Kontrak Pelaksanaan Program Penelitian Lanjutan
Skema Penelitian Dasar Kemitraan
TAHUN ANGGARAN 2023
Nomor : 612/UNWAR/LEMLIT/PD-13/2023

Pada hari ini **Sabtu** tanggal **Enam** bulan **Mei** tahun **Dua Ribu Dua Puluh Tiga**, kami yang bertanda tangan dibawah ini :

1. **Prof. Dr. I Made Suwitra, SH., MH.**
   NIP: 196012311985031024
   : Kepala Lembaga Penelitian Universitas Warmadewa selanjutnya di sebut **PIHAK PERTAMA**

2. **Dr. dr. Dewa Ayu Putri Sri Masyeni, Sp.PD-KPTI.**
   NIDN: 0827116503
   : Ketua Peneliti selanjutnya disebut **PIHAK KEDUA**

PIHAK PERTAMA dan **PIHAK KEDUA** secara bersama-sama selanjutnya disebut **PARA PIHAK**

PARA PIHAK sepakat menandatangani Kontrak Pelaksanaan Program Penelitian Lanjutan Tahun 2023, dengan ketentuan dan syarat sebagai berikut:

PASAL 1
DASAR HUKUM

1. Undang-Undang Republik Indonesia Nomor 20 Tahun 2003, tentang Sistem Pendidikan Nasional;
2. Undang-Undang Republik Indonesia Nomor 12 Tahun 2012, tentang Pendidikan Tinggi;
3. Peraturan Menteri Riset, Teknologi dan Pendidikan Tinggi Republik Indonesia Nomor 38 Tahun 2019 tentang Prioritas Riset Nasional Tahun 2020-2024;
4. Peraturan Menteri Pendidikan dan Kebudayaan Republik Indonesia Nomor 3 Tahun 2020, tentang Standar Nasional Pendidikan Tinggi;
5. Surat Direktur Riset, Teknologi, dan Pengabdian kepada Masyarakat Kementerian Pendidikan, Kebudayaan, Riset dan Teknologi Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Nomor: 0162/E5.4/DT.05.00/2023 tanggal 6 Maret 2023 perihal Pengumuman Program Penelitian Lanjutan (*on going*) Tahun Anggaran 2023.
6. Kontrak Penelitian antara LLDIKTI Wilayah VIII dengan Universitas Warmadewa tentang Pelaksanaan Program Penelitian Lanjutan Penelitian Dasar Kompetitif Nasional Tahun Anggaran 2023 Nomor: 078/E5/PG.02.00.PL/2023 dan Nomor LLDIKTI: 1751/LL8/AL.04/2023.

PASAL 2
RUANG LINGKUP

(1) Ruang lingkup Kontrak Penelitian ini meliputi pelaksanaan penelitian tahun anggaran 2023.

(2) Penelitian sebagaimana dimaksud pada ayat (1) beserta nama pelaksana penelitian, skema, luaran tambahan, jangka waktu penelitian, dan besarnya biaya penelitian sebagaimana tercantum dalam Kontrak Penelitian ini.

PASAL 3
SUMBER DANA

(1) **PIHAK PERTAMA** memberikan pendanaan Pelaksanaan Program Penelitian lanjutan dengan judul **Identification of Molecular and Cellular Pathways Predicting Susceptibility or Resistance to Severe Dengue Fever** yang bersumber pada Daftar Isian Pelaksanaan Anggaran Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Direktorat jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, kebudayaan, Riset, dan Teknologi Tahun Anggaran 2023, Nomor SP DIPA-023.17.1.690523/2023 revisi ke-4 tanggal 31 Maret 2023.

(2) **PIHAK KEDUA** bertanggungjawab penuh atas pelaksanaan, administrasi dan keuangan atas pekerjaan sebagai dimaksud pada ayat (1) dan berkewajiban menyimpan semua bukti-bukti pengeluaran serta dokumen pelaksanaan lainya.

PASAL 4
NILAI KONTRAK

(1) **PIHAK KESATU** memberikan pendanaan dengan nilai Kontrak sebesar: Rp. 301.330.000 kepada **PIHAK KEDUA**.
(2) Nilai kontrak sebagaimana dimaksud pada ayat (1) digunakan untuk pembiayaan pelaksanaan program penelitian lanjutan, pajak, dan biaya lain yang sah.
(3) Pembayaran nilai kontrak sebagaimana dimaksud ayat (1) dilakukan melalui Kantor Pelayanan Perbendaharaan Negara dengan detail rekening sebagai berikut:

Nama Institusi	:	Universitas Warmadewa
Nomor Rekening	:	0284359204
Nama Penerima pada Rekening	:	Ibu DEWA AYU PUTRI SRI MASYENI
Nama Bank	:	BNI
Alamat Bank	:	RENON
Kota	:	DENPASAR
NPWP	:	68.542.219.8-906.000

(4) **PIHAK KESATU** tidak bertannggungjawab atas keterlambatan dan/atau tidak terbayarnya sejumlah dana, yang disebabkan oleh kesalahan **PIHAK KEDUA** dalam menyampaikan informasi detail rekening institusi sebagaimana dimaksud pada ayat (3).

