methods or 2 rapid tests by different and 1 time test by ELISA method in participating. We excluded severe cases.

The selection of the subjects by purposive sampling method. There were 490 HIV-infected persons included in this study. Interviewers and data collection for 1 week by researcher’s personnel of the Center for Biomedical and Basic Health Technology.

The data was obtained by interview using a structured questionnaire and analyzed to obtain a percentage of the variables studied.

All of the HIV-infected persons asked for the Opportunistic infection (TB OI) during the HIV infections. Especially for TB as OI, they had to inform how the diagnosis have been enforced such as the laboratory confirm or Rontgen. The subjects were asked about the TB medication. Interviewers
and data collection for 1 week by researcher of the Center for Biomedical and Basic Health Technology.

The logistic regression method were done using STATA 9.0 version to identify the dominant risk factors related to TB OI.

This study received ethical clearance from the Ethical Committee of the Litbangkes, and informed consent available from the subjects.

Logistic regression was done using STATA 9.0 version to identify the dominant risk factors related to TB OI.

This study received ethical clearance from the Ethical Committee of the Health Research and Development (Badan Penelitian dan Pengembangan Kesehatan), and informed consent were obtained from the subjects.

RESULTS

All of the 490 patients were analyzed. Table 1 showed that the proportion of active tuberculosis OI in HIV infected subjects was 48.6%. In terms of gender, the number of HIV-infected persons was higher in men compared to women (53%). While the proportion of tuberculosis OI was greater in the younger age group (64%). It appeared that active TB developed very rapidly in HIV-positive cases.

Table 1 showed that persons who had and did not have opportunistic TB infection were similarly distributed in terms of age and persons with CD4 counts.

HIV-infected persons residing in the Papua province had the highest risk for opportunistic TB infection which was equal to 117% compared to those living in the province of North Sumatera. The risk for opportunistic TB infections among six other provinces was lowest in West Sumatra province or 13% compared to those who live in North Sumatera.

Table 2, the final model, gender and the stage of HIV infection were the dominant risk factors for OI TB. In

Table 1. Several demographic, clinical characteristic and the risk of opportunistic TB infection

<table>
<thead>
<tr>
<th></th>
<th>Opportunistic TB infection</th>
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<th>Crude relative risk</th>
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<td></td>
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</tr>
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<td></td>
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<td>Age</td>
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<td>0-14 years</td>
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<td>35.7</td>
<td>9</td>
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<td>15-30 years</td>
<td>95</td>
<td>48.5</td>
<td>101</td>
<td>51.5</td>
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<tr>
<td>31-65 years</td>
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<td>128</td>
<td>45.7</td>
<td>0.71</td>
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<td>West-Sumatera</td>
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<td>351-1523/mm3</td>
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<td>0-350/mm3</td>
<td>178</td>
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<td>181</td>
<td>50.4</td>
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Table 2. The relationship between gender, stage of HIV and risk of opportunistic TB infection

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<td>Gender</td>
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<tr>
<td>Female</td>
<td>126</td>
<td>58.1</td>
<td>91</td>
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<tr>
<td>Male</td>
<td>126</td>
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<td>Stage 4</td>
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<td>29.2</td>
<td>46</td>
<td>70.8</td>
<td>1.52</td>
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</table>

HIV and opportunistic tuberculosis infection 123
terms of gender, male had a 24% higher risk for OI TB. Furthermore stage 4 HIV cases had a 52% higher risk for OI TB compared with stages 1-3 of the HIV infection.

DISCUSSIONS

The limitation of this study was that CD4 count of < 200 cells/μl was not analyzed. A study conducted by Masur et al, reported that HIV-infected persons with CD4 count of < 200 cells/μl were easily infected with the bacteria that caused pneumonia, such as Mycobacterium Avium-intracellulare.11

Treatment to improve quality of life and prolong life in people with HIV is currently booming and is progressively getting better. However, infectious complications still remains a threat to people with HIV. Most complications causing hospitalization of people with HIV are pulmonary complications (30-40%). Several previous studies mentioned that the main cause of lung infection complications is caused by P. carinii, followed by Tuberculosis.9 Currently, the major complication based on the results of this study showed Tuberculosis.

This study showed that the increase of active TB is proportionately parallel to the severity of HIV. This finding correspond to those reported by other studies, which showed that the OI TB developed actively among HIV-positive cases especially with those with lower CD4 counts. The risk of progressing from latent to active TB is estimated to be between 12 and 20 times greater in people living with HIV than among those without HIV infection.9 The best way to prevent the OI TB was by avoiding exposure of HIV-infected persons to TB. This was rather difficult since TB is endemic in Indonesia.9 Knowing the early presence of TB infection in people with HIV is to do a tuberculin skin test (TST) using 5-TU purified protein derivative (PPD) by the Mantoux method (AI) at the time the person is diagnosed with HIV. If the result is a positive TST (≥ 5 mm of induration), then a chest x-ray should be done immediately and treatment started for active TB. All people with HIV and a positive TST result, even without any symptoms of active TB or TB treatment history, should be as latent TB.10

Generally, the symptoms of pulmonary tuberculosis in HIV-infected persons have no symptoms if the CD4 count is > 350 cells/μL. But if CD4 count is down to 50 cells/μL, it will be accompanied by extrapulmonary TB.9 In this study, it appeared that HIV-infected persons with CD4 < 350 cells/μL was at a higher risk for TB infection compared to HIV-infected persons with CD4 > 350 cells/μL.

In stages 1 and 2 of positive HIV cases, there were no TB infection reported. This was probably due to the lack of chest x-rays done among HIV 1 and 2 stages, since no TB symptoms were present. The majority (76%) cases of active TB in HIV-positive patients had more than one types of OI. The risk of HIV-infected persons developing TB infection is higher since HIV and TB are closely related. Globally, 50% of HIV-infected persons developing TB. The development of TB among HIV-infected persons is related to immunosuppression. The risk was increased with the increasing degree of the immunosuppression.12 In this study, the same pattern was found, where stage 4 of HIV-infected persons with immunosuppression have higher risk to develop TB infection than those without immunosuppression.

In conclusion, patients with stage 4 of HIV infection and males were more at risk for developing TB IO. Therefore for early detection of TB should be a concern. Opportunistic infection can occur at the different stages of HIV infections. The incidence of TB as the OI among HIV patients in Indonesia were high and it is very important to test for TB in every HIV patients.

Acknowledgment

This study was supported by the Indonesian Government. We express our gratitude to the National Laboratory staff, the Sentinel Hospital team, and the District Health Officers for their assistance in the completion of this study.

REFERENCES


Some epitopes conservation in non structural 3 protein dengue virus serotype 4

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Abstract

Background: Non Structural 3 (NS3) protein of dengue virus (DENV) is known to induce antibody, CD4+ and CD8+ T cell responses, and playing role in viral replication. NS3 protein has T and B cell epitopes, which has conservation difference between DENV-4 strains. This study aimed to identify conservation of T and B cell epitope in NS3 protein among DENV-4 strains and four serotypes DENV of Indonesia strains.

Methods: Research was held at the Department of Microbiology, Faculty of Medicine, Universitas Indonesia, June 2013 to April 2014. NS3 amino acid sequence of DENV-4 081 strain was obtained after NS3 gene of DENV-4 081 PCR products were sequenced. T and B cell epitopes of NS3 protein of DENV-4 081 strain were analysed and compared to NS3 proteins of 124 DENV-4 strains around the world and four serotypes of Indonesia strains. World strains were isolated from America (i.e. Venezuela, Colombia, etc.) and Asia (i.e. China, Singapore, etc.). For the comparison, T and B cell epitope positions of NS3 protein were obtained from published report.

Results: Eight positions of T cell epitopes and two positions of B cell epitope of NS3 DENV-4 081 were identical and conserved to NS3 protein of 124 DENV-4 strains around the world. B cell epitope of NS3 DENV-4 081 protein at aa 537-544 was found identical and conserved to four serotypes DENV of Indonesia strains.

Conclusion: This wide conservation of T and B epitopes in almost all DENV-4 strains around the world and all serotypes of Indonesia strains. (Health Science Journal of Indonesia 2015;6:126-31)

Keywords: dengue virus, NS3 protein, T cell epitope, B cell epitope
Dengue virus (DENV) infection remains a public health problem, either in the world or in Indonesia. According to data from World Health Organization (WHO), 50 to 100 million infections were estimated to occur each year, including 500,000 cases of dengue fever and 22,000 deaths, mostly among children.\(^1\)

Based on data from Indonesian Ministry of Health, the number of patients with DHF increased annually, in 2013 there were 112,511 of DHF cases in 32 provinces (incidence rate of 45.85 per 100,000 population).\(^2\)

The virus has three structural proteins, the capsid protein (C), envelope (E) and membrane (M). These structural proteins along with seven non-structural proteins (NS) are encoded by the viral genome in the form of single-stranded RNA with positive polarity. DENV genome is a sequence of 5′UTR-C-PRM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5′-3′UTR.\(^3\)

NS3 protein plays an important role in DENV life cycle.\(^4,5\)

The functions of NS3 includes a viral protease that plays a role in cutting the DENV polyprotein after translation, and plays a role in viral RNA replication through the activities as nucleotide triphosphatase (NTPase), RNA 5′triphosphatase (RTPhase) and helicase.\(^3,6\)

Non-Structural (NS) 3 protein has the ability to induce antibody responses,\(^7\) CD4\(^+\) and CD8\(^+\) T cells, T cells activated by NS3 can cross-react between serotypes.\(^8\)

Anti-NS3 antibodies were proven in vivo to protect and delay the time of death (survival time) of mice challenged with DENV at doses of 100 LD\(_{50}\).\(^9\) NS3 sized 618 amino acid/ 69 kD, is the second largest gene/protein of DENV and its primary amino acid sequence is the most conserved.