PASAL 5
NILAI DAN TAHAPAN PEMBAYARAN

(1) Nilai kontrak sebagaimana dimaksud dalam Pasal 3 ayat (1) dibayarkan oleh **PIHAK PERTAMA** kepada **PIHAK KEDUA** secara bertahap melalui mekanisme transfer, dengan ketentuan sebagai berikut:
   a. Nilai kontrak keseluruhan sebesar Rp. 301.330.000 (*Tiga Ratus Satu Juta Tiga Ratus Tiga Puluh Ribu Rupiah*)
   b. Pembayaran tahap pertama sebesar 70% (Tujuh puluh persen) dari jumlah keseluruhan bantuan dana penelitian yaitu Rp. 301.330.000 x 70% = Rp. 210.931.000 (*Dua Ratus Sepuluh Juta Sembilan Ratus Tiga Puluh Satu Ribu Rupiah*) setelah PIHAK KEDUA menandatangani Kontrak Pelaksanaan Program Penelitian Lanjutan;
   c. Pembayaran tahap kedua sebesar 30% (Tiga puluh persen) dari jumlah keseluruhan bantuan dana penelitian yaitu Rp. Rp. 301.330.000 x 30% = Rp. 90.399.000 (*Sembilan Puluh Juta Tiga Ratus Sembilan Puluh Sembilan Ribu Rupiah*).

(2) Pembayaran tahap pertama sebagaimana dimaksud pada ayat (1) huruf b, akan dibayarkan setelah revisi proposal penelitian dan surat pernyataan kesanggupan pelaksanaan penelitian diunggah oleh **PIHAK KEDUA** ke laman yang ditentukan;

(3) Apabila pembayaran tahap pertama sebagaimana dimaksud pada ayat (1) huruf b cair setelah tanggal 16 Agustus 2023, pelaksana penelitian mengunggah Surat Pernyataan Tanggung Jawab Belanja (SPTB) ke laman yang ditentukan paling lambat 2 (dua) minggu setelah dana cair.

(4) Pembayaran tahap kedua sebagaimana dimaksud pada ayat (1) huruf c dibayarkan setelah pelaksana penelitian mengunggah Laporan Kemajuan/Antara Penelitian dan Surat Pernyataan Tanggung Jawab Belanja (SPTB) ke laman yang ditentukan paling lambat tanggal 23 Agustus 2023.

(5) **PIHAK KEDUA** menyetorkan bukti unggah surat pernyataan telah menyelesaikan seleruh pekerjaan yang diunggah pada laman yang ditentukan dan menyampaikan kepada **PIHAK PERTAMA** paling lambat tanggal 10 Desember 2023, dengan melampirkan dokumen sebagai berikut:
   a. Surat Pernyataan Tanggung Jawab Belanja (SPTB); dan
   b. Laporan akhir tahun untuk pendanaan multitahun yang dilaksanakan pada tahun berjalan; atau
   c. Laporan akhir pelaksanaan penelitian untuk pendanaan monotahun dan multitahun pada tahun terakhir.

(6) Apabila pembayaran tahap kedua sebagaimana dimaksud pada ayat (1) huruf c cair setelah tanggal 1 Desember 2023, pelaksana penelitian mengunggah Surat Pernyataan Tanggung Jawab Belanja (SPTB) ke laman yang ditentukan paling lambat 2 (dua) minggu setelah dana cair.

PASAL 6
HAK DAN KEWAJIBAN

(1) **PIHAK PERTAMA** mempunyai hak menerima dokumen hasil unggahan dari penerima dana penelitian di laman yang ditentukan sebagai berikut:
   a. Menerima catatan harian penelitian;
   b. Menerima laporan kemajuan penelitian;
   c. Menerima laporan akhir tahun atau laporan akhit pelaksanaan penelitian;
   d. Melakukan pemantauan dan evaluasi;
   e. Menerima surat Pernyataan Tanggungjawab Belanja (SPTB) atas dana penelitian yang telah ditetapkan;
   f. Menerima hasil laporan pemantauan dan evaluasi dari **PIHAK KEDUA**.

(2) **PIHAK KEDUA** mempunyai hak mendapatkan dana penelitian dari **PIHAK PERTAMA**.

(3) **PIHAK PERTAMA** mempunyai kewajiban:
   a. Memberikan pendanaan penelitian kepada **PIHAK KEDUA**;
   b. Melakukan pemantauan dan evaluasi;
   c. Melakukan penilaian luaran penelitian; dan
   d. Melakukan validasi luaran tambahan.
   e. Membuat Kontrak Pelaksanaan Penelitian dengan ketua pelaksana penelitian yang paling sedikit memuat:
      1. Nama pelaksana;
      2. Judul Penelitian;
      3. Ruang lingkup penelitian;
      4. Sumber dana penelitian;
      5. Nilai kontrak penelitian;
      6. Tata cara dan tahapan pembayaran;
      7. Jangka waktu pelaksanaan dan penyelesaian;
      8. Hak dan kewajiban para pihak;
      9. Batas akhir pelaporan;
      10. Pencantuman pemberi dana penelitian dalam publikasi ilmiah;
      11. Luaran penelitian;
      12. Serah terima hasil penelitian;
      13. Kesanggupan pelaksanaan penelitian; dan
      14. Sanksi.

(4) **PIHAK KEDUA** mempunyai kewajiban:
   a. Mengkoordinir dan bertanggungjawab penuh atas terlaksananya Kontrak Penelitian ini, administrasi dan keuangan;
   b. Melakukan koordinasi dengan penerima dana penelitian apabila dalam pelaksanaan penelitian terdapat sisa dana serta melaporkan kepada PIHAK PERTAMA
   c. Menyimpan semua bukti-bukti pengeluaran serta dokumen pelaksanaan lainnya;

PASAL 7
PENGGANTIAN KEANGGOTAAN

(1) Apabila terjadi perubahan susunan tim pelaksana penelitian karena tidak dapat menyelesaikan penelitian atau mengundurkan diri, maka **PIHAK KEDUA** wajib menunjuk pengganti serta mengirimkan surat permohonan perubahan kepada Direktorat Riset dan Pengabdian Kepada Masyarakat Kemendikbudristek dengan diketahui **PIHAK PERTAMA**.
(2) Perubahan dapat dilakukan setelah mendapat persetujuan Direktorat Riset dan Pengabdian Kepada Masyarakat Kemendikbudristek.
(3) Dalam hal tidak terdapat pengganti ketua tim pelaksana penelitian sesuai dengan syarat dan ketentuan dalam panduan penelitian, maka penelitian dibatalkan dan dana dikembalikan ke Kas Negara.