This study aimed to obtain information about the similarity of T cell and B cell epitopes of NS3 DENV-4 081 Indonesian strain in comparison to DENV-4 world strains, as well as the similarity to the NS3 of the other serotypes Indonesia strains.

**METHODS**

The research was held in Department of Microbiology, Faculty of Medicine, Universitas Indonesia from June 2013 to April 2014. DENV-4 strain 081 was isolated in year 2013, a collection of National Institute of Health Research and Development, Ministry of Health, Indonesia. To get DNA sequence from DENV-4 strain 081, first viral RNA was extracted from culture supernatants of infected-C6/36 cell using QIAamp viral RNA extraction kit according to the manufacturer’s instruction (Qiagen, Hilden, Germany). Complementary DNA (cDNA) strands were reverse-transcribed using Super Script II First Strand Synthesis System with Random hexanucleotide primer according to the manufacturer’s instructions (Invitrogen, Massachusetts, USA). The entire NS3 gene was then amplified by polymerase chain reaction (PCR) using Platinum Taq Polymerase (Invitrogen, Massachusetts, USA). After purification of the PCR products using QIAquick PCR purification Kits (Qiagen GmbH, Hilden, Germany), the samples were sent to PT Genetika Science Indonesia for direct sequencing. The sequencing processes used Sanger method with Taq Big Dye Deoxy Terminator Cycle sequencing kits (Applied Biosystems, Foster City, CA USA). Nucleotide and amino acid sequence analysis was performed using GENETIC MAC- and BLAST program. Codon positions described in this study were based on the data of DENV-4 Singapore strain (GenBank accession no.GQ398256.1). Primer sequences for DNA synthesis and sequence were designed using PRIMER program and obtained from the published data of DENV4 Singapore strain (GenBank accession no.GQ398256.1).

Analysis of T and B cell epitope on NS3 protein were done by comparing the amino acid sequence of NS3 protein DENV4 081 to the NS3 protein of DENV-4 world strains and serotypes of Indonesia strains which obtained from the Genbank. Total world strains were 124 strains which 25 strains were from Asia (China, Singapore, India, Malaysia, Cambodia, India, Thailand, Philippines, Pakistan, and Taiwan); 99 strains were from America (Venezuela, Dominica, Colombia, US, Haiti, and Brazil) isolated between 1961 and 2011; and twelve Indonesian strains. The references for analyzing NS3 T\(^8,10,17\) and B cell epitopes\(^18-20\) were listed in Table 1. NS3 protein epitopes of DENV-4 081, 124 DENV-4 world strains and other serotypes of Indonesia strains were analysed using Bioedit version 7.0.5.3 year 2005 (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

**RESULTS**

Homology analysis of T cell epitopes of NS3 protein of DENV-4 081 to the NS3 protein of 124 DENV-4 world strains (25 strains from Asia and 99 strains from America) was shown in Table 2.

From fourteen T cell epitopes of the NS3 protein of DENV-4 081 compared to NS3 protein of 124 DENV-4 world strains, different amino acid at six epitopes were found in nine strains (Table 2), while the other eight epitopes were well conserved in all 124 world strains. T cell epitopes that have different
Table 1. B and T cell epitopes in NS3 DENV-4 protein

<table>
<thead>
<tr>
<th>No</th>
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<th>AA sequence</th>
<th>Reference</th>
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<tr>
<td>1</td>
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<tr>
<td>3</td>
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<td>VPNYNLIIMDEAHFT</td>
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</tr>
<tr>
<td>10</td>
<td>296-310</td>
<td>DENV4 814669 strain</td>
<td>ARGYISTRVEMGAA</td>
<td>4, 15</td>
</tr>
<tr>
<td>11</td>
<td>336-350</td>
<td>DENV2 2005 Singapore strain</td>
<td>EREIPERSWNSGHG</td>
<td>16</td>
</tr>
<tr>
<td>12</td>
<td>495-509</td>
<td>DENV2 16681 strain</td>
<td>LDNINTPEGIIPSMF</td>
<td>10</td>
</tr>
<tr>
<td>13</td>
<td>526-540</td>
<td>-</td>
<td>RGEQRKTFVEMRGG</td>
<td>17</td>
</tr>
<tr>
<td>14</td>
<td>596-610</td>
<td>DENV22005 Singapore strain</td>
<td>LDARIYSDPLALKEF</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td><strong>B cell epitopes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>425-432</td>
<td>DENV3</td>
<td>PRRCLKPV</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>460-469</td>
<td>DENV2 Tr1751 strain</td>
<td>RVGRNPKNE</td>
<td>19</td>
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<tr>
<td>3</td>
<td>521-532</td>
<td>DENV2 Eden 3295 strain</td>
<td>DETPMRGEITK</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>537-544</td>
<td>DENV3</td>
<td>MRRGDLPV</td>
<td>18</td>
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</tbody>
</table>

Table 2. T and B cell epitopes analysis of NS3 DENV4 protein. Bold letters show amino acid mutation

<table>
<thead>
<tr>
<th>No</th>
<th>NS3 Epitope position</th>
<th>World strains</th>
<th>Amino acid sequence</th>
<th>World strain</th>
<th>081 strain</th>
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<tbody>
<tr>
<td></td>
<td><strong>T cell epitopes</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>45-59</td>
<td>DENV4 Cina 2010 (JF741967)</td>
<td>VFHTVGHVTRGVSVC</td>
<td>VFHTMWHVTRGVSVC</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>145-159</td>
<td>DENV4 India 1961 (JF262783)</td>
<td>RVIQLYGNGVTRSG</td>
<td>RVIQLYGNGVTRSG</td>
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</tr>
<tr>
<td>3</td>
<td>231-245</td>
<td>DENV4 Venezuela 2007 (GQ199876)</td>
<td>MEEALRGLPVRYQTP</td>
<td>MEEALRGLPVRYQTP</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>495-509</td>
<td>DENV4 Pakistan 2009 (KF041260)</td>
<td>LDNIHTPEGIIPTLF</td>
<td>LDNIHTPEGIIPTLF</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>526-540</td>
<td>DENV4 Singapore 2005 (GQ398256)</td>
<td>RGEQRKTFVEMRGG</td>
<td>RGEQRKTFVEMRGG</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>596-610</td>
<td>DENV4 Colombia 2004 (GQ868583)</td>
<td>LDARVYADPMALQDF</td>
<td>LDARVYADPMALQDF</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>DENV4 Pakistan 2009 (KF041260)</td>
<td>LDARVYADPMALKDF</td>
<td>LDARVYADPMALKDF</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>B cell epitopes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>460-469</td>
<td>DENV4 Venezuela 2007 (EU854300)</td>
<td>RGRNLAQED</td>
<td>RGRNPQAQED</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DENV4 Puerto Rico 1998 (FJ024424)</td>
<td>RGRNPTQED</td>
<td>RGRNPQAQED</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DENV4 Venezuela 2007 (FJ882581)</td>
<td>RGRNLAQED</td>
<td>RGRNPQAQED</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DENV4 Venezuela 2007 (FJ882582)</td>
<td>RGRNLAQED</td>
<td>RGRNPQAQED</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>DENV4 Venezuela 2007 (FJ882588)</td>
<td>RGRNLAQED</td>
<td>RGRNPQAQED</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DENV4 Puerto Rico 1999 (FJ882599)</td>
<td>RGRNPTQED</td>
<td>RGRNPQAQED</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DENV4 Haiti 1994 (JF262782)</td>
<td>RGRNLAQED</td>
<td>RGRNPQAQED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>537-544</td>
<td>DENV4 Singapore 2005 (GQ398256)</td>
<td>MKRGLDLPV</td>
<td>MKRGLDLPV</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DENV4 China 2012 (KQ333651)</td>
<td>MKRGLDLPV</td>
<td>MKRGLDLPV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DENV4 Thailand 1997 (AY618988)</td>
<td>MKRGLDLPV</td>
<td>MKRGLDLPV</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>DENV4 Thailand 1997 (AY618989)</td>
<td>MKRGLDLPV</td>
<td>MKRGLDLPV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
amino acids were at aa 45-59, aa 145-159, aa 231-245, aa 495-509, aa 526-540 and aa 596-610 (Table 2).

From analysis of four B cell epitopes of the NS3 protein of DENV-4 081 and 124 DENV-4 world strains, different amino acid at two epitopes were found in several strains of 124 DENV-4 world strains, i.e. aa 460 to 469 and aa 537 to 544 (Table 2). The other two B cell epitopes were well conserved in all 124 DENV-4 world strains. However, B cell epitope at aa 537 to 544 was well conserved in all four serotypes of Indonesia strains.