PASAL 8
PAJAK

Ketentuan pengenaan pajak pertambahan nilai dan/atau pajak penghasilan dalam rangka pelaksanaan kegiatan penelitian ini wajib dilaksanakan oleh **PIHAK KEDUA** sesuai dengan ketentuan peraturan perundang-undangan di bidang perpajakan.

PASAL 9
KEKAYAAN INTELEKTUAL

(1) Hak Kekayaan Intelektual yang dihasilkan dari pelaksanaan penelitian diatur dan dikelola sesuai dengan ketentuan peraturan dan perundang-undangan.
(2) Setiap publikasi, makalah, dan/atau ekspos dalam bentuk apapun yang berkaitan dengan hasil penelitian wajib mencantumkan PIHAK DRTPM sebagai pemberi dana.
(3) Pencantuman nama DRTPM sebagaimana dimaksud pada ayat (2), paling sedikit mencantumkan nama Direktorat Riset, Teknologi dan Pengabdian kepada Masyarakat Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi.

PASAL 10
INTEGRITAS AKADEMIK

(1) Pelaksana penelitian wajib menjunjung tinggi integritas akademik yaitu komitmen dalam bentuk perbuatan yang berdasarkan pada nilai kejujuran, kredibilitas, kewajaran, kehormatan, dan tanggung jawab dalam kegiatan penelitian yang dilaksanakan.
(2) Penelitian dilakukan sesuai dengan kerangka etika, hukum, dan profesionalitas serta kewajiban sesuai dengan ketentuan peraturan perundang-undangan.
(3) Penelitian dilakukan dengan menjunjung tinggi standar ketelitian dan integritas tertinggi dalam semua aspek penelitian.

PASAL 11
KEADAAN KAHAR

(1) Apabila terjadi keadaan kahar (*force majeure*) suatu keadaan yang terjadi di luar kehendak **PARA PIHAK** dalam kontrak, dan tidak dapat diperkirakan sebelumnya, sehingga kewajiban yang ditentukan dalam kontrak menjadi tidak dapat dipenuhi, maka **PARA PIHAK** sepakat tidak akan saling menuntut pelaksanaan pemenuhan ketentuan dalam Kontrak Penelitian ini.
(2) Peristiwa atau kejadian yang dapat digolongkan keadaan kahar (*force majeure*) sebagaimana dimaksud pada ayat (1) meliputi bencana alam, wabah penyakit, kebakaran, perang, blokade, peledakan, sabotase, revolusi, pemberontakan, huru-hara, serta adanya tindakan pemerintah dalam bidang ekonomi dan moneter yang secara nyata berpengaruh terhadap pelaksanaan Kontrak Penelitian ini.
(3) Apabila terjadi keadaan kahar (*force majeure*) sebagaimana dimaksud pada ayat (2), maka pihak yang mengalami wajib memberitahukan kepada pihak lainnya secara tertulis, selambat-lambatnya dalam waktu 7 (tujuh) hari kerja sejak terjadinya keadaan kahar (*force majeure*), disertai dengan bukti-bukti yang sah dari pihak yang berwajib, dan **PARA PIHAK** dengan itikad baik akan segera membicarakan penyelesaiannya.

PASAL 12
JANGKA WAKTU PENYELESAIAN

(1) Kontrak ini berlaku sejak tanggal 6 Mei sampai dengan tanggal 10 Desember 2023.
(2) Kontrak ini dapat diubah berdasarkan kesepakatan tertulis **PARA PIHAK** yang dituangkan dalam suatu adendum dan merupakan bagian yang tidak terpisahkan dari Kontrak ini.

PASAL 13
PEYELESAIAN PERSELISIHAN

(1) Dalam hal terjadi perselisihan atau perbedaan penafsiran terkait Kontrak Penelitian ini, **PARA PIHAK** sepakat untuk menyelesaikannya secara musyawarah dan mufakat.
(2) Dalam hal musyawarah dan mufakat sebagaimana dimaksud pada ayat (1) tidak tercapai, **PARA PIHAK** sepakat untuk menyelesaikannya melalui Pengadilan setempat

PASAL 14
SANKSI

(1) Apabila sampai dengan batas waktu yang telah ditetapkan untuk melaksanakan Pelaksanaan Program Penelitian lanjutan telah berakhir, **PIHAK KEDUA** tidak melaksanakan kewajiban sebagaimana dimaksud dalam Pasal 6 ayat (4), maka **PIHAK KEDUA** dikenai sanksi administratif.
(2) Apabila dikemudian hari terbukti bahwa judul penelitian yang diajukan pada program penelitian sebagaimana dimaksud dalam Pasal 3 ayat (1) ditemukan adanya duplikasi

dan/atau ditemukan adanya ketidak jujuran/itikad buruk yang tidak sesuai dengan kaidah ilmiah, maka kegiatan penelitian tersebut dinyatakan batal dan **PIHAK KEDUA** dikenai sanksi administratif.

(3) Sanksi administratif sebagaimana dimaksud pada ayat (1) dan (2) dapat berupa penghentian pembayaran dan/atau Ketua Tim Pelaksana Penelitian tidak dapat mengajukan proposal penelitian dalam kurun waktu 2 (dua) tahun berturut-turut.

PASAL 15
PENUTUP

Kontrak Penelitian ini dibuat dan ditandatangani oleh **PARA PIHAK** dalam rangkap 2 (dua) asli bermeterai cukup yang biayanya dibebankan kepada **PIHAK KEDUA**, untuk tiap-tiap PIHAK dan memiliki kekuatan hukum yang sama.

PIHAK PERTAMA,	PIHAK KEDUA,
	
Prof. Dr. I Made Suwitra, SH., MH.	Dr. dr. Dewa Ayu Putri Sri Masyeni, Sp.PD-KPTI.
NIP. 196012311985031024	NIDN: 0827116503

Direktorat Riset dan Pengabdian Masyarakat Direktorat Jenderal Riset dan Pengembangan
Kementerian Riset, Teknologi, dan Pendidikan Tinggi
Gedung BPPT II Lantai 19, Jl. MH. Thamrin No. 8 Jakarta Pusat
https://simlitabmas.ristekdikti.go.id/

LAPORAN AKHIR PENELITIAN MULTI TAHUN

ID Proposal: 8a4c8d66-3af1-49e4-b947-c773eb99ab5e
laporan akhir Penelitian: tahun ke-2 dari 2 tahun

1. IDENTITAS PENELITIAN

A. JUDUL PENELITIAN

Identification of molecular and cellular pathways predicting susceptibility or resistance to severe dengue fever

B. BIDANG, TEMA, TOPIK, DAN RUMPUN BIDANG ILMU

Bidang Fokus RIRN / Bidang Unggulan Perguruan Tinggi	Tema	Topik (jika ada)	Rumpun Bidang Ilmu
Kesehatan	-		

C. KATEGORI, SKEMA, SBK, TARGET TKT DAN LAMA PENELITIAN

Kategori (Kompetitif Nasional/ Desentralisasi/ Penugasan)	Skema Penelitian	Strata (Dasar/ Terapan/ Pengembangan)	SBK (Dasar, Terapan, Pengembangan)	Target Akhir TKT	Lama Penelitian (Tahun)
Penelitian Penugasan			SBK Riset Dasar	3	2

2. IDENTITAS PENGUSUL

Nama (Peran)	Perguruan Tinggi/ Institusi	Program Studi/ Bagian	Bidang Tugas	ID Sinta	H-Index
DEWA AYU PUTRI SRI MASYENI - Ketua Pengusul	Universitas Warmadewa	Profesi Dokter	- Membuat konsep proposal - Mengurus ijin penelitian - Mengorganisir dan	5978655	12

			melaksanakan penelitian pada hewan coba - Melakukan pemeriksaan klinis hewan coba - Menganalisis data, menulis dan submit manuskript untuk publikasi		

3. MITRA KERJASAMA PENELITIAN (JIKA ADA)

Pelaksanaan penelitian dapat melibatkan mitra kerjasama, yaitu mitra kerjasama dalam melaksanakan penelitian, mitra sebagai calon pengguna hasil penelitian, atau mitra investor

Mitra	Nama Mitra
Mitra Pelaksana Penelitian	Diana Hansen

4. LUARAN DAN TARGET CAPAIAN

Luaran Wajib

Tahun Luaran	Jenis Luaran	*Status target capaian (accepted, published, terdaftar atau granted, atau status lainnya)*	*Keterangan (url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya)*
1	Artikel di Jurnal Internasional Terindeks di Pengindeks Bereputasi	Published	Virology Journal
2	Video Kegiatan		
2	Artikel di Jurnal Internasional Terindeks di Pengindeks Bereputasi	Sedang direview	Cytokine Journal
2	Artikel di Jurnal Internasional Terindeks di Pengindeks Bereputasi	Submited	Cytokine Journal

Luaran Tambahan

Tahun Luaran	Jenis Luaran	*Status target capaian (accepted, published, terdaftar atau granted, atau status lainnya)*	*Keterangan (url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya)*
1	Artikel pada Conference/ Seminar Internasional di Pengindeks Bereputasi		ASM meeting
2	Artikel pada Conference/ Seminar Internasional di Pengindeks Bereputasi		All about dengue meeting

5. ANGGARAN

Rencana anggaran biaya penelitian mengacu pada PMK yang berlaku dengan besaran minimum dan maksimum sebagaimana diatur pada buku Panduan Penelitian dan Pengabdian kepada Masyarakat

Total RAB 2 Tahun Rp. 0

Tahun 1 Total Rp. 0

Jenis Pembelanjaan	Komponen	Item	Satuan	Vol.	Biaya Satuan	Total

Tahun 2 Total Rp. 0

Jenis Pembelanjaan	Komponen	Item	Satuan	Vol.	Biaya Satuan	Total

Tahun 3 Total Rp. 0

Jenis Pembelanjaan	Komponen	Item	Satuan	Vol.	Biaya Satuan	Total

6. KEMAJUAN PENELITIAN

A. RINGKASAN

Penyakit infeksi virus Dengue merupakan masalah kesehatan global dunia khususnya di negaranegara tropis dan sub-tropis. Prevalensi infeksi virus Dengue (VD) diperkirakan meningkat 3 kali lipat

dari prediksi World Health Organization (WHO) yaitu mencapai 390 juta infeksi per tahun, dengan 96 juta kasus simptomatis. Angka tersebut menunjukkan bahwa infeksi VD adalah infeksi arbovirus terbanyak yang ditemukan di seluruh dunia, dimana diperkirakan sebanyak 3,6 milyar penduduk dunia berisiko terinfeksi VD. Dalam kawasan Asia Tenggara, Negara Indonesia menduduki peringkat ketiga tertinggi dalam hal kasus DBD menurut data dari World Health Organization. Virus Dengue adalah suatu RNA virus terdiri dari 4 serotipe, yaitu: dengue virus (DENV)-1, DENV-2, DENV-3, dan DENV-4. Keempat serotipe virus dengue ditemukan bersirkulasi di Bali, dan tidak hanya menginfeksi penduduk