The T and B cell epitopes in the NS3 DENV-4 081 protein were compared to NS3 protein of DENV 1, 2, 3, and 4 Indonesia strains to identified similarity between T and B cell epitopes of NS3 protein DENV-4 and four DENV serotypes. The T cell epitopes of NS3 protein DENV-4 081 at aa 221-235, aa 231-245 and aa 296-310 were found 100% identical to the other NS3 protein DENV-4 Indonesia strains and DENV-2 Indonesia strains. The B cell epitope of NS3 protein DENV-4 081 at aa 425-432 was found 100% identical to the NS3 protein of DENV 1, 3 and 4 Indonesia strains. From all B cell epitopes, the epitope at position 537-544 was found conserved and identical in all serotypes of DENV Indonesia strains.

The characteristics of different amino acids in the six T cell epitopes which showed variability in DENV-4 081 and several 124 DENV-4 strains (Table 2) were identified by their hydrophobicity. The result showed that only at aa 145-159, aa 526-540 and aa 596-610 of NS3 protein different property of amino acid were found (Table 3), i.e. T cell epitopes of NS3 DENV-4 081 at aa 526-540 have arginine which is hydrophilic, compared to the same epitope positions of several DENV-4 strains (Table 2) have lysine which is hydrophobic. On the other T cell epitope of NS3 DENV-4 081 at aa 596-610 compared to several DENV-4 world strains there is amino acid changing from lysine (hydrophobic) to glutamine (hydrophilic).

Analysis of B cell epitopes of NS3 DENV-4 081 at aa 460-469 and aa 537-544 and several DENV-4 strains (Table 2) showed different properties of amino acids as shown in Table 3.

The B cell epitope at aa 460-469 of NS3 protein DENV-4 081 have proline which is hydrophilic, compared to the same epitope position of several DENV-4 world strains (Table 2) have leucine which is hydrophobic. The B cell epitope of NS3 protein DENV-4 081 at aa 537-544 have arginine (hydrophilic) compared to several 124 DENV-4 world strains, there is amino acid changing from arginine (hydrophilic) to lysine (hydrophobic).

### DISCUSSION

From total fourteen T cell epitopes analysed, eight T cell epitopes were 100% identical and conserved to NS3 protein of 124 DENV-4 world strains (Table 2). Two of four B cell epitopes were well also conserved in 124 DENV-4 strains around the world (Table 2). This analysis also showed that from total 25 strains from Asia and 99 strains from America, 68% of Asia strains and 98% of America strains have similarity in

<table>
<thead>
<tr>
<th>No</th>
<th>NS3 epitope position</th>
<th>AA change</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cell epitopes</td>
<td>45-59</td>
<td>Met Val</td>
<td>No Change, both are hydrophobic</td>
</tr>
<tr>
<td></td>
<td>145-159</td>
<td>Lys Arg Val Ile</td>
<td>Lys hydrophobic, Arg hydrophilic</td>
</tr>
<tr>
<td></td>
<td>231-245</td>
<td>Ile Val</td>
<td>No Change, both are hydrophobic</td>
</tr>
<tr>
<td></td>
<td>495-509</td>
<td>Tyr His</td>
<td>No Change, both are hydrophobic</td>
</tr>
<tr>
<td></td>
<td>526-540</td>
<td>Arg Lys</td>
<td>Lys hydrophobic, Arg hydrophilic</td>
</tr>
<tr>
<td></td>
<td>596-610</td>
<td>Met Val Lys Gln</td>
<td>Lys hydrophobic, Gln hydrophilic</td>
</tr>
<tr>
<td>B cell epitopes</td>
<td>460-469</td>
<td>Pro Leu Ala Thr</td>
<td>Pro hydrophilic, Leu hydrophobic</td>
</tr>
<tr>
<td></td>
<td>537-544</td>
<td>Arg Lys</td>
<td>Arg hydrophilic, Lys hydrophobic</td>
</tr>
</tbody>
</table>
all fourteen T cell epitopes of NS3 protein DENV-4. In total, 84% of Asia strains and 93% of America strains showed similarity in all four B cell epitopes of NS3 protein DENV-4. From total 25 DENV-4 strains from Asia, there were eight strains showed genetic variation in the amino acid sequence of NS3 protein. The 68% similarity in all fourteen epitopes analysed in DENV-4 strains from Asia needs further study, because only 25 DENV-4 strains were obtained of total 124 world strains. The variations in the amino acid sequence of T cell epitopes of NS3 protein DENV-4 were about one or two amino acid difference (Table 2 and Table 3).

It is known that the amino acid changes would not alter the preservation area epitope recognition by antibodies and lymphocytes as long as it still have same tertiary structure. Tertiary structure of a protein is determined by various factors, one of it is hydrophobic interaction of the protein-building amino acids. Amino acid changes can cause conformational change of the protein, which may change epitope recognition by antibodies. Mutated amino acids of T cell and B cell epitopes affect hydrophobicity of epitopes so further analysis is required to get three-dimensional structure of NS3 protein.