lokal akan tetapi juga menginfeksi wisatawan internasional yang sedang berwisata di Bali. Manifestasi infeksi dengue sangat bervariasi dari ringan hingga berat mengancam nyawa. Sampai saat ini terapi antivirus belum ditemukan untuk tata laksana infeksi dengue. Begitu pula dengan vaksin dengue sampai saat ini baru dicoba pada anak- anak dan dewasa muda dengan persyaratan yang cukup rumit yang harus diperiksa sebelum pemberian vaksin dengue. Oleh karena itu dapat memprediksi infeksi dengue berat merupakan tantangan bagi klinisi untuk dapat mengenali infeksi dengue berat lebih dini sehingga dapat diberikan tata laksana yang adekuat untuk mengurangi mortalitas. Menemukan biomarker infeksi dengue berat merupakan tugas peneliti dalam membantu para klinisi. Patogenesis infeksi dengue berat didasari dengan adanya kebocoran plasma pada endotel vaskular sehingga deteksi beberapa protein penanda kebocoran plasma dapat dideteksi untuk menangkap kasus-kasus infeksi dengue berat. Beberapa biomarker yang masih belum banyak diteliti adalah platelet activating factor (PAF), matrix metaloprotein 9, serta komponen endotel vaskular syndecan-1 serta heparan sulfat.
Penelitian ini merupakan penelitian analitik cross- sectional pada penderita infeksi dengue. Kadar biomarker kebocoran plasma akan diukur dengan metode ELISA. Hasil penelitian akan dipublikasi pada jurnal internasional bereputasi/terindex Scopus. TKT penelitian adalah TKT 3

B. KATA KUNCI

biomarker; kebocoran_plasma;endotel_vaskular

Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. **HASIL PELAKSANAAN PENELITIAN:** Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian meliputi data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

Hasil penelitian:

Short communication

Cytokines differences and their correlation in dengue infection

(preliminary study)

Sri Masyeni[1], RT Sasmono[2], Diana Hansen[3]

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[3] Monash University, Australia

masyeniputu@yahoo.com

Abstract

Background: Dengue infection was one of several reemerging neglected tropical diseases with the highest-burden worldwide. This study aims to determine the differences and correlations of various cytokines in dengue infection.

Methods: This study was conducted in Bali, at the second semester of 2022 and gathered more than the minimum number of patients required. Laboratory examinations were used to find out several biomarkers such as Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels. Serological tests and RT-PCR were used to confirm dengue infection.

Results: The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in these patients were 208.36 ± 186.73 pg/mL, 184 ± 101.51 pg/mL, 130.12 ± 74.12 pg/mL, and 100.45 ± 72.58 pg/mL, respectively. Dengue patients were then grouped into several categories. The first category was based on severity which consists of DF and DHF, and the second category was based on infection status which consists of primary and secondary infection. There were differences in cytokines levels between these groups with p <0.05 and moderate to strong correlations with r values spanning 0.37 to 0.71. There were also significant correlations between these cytokines and symptoms of dengue infection.

Conclusions: PAF, Syndecan-1, hyaluronan, and MMP-9 levels were found to vary and associated with severity or infection status in dengue infection. These cytokines were also tied with dengue infection symptoms.
Keywords: biomarker, dengue, severity, infection status

Introduction

Dengue continues to be one of numerous neglected diseases in tropical countries, with cases increasing nearly 30-fold in the previous decade.[1] Dengue fever is endemic in Southeast Asia, but it has spread to non-endemic countries in Europe and the United States. Dengue virus (DENV) infection puts 3.9 billion people at risk across the world, with Asia having the highest incidence.[2] The worldwide economic impact of dengue infection is unknown. However, it is believed to be roughly $8.9 billion USD each year.[3]

Dengue infection is caused by one of four serotypes of DENV (DENV 1-4), each of which has a unique immunoreactivity.[4] There are no distinguishing clinical characteristics for each serotype because they can all present with symptoms that range from mild dengue fever (DF) to more severe dengue haemorrhagic fever (DHF), which can include dengue shock syndrome (DSS).[5] Primary DENV infection often results in a moderate to asymptomatic disease. However, secondary infection may result in a more severe condition.[6] This condition is well explained by antibody-dependent enhancement (ADE) theory and alteration of pro- and anti-inflammatory mediators known as cytokines storm.[7] Several studies showed that increased dysregulations of cytokines have an important role in the pathogenesis of DHF and DSS.[8] Cytokines that have major implications in the severity of dengue infection are Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels.[9] Pro-inflammatory cytokines group consists of Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels, while the anti-inflammatory cytokine only consists of IL-10. IL-6 has a unique property where it can be both pro- and anti-inflammatory cytokine accordingly.[10]

The study aims to determine the differences and correlations of several cytokine levels Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels with severity and infection status, as well as symptoms of dengue infection.

Materials and methods

Study design

This was a prospective cross-sectional study that comprised confirmed dengue infection patients in Bali. Written informed consent was obtained from all subjects. Patients admitted to the hospital were asked to participate in the study, and recruitment took place from June 2022 to December 2022. Patients above 18 years old who were admitted to the emergency room with a fever > 38^0C accompanied by at least one other symptom like headache, myalgia, retroorbital pain, or vomiting were enrolled. Patients with known malignancy, autoimmune disease, coinfections or other inflammatory disorders were excluded from this study.

$$n = \left\{ \frac{Z\alpha + Z\beta}{0{,}5 \ln \left[\frac{1+r}{1-r}\right]} \right\}^2 + 3$$

The number of patients required for this study was calculated using the above equation with Zα or standard deviation α (1.64), Zβ or standard deviation β (1.28), and r as the minimum accepted correlation coefficient (0.4). Based on these calculations, we determined the minimum number of patients was 51.

Patients were then grouped into two categories based on severity and infection status. The first category consists of dengue fever (DF) and dengue haemorrhagic fever (DHF) were based on the severity at the time of admission depending on the presentation of haemoconcentration (haematocrits level more ≥ than 50% or any decrease of haematocrits level ≥ 20% after fluid administration). The second category was based on infection status which consists of primary and secondary infection determined by serology and RT-PCR findings.

Laboratory analysis

Serum samples were tested at admission using a dengue NS1 antigen rapid test, Dengue Duo IgM/IgG Capture Elisa (Standard Diagnostics/SD, Bio line, Korea) and RT-PCR to confirm dengue infection. Dengue viral RNA was extracted from acute serum samples using the QI Amp Viral RNA mini kit (Qiagen, Hilden, Germany), according to the Qiagen instructions. To determine the level of cytokines, samples were tested using enzyme-linked immunosorbent assay using reagents from Elabscience® for Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels, according to the manufacturer's instructions. Complete blood counts (CBC) such as haemoglobin, haematocrit, erythrocytes, platelets and leucocytes were also documented on the day of admission. To determine the

infection status, the study combined the results of serological and RT-PCR tests. Patients with negative serological and positive RT-PCR tests were considered to have a primary infection, while those with positive serological and positive RT-PCR were considered to have a secondary infection.

Statistical analysis

SPSS Version 25 (SPSS Inc., Chicago, IL) was used to statistically analyse the data that were collected. A probability value of $p \leq 0.05$ was considered statistically significant. Numerical data was compared using the Independent-Samples or Mann-Whitney's test according to its distribution, and Chi-square was used for categorical data. As for correlation, Pearson's test or Spearman's test was used to find the relationship between this data and their associated correlation coefficient.

Result

Dengue patients characteristics

The study successfully enrolled 64 patients within six months. Most patients were female patients with a median age of 26 years. The median day of fever onset was on day four. There were four different dengue virus serotypes present in the patients, with DENV-3 having the highest prevalence. More than half of dengue patients had symptoms like headaches, retroorbital pain and nausea. For further analysis, patients are then grouped into 2 categories according to severity and infection status. Based on severity, there were no differences between DF and DHF in terms of sex, age, fever onset, and serotypes. DHF patients had longer lengths of stay in the hospital and more severe symptoms than DF. There were more patients in secondary infection than primary infection with most of them being female. Based on infection status, secondary infection had longer lengths of stay and more severe symptoms than primary infection. Detailed information on patients characteristics was shown in table 1.

Table 1. Dengue patients characteristics

	Total (N =64)	DF (N=32)	DHF (N=32)	*p*	1st (N=22)	2nd (N=42)	*p*
Sex (%)				0.8			0.048*
Male	27 (42.2)	13 (48.1)	14 (51.9)		13 (48.1)	14 (51.9)	
Female	37 (57.8)	19 (51.4)	18 (48.6)		9 (24.3)	28 (75.7)	
Age (year)	26 (18-84)[a]	31 (18-84)[a]	25 (18-49)[a]	0.12[b]	30 (18-52)[a]	25 (18-84)[a]	0.63[b]
Fever onset (day)	4 (2-6)[a]	4 (2-5)[a]	4 (2-6)[a]	0.22[b]	4 (3-5)[a]	4 (2-6)[a]	0.11[b]
Length of stay (days)	6 (1-10)[a]	5 (1-8)[a]	7 (3-10)[a]	0.001[b]*	4 (1-6)[a]	7 (3-10)[a]	<0.001[b]*
Serotype (%)				0.66			0.21
DENV-1	6 (9.4)	4 (66.7)	2 (33.3)		4 (66.7)	2 (33.3)	
DENV-2	10 (15.6)	4 (40)	6 (60)		4 (40)	6 (60)	
DENV-3	44 (68.8)	22 (50)	22 (50)		12 (27.3)	32 (72.7)	
DENV-4	1 (1.6)	1 (100)	0 (0)		1 (100)	0 (0)	
NA	3 (4.7)	1 (33.3)	2 (66.7)		1 (33.3)	2 (66.7)	
Symptoms (%)							
Headache	56 (87.5)	26 (46.4)	30 (53.6)	0.13	16 (28.6)	40 (71.4)	0.01*
Retroorbital pain	30 (46.9)	9 (30)	21 (70)	0.004*	6 (20)	24 (80)	0.045*
Nausea	43 (67.2)	14 (32.6)	29 (67.4)	<0.001*	6 (14)	37 (86)	<0.001*
Vomiting	23 (35.9)	3 (13)	20 (87)	<0.001*	0 (0)	23 (100)	<0.001*
Bleeding	16 (25)	0 (0)	16 (100)	<0.001*	0 (0)	16 (100)	0.001*

Note: 1st = Primary Infection, 2nd = Secondary Infection

[a] Presented as the median (minimum-maximum)

[b] The Mann-Whitney test was used to compare the group

* Significant value at $p \leq 0.05$

DHF and secondary infection had higher cytokine level

Cytokine levels in patients with DF and DHF differed markedly. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in DF patients were 126.41 ± 52.69 pg/mL, 129.79 ± 50.06 pg/mL, 173.14 ± 83.37 pg/mL, and 58.62 ± 36.48 pg/mL, respectively. PAF, Syndecan-1, and hyaluronan levels were higher in DHF patients compared to DF patients, whereas MMP-9 levels were lower. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in DHF patients were 282.62 ± 230.22 pg/mL, 233.69 ± 111.27 pg/mL, 91.15 ± 32.49 pg/mL, and138.37 ± 76.64 pg/mL, respectively.