Table 4. T dan B cell epitopes position of NS3 DENV4 081 protein which is identical to NS3 DENV 1, 2, 3 dan 4 protein Indonesia strain

<table>
<thead>
<tr>
<th>No</th>
<th>T cell epitope position</th>
<th>B cell epitope position</th>
<th>AA epitope sequence of DENV4 081 strain</th>
<th>DENV1</th>
<th>DENV2</th>
<th>DENV3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>221-235</td>
<td>LAPTRVVAEMEAL</td>
<td>Identical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>231-245</td>
<td>MEEALRGPLPYQTP</td>
<td>Identical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>296-310</td>
<td>ARGYISTRVEMGEEA</td>
<td>Identical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>425-432</td>
<td>PRRCLKPV</td>
<td>Identical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>537-544</td>
<td>MRRGDLPV</td>
<td>Identical</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

epitopes were found in almost all DENV-4 strains around the world and all serotypes of Indonesia strains.

Acknowledgments

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REFERENCES


Type of female sex worker and other risk factors of syphilis

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Abstract

Background: Syphilis is one of the chronic sexual transmission diseases which is caused by Treponema pallidum bacteria that can cause disability in patients and babies born. This analysis aims at looking at the relationship type and work duration as Female Sex Workers (FSW) and the syphilis cases within 7 cities in Indonesia.

Methods: The data was taken from Survey on FSW using a structured questionnaire in 7 cities (Kupang, Samarinda, Pontianak, Yogyakarta, Timika, Makassar and Tangerang) in Indonesia in 2007, the cross-sectional design and respondents are selected by cluster random sampling directly and indirectly towards the WPS who fulfill the operational definition criteria. Syphilis diagnosis was confirmed by laboratory tests Rapid Plasma Reagents (RPR) and Treponema pallidum Haemaglutination Assay (TPHA).

Results: There were 1750 respondents who participated in the study and about 12.2% were infected with the syphilis. Makassar has the highest prevalence about 55.2%. The WPS who are located outside of Java have the syphilis infection risk about 3.16 times higher than the WPS located in Java [Adjusted Relative Risk (RRa) = 3.16; P = 0.000]. The indirect WPS had 46% more risk for syphilis infection compared to direct WPS (RRa = 1.46; P = 0.002), whereas the FSW who seek treatment from doctor have a risk about 58% more risk compared to the direct health facilities treatment (RRa = 1.58; P = 0.006).

Conclusion: The location of FSW which is outside of Java, the FSW does not directly have a higher risk of being infected with syphilis. Female sex workers who seek the doctor treatment are able to be indicated earlier rather than they are who seek treatment to other health care facilities. (Health Science Journal of Indonesia 2015;6:132-6)

Keyword: Syphilis, Female Sex Worker, Indonesia
The high prevalence of Sexual Transmitted Infections (STI) and Reproductive Tract infections (RTI) for population in a region, it is known to be the first sign of the spreading risk of HIV, though HIV prevalence in a region is very low. Diseases which belong to IMS or ISR are like, syphilis, gonorrhea, trichomoniasis, clamidiasis, bacterial vaginosis, candidosis, HSV2. Some clinical manifestations of STI are genital sores on syphilis, cancroids, and herpes simplex virus type – 2. STI which can lead HIV transmission. This is proved that the STI control programs have effectively reduced the HIV in population.1,2

The total prevalence of syphilis on high-risk groups based on a survey in 2005 within 10 cities in Indonesia is about 9%, while the prevalence in each city varies.3 According to the research on rural population in Africa in 1991-1994, it showed the prevalence of syphilis in men about 7.5% while women are about 9.1%.4

The huge problem caused by the disease requires special attention to overcome. Early detection by serology accompanied by the management of the patient is expected to stop the chain of transmission and reduce the prevalence of syphilis in Indonesia. Epidemiologically, some demographic factors affect the syphilis case. The education level leads the FSW to have basic knowledge to quickly understand about healthy life, clean, and safe behavior. Therefore, a risk of being infected with sexually transmitted diseases will certainly be reduced.

The study aims at identifying the risks of the dominant factors for syphilis in Female Sex Workers, within seven cities in Indonesia in 2007.

METHODS

Data is taken from a complete study on FSW survey within 7 cities in Indonesia in 2007, cross-sectional design and the respondents were selected by random cluster sampling directly and indirectly towards the FSW who fulfill operational definitions criteria. Data were collected from seven cities; that is Kupang, Samarinda, Pontianak, Yogyakarta, Timika, Makassar and Tangerang. Total sample collected in 1750 respondents consist of 1286 respondents (73.5%) direct FSW and 464 respondents (26.5%) indirect FSW. The direct FSW means their profession is only as female sex workers. Whereas an indirect FSW is a person who have other profession rather than female sex workers, for example barkeeper, escort girl at karaoke, or a masseur in a massage parlor. The 2007 survey was actually the continuation from the previous one conducted in 2003 and 2005 in 13 different cities; hopefully it can complete the previous data.