The study also discovered that secondary infection had notable cytokine differences from primary infection. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in primary infection were 126.08 ± 62.43 pg/mL, 119.60 ± 42.09 pg/mL, 172.43 ± 68.17 pg/mL, and 50.74 ± 19.27 pg/mL, respectively. Meanwhile, the cytokines levels in secondary infection were 245.58 ± 211.56 pg/mL, 213.57 ± 213.57 pg/mL, 110.99 ± 69.24 pg/mL, and 122.94 ± 76.70 pg/mL, respectively. This result shown in Table 2 confirmed secondary infection had higher PAF, Syndecan-1, hyaluronan, levels, while the level of MMP-9 was lower.

Table 2. Complete blood count and cytokines result of the dengue patients

	Total (N=64)	DF (N=32)	DHF (N=32)	*p*	1st (N=22)	2nd (N=42)	*p*
CBC at admission							
Haemoglobin (g/dL)	14.27 ± 1.57	13.58 ± 1.29	14.89 ± 1.55	0.001*	13.58 ± 1.17	14.58 ± 1.64	0.01*
Haematocrit (%)	43.11 ± 5.26	40.58 ± 4.32	45.41 ± 5.03	<0.001*	40.25 ± 4.12	44.40 ± 5.25	0.002*
Leucocyte (x10^3/mL)	3.05 ± 1.14	2.90 ± 1.04	3.18 ± 1.23	0.61	3.30 ± 1.10	2.93 ± 1.15	0.09
Thrombocyte	93.66 ± 44.61	99.07 ± 42.42	88.75 ± 46.62	0.16	119.11 ±	82.14 ± 41.26	<0.001*

($x10^3$/mL)	4.96 ± 0.65	4.72 ± 0.59	5.17 ± 0.64	0.008*	41.98	5.02 ± 0.67	0.45
RBC ($x10^6$/mL)					4.83 ± 0.61		
PAF (pg/mL)	208.36 ± 186.73	126.41 ± 52.69	282.62 ± 230.22	0.001*	126.08 ± 62.43	245.58 ± 211.56	0.003*
Synde-1 (pg/mL)	184 ± 101.51	129.79 ± 50.06	233.69 ± 111.27	<0.001*	119.60 ± 42.09	213.57 ± 213.57	<0.001*
Hyaluronan (pg/mL)	130.12 ± 74.12	173.14 ± 83.37	91.15 ± 32.49	<0.001*	172.43 ± 68.17	110.99 ± 69.24	<0.001*
MMP-9 (pg/mL)	100.45 ± 72.58	58.62 ± 36.48	138.37 ± 76.64	<0.001*	50.74 ± 19.27	122.94 ± 76.70	<0.001*

Note: 1st = Primary Infection, 2nd = Secondary Infection
* Significant values at $p \leq 0.05$

Cytokines correlations with symptoms, severity, and infection status

The level of cytokines was found to be significantly related to many symptoms such as nausea, vomiting, and bleeding. The correlation coefficients varied from weak to strong. These symptoms were correlated with higher PAF, Syndecan-1, hyaluronan, but not with MMP-9 levels.

Cytokines also had a notable association with the severity of infection. Higher levels of PAF, Syndecan-1, and hyaluronan, correlated with more severe disease. MMP-9 levels were higher in mild conditions. The correlation coefficient in this category was varied from moderate to strong. There was a marked relationship between PAF, Syndecan-1, hyaluronan, and MMP-9 with infection status. The correlation coefficient in this category varied from weak to moderate. These findings were shown in Table 3.

Table 3. Correlation between cytokines and several variables in dengue infection

	Headache		Retroorbital pain		Nausea		Vomiting		Bleeding		Severity of Infection		Infection Status	
	p	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r
PAF	0.12	0.19	0.46	0.09	0.01*	0.31	0.01*	0.32	<0.001*	0.45	<0.001*	0.43	0.003*	0.37
Synde-1	0.08	0.22	0.13	0.19	<0.001*	0.47	0.001*	0.41	0.003*	0.36	<0.001*	0.60	<0.001*	0.55
Hyalu	0.95	-0.01	0.03*	-0.27	0.02*	-0.29	<0.001*	-0.43	0.011*	-0.32	<0.001*	-0.58	<0.001*	-0.47
MMP-9	0.26	0.14	0.01*	0,33	<0.001*	0.47	<0.001*	0.62	<0.001*	0.64	<0.001*	0.71	<0.001*	0.59

Note: * Significant values at $p \leq 0.05$

Discussion

This cross-sectional study, which included 64 patients from multiple hospitals in Bali, added further evidence of the important role cytokines played in dengue infection. The main cytokines that have been examined in this study were PAF, Syndecan-1, hyaluronan, and MMP-9. These cytokine levels were evaluated based on the severity and status of dengue infection. The study also identified a connection between cytokine levels and dengue symptoms.

Clinical classifications of dengue infection severity comprise DF, DHF, and DSS.[1] The severity in this study was classified into DF and DHF, and the study found that there were significant differences in cytokine levels between this group. PAF, Syndecan-1, and hyaluronan levels

were higher in DHF patients compared to DF patients, although MMP-9 level was lower. This corresponds to higher pro-inflammatory along with lower anti-inflammatory cytokines activity in DHF patients. PAF, Syndecan-1, hyaluronan, and MMP-9 were endothelial activation products formed by CD4+ T helper 2 cells.[11,12] Although previous studies indicated that PAF, Syndecan-1, hyaluronan, and MMP-9 were associated with plasma leakage in dengue infection, they frequently fell short of explaining the severity of the disease.[13,14] These findings were in accordance with our results and the study was able to establish a significant correlation between each cytokine and severity. In contrast to other research where severe dengue was associated with increased MMP-9 levels, our DHF patients had lower levels of MMP-9. As the study was unable to identify the source of MMP-9 in these patients, several sources of MMP-9, including T helper cells, monocytes, as well as a wide variety of immune effector cell types, including B cells, cytotoxic T cells, and granulocytes, such as neutrophils, could explain this finding.[15] PAF, Syndecan-1, and hyaluronan, and MMP-9 had moderate correlations with severity, whereas IL-17 had strong correlations. This indicates that these cytokines significantly contribute to the severity of the dengue infection.

Infection status provided the basis for the second classification in this study. The study classified dengue infections as either primary or secondary. Cytokine levels differ markedly between primary and secondary infections. The secondary infection had higher PAF, Syndecan-1, hyaluronan, and MMP-9. These findings were linked to two well-known concepts in dengue infection: the antibody-dependent enhancement (ADE) phenomena and the cytokine storm.[6,7] ADE increases dengue virus protein synthesis, viral RNA generation and release. Although PAF and gamma interferon were not generated in the presence of dengue virus, Syndecan-1 and hyaluronan were produced at elevated titers, indicating a signalling component of ADE.[16] Cytokine storm was a condition in which cytokine levels shifted, causing endothelial cells to break down and subsequent plasma leakage.[7] PAF showed a weak correlation, while Syndecan-1, hyaluronan, and MMP-9 had moderate correlations with infection status. This result also supported the previously mentioned concepts.

Dengue infection has several unspecific symptoms like headache, retroorbital pain, nausea, vomiting, and bleeding.[1] The study found that some of these symptoms were associated with cytokine levels. The most notable finding was the strong correlation between MMP-9 with bleeding. MMP-9 functions as a protective mediator in barrier immunity through a variety of methods.[17] MMP-9 supports epithelial integrity by modulating tight junction proteins, which link and stabilize epithelial cell connections to maintain the barrier and keep out gut luminal contents and commensal microbes.[18] The findings vary from those of a recent study, which

found that IL-8 levels were considerably greater in DF with bleeding than in DF without bleeding during the acute phase of infection. IL-8 features contribute to platelet activation and endothelial permeability, resulting in thrombocytopenia and worsening bleeding in dengue infection.[20] Other notable findings in this study were weak to strong correlation between PAF, Syndecan-1, hyaluronan, and MMP-9 with retroorbital pain, nausea and vomiting.

This study has several limitations. This study had restricted resources and funding, which limited the cytokines that were planned to be included, such as IL-8, interferon, and other cytokines. Following that, the study was unable to determine the serotype of three dengue-infected individuals. We suspected that it was a simple human error. Also, the study was only able to include symptomatic individuals who were seeking treatment at a hospital. Finally, because this study was conducted during the COVID-19 pandemic, numerous patients withdrew for various reasons such as coinfections and personal concerns. Despite these constraints, the study yielded substantial results.

Conclusions

The study found considerable differences and correlations between cytokine levels in dengue infection based on severity and infection status. PAF, Syndecan-1, and MMP-9 levels were considerably greater in DHF and secondary infection, but hyaluronan levels were substantially lower. The study additionally found a link between cytokines and dengue symptoms, with particular emphasis on bleeding.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Source

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Ethical Approval Statement

The study protocol was approved by the Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Bali (document number 46/Unwar/FKIK/EC-KEPK/VI/2022).

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D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta mengunggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui BIMA.

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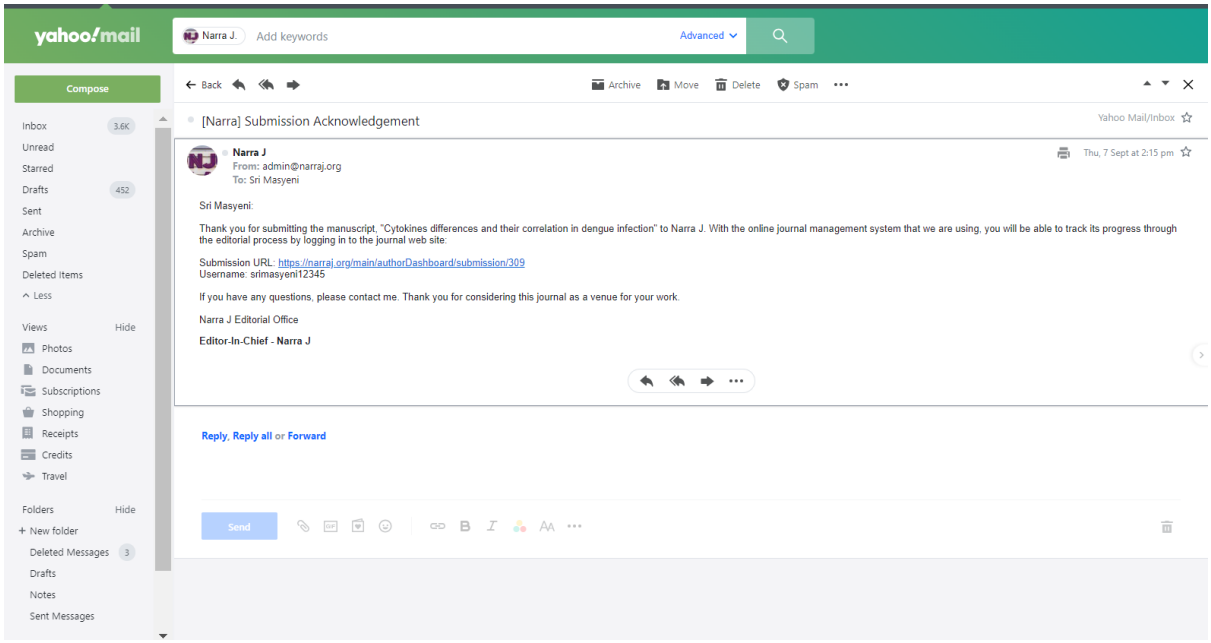