The inclusion criteria of respondents who participated in this study, mainly direct and indirect female sex workers, aged about 15-50 years, not being menstruation and pregnant.

Before conducting the study, the preparation of the samples was done by using secondary data from the Local Health Office and non-governmental organizations that had been working as an outreach localization. If the mapping results obtained more respondents than expected, then we invited 250 female sex workers to be the respondent who came first and fulfill the qualifications.

The implementation of survey was preceded by interviews with the respondents, followed by a physical examination. Blood samples were screened for syphilis using Rapid Plasma Reagin (RPR) and Treponemal Pallidum Haemaglutination Antibody (TPHA), as a non-treponemal serological test and treponemal serologic test respectively. Respondents was diagnosed as syphilis when the results of RPR titer ≥ 1: 2 and positive TPHA. Once the laboratory test results obtained, the respondents received treatment as well as counselling sessions.

Information on risk factors was obtained through interviews with a structured questionnaire. Exploring the risk factors includes the factors related to the type and work duration as FSW, clinical history, and behavioral treatment. Data were analyzed using STATA version 9 with Cox regression.

This study has obtained permission from the Ethics Commission, NIHRD and the Ministry of Home Affairs.
RESULTS

Table 1 shows that the Syphilis prevalence in 7 cities is 12.3% (214), and the highest one occurs in indirect FSW which is about 19.2% (89). The greatest proportion of syphilis was found in Makassar which is about 55.2% (138) and mostly occurs in indirect FSW about 53% (87). Other cities have lower prevalence of syphilis around below 10% and the lowest was found in Samarinda which about 0.8% (2). While in Tangerang city, all respondents are direct FSW.

Furthermore, table 1 shows the risk of FSW who have multiple anonymous sexual partners 7% higher compared with FSW who do not have multiple anonymous sexual partners. The FSW who has been working less than 3 months have higher risk of syphilis compared to the WPS who has been working more than 3 months.

In Table 2, it shows a model of analysis results, about how to seek the treatment, type of FSW, and the location of FSW become the dominant factor on the risk of syphilis.

About the way the FSW get the treatment, for those who come to the health facilities have about 1.49 times higher risk of syphilis rather than those who seek treatment to a special treatment for syphilis [adjusted relative risk (RRa) = 1.49; Confident Interval (CI) = 1.07 to 2.06 P value = 0.017].

Furthermore, indirect FSW have a risk of 85% higher to suffer syphilis rather than direct FSW (RRa = 1.85; CI = 1.45 to 2.36; P value = 0.000).

Table 1. Correlation of sexual partners, periods of being FSW with the risk of syphilis

<table>
<thead>
<tr>
<th>Cities</th>
<th>Negative (N=1536)</th>
<th>Positive (N=214)</th>
<th>Crude relative risk</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
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</tr>
<tr>
<td>Samarinda</td>
<td>248</td>
<td>99.2</td>
<td>2</td>
<td>0.8</td>
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<tr>
<td>Timika</td>
<td>245</td>
<td>98.0</td>
<td>5</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Pontianak</td>
<td>243</td>
<td>97.2</td>
<td>7</td>
<td>2.8</td>
<td>3.5</td>
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<td>Yogyakarta</td>
<td>240</td>
<td>96.0</td>
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<tr>
<td>Tangerang</td>
<td>224</td>
<td>89.6</td>
<td>26</td>
<td>10.4</td>
<td>13</td>
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<tr>
<td>Kupang</td>
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<td>89.6</td>
<td>26</td>
<td>10.4</td>
<td>13</td>
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<td>138</td>
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<tr>
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<td>103</td>
<td>11.2</td>
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<tr>
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<td>87.3</td>
<td>111</td>
<td>12.7</td>
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<td>Periods of being FSW</td>
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<td></td>
<td></td>
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<tr>
<td>0-3 months</td>
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<td>90.9</td>
<td>21</td>
<td>9.1</td>
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<td>3-12 months</td>
<td>411</td>
<td>89.2</td>
<td>50</td>
<td>10.8</td>
<td>1.19</td>
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<tr>
<td>&gt;12 months</td>
<td>916</td>
<td>86.5</td>
<td>143</td>
<td>13.5</td>
<td>1.48</td>
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</table>

Table 2. Relationship among dominant factor, and risk of syphilis

<table>
<thead>
<tr>
<th>Seeking treatment</th>
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<th>95% confidence interval</th>
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<td></td>
<td>RRa</td>
<td>%</td>
<td>RRa</td>
<td>%</td>
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<tr>
<td>Public Health Facilities</td>
<td>1217</td>
<td>88.5</td>
<td>158</td>
<td>11.5</td>
<td>1.00</td>
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<tr>
<td>Clinic</td>
<td>132</td>
<td>81.0</td>
<td>31</td>
<td>19.0</td>
<td>1.49</td>
</tr>
<tr>
<td>Get own medicine</td>
<td>92</td>
<td>92.9</td>
<td>7</td>
<td>7.1</td>
<td>0.45</td>
</tr>
<tr>
<td>Without treatment</td>
<td>95</td>
<td>84.1</td>
<td>18</td>
<td>15.9</td>
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<td>Type FSW</td>
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<td>207</td>
<td>16.6</td>
<td>11.61</td>
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</table>

*Adjusted each other among variables listed on this table
Meanwhile, in terms of the location, compared with FSW located in Java, the FSW located outside Java has a 11.61-fold risk for syphilis infection (ARR = 11.61; CI = 5.50 to 24.49, P = 0.000).

DISCUSSION

In this research, the region of study only covers 7 selected cities so that the analysis is not sufficient to represent Indonesia. Another limit is about the serological examination of syphilis which is not able to distinguish the stages of syphilis, though it has been recognized to have a high sensitivity and specificity.\(^2,5\)

Syphilis prevalence found in this study is about 12.3%. That was a bit higher than the prevalence of syphilis in direct WPS based on the Integrated Biological and Behavioral Survey (IBBS) in 2011 and 2009 which were carried out in several different cities in Indonesia. According to IBBS prevalence, direct FSW is greater than indirect FSW. The syphilis prevalence in this study is different with prevalence of syphilis on IBBS due to the location and time of research.\(^6,7\)

According to the various results of analysis, it shows that the respondents who try to find treatment to health care facilities have higher risk of syphilis about 11.5 times than other respondents (ARR = 11.5; Confident Interval (CI) = 1.14 to 2.20 P value = 0.006). Research in Ghana and Uganda shows the severity and symptoms of disease will affect people with syphilis to seek medical facilities.\(^8\) The primary and secondary syphilis shows the symptoms that may cause the respondent to visit health facilities including genital ulcers and eruption of the skin.\(^2,5\)

Most women suspend their willing to seek treatment since they thought that they suffered normal limits of symptoms.\(^9\) The painless lesions in the genital will arouse them seeking the treatment. The research conducted by Malek et al. in Pittsburg in 1999-2002 showed the most IMS patients suspended the examination after 7 days of suffering the symptoms.\(^9\)

In this study, it is found that type of FSW also affects the syphilis cases significantly. Indirect female sex workers (FSW) have the risk about 19.2 times of suffering from syphilis compared to direct FSW (RRa = 19.2; CI = 1.14 to 1.86; P = 0.002). Several studies in other countries show some different patterns. Research conducted by Li et al. Liuzhou, China showed a greater risk of syphilis found in FSW who work to offer their service on the street rather than the FSW who only receive calls for services over the phone.\(^11\)

However, during 2009-2012 it showed different results from the study conducted in Shenzhen. In the study, it did not reveal any significant relationships between the types of FSW with the syphilis cases. This is due to the research that claims that the direct FSW who works on the street are those who work in a salon and a small club, while indirect FSW are largely a group of FSW who work in hotels, karaoke bars and nightclubs, so it is not be able to be compared directly in this study.\(^1\)

The indirect FSW have the greatest risk of syphilis rather than the direct one since they are not reached by the STI control program in Indonesia. The prostitution covered by other profession, like masseur and escort bar and karaoke, cause difficulties to be recognized by health professionals and outreach.\(^10\)

Based on this study, it is found that the respondents from outside Java have the high risk of suffering from syphilis about 3.16 times rather than the respondents who live in Java. This pattern is similar to the studies found in China, which indicates that the respondents in a region also give influence towards the syphilis case related to the FSW mobility and the availability of health facilities.\(^11\)

The FSW group is one of the high-risk populations which becomes a source of infection transmitted diseases including syphilis. The data obtained from population studies can be used to overcome the STI.\(^12\)

In conclusion, the location of work for FSW which are outside Java shows the high risk of being infected with syphilis rather than those who work in Java. Then, it also about comparison for direct and indirect FSW, those
who seek treatment immediately to the doctor may be indicated easily rather than those who seek treatment in health care facilities.

Acknowledgment

The authors wish to thank all subjects who participated in this study. Syphilis research team laboratory, data management and data collection, and Center for Biomedical and Basic Tecnology of Health for funding this research.

REFERENCES

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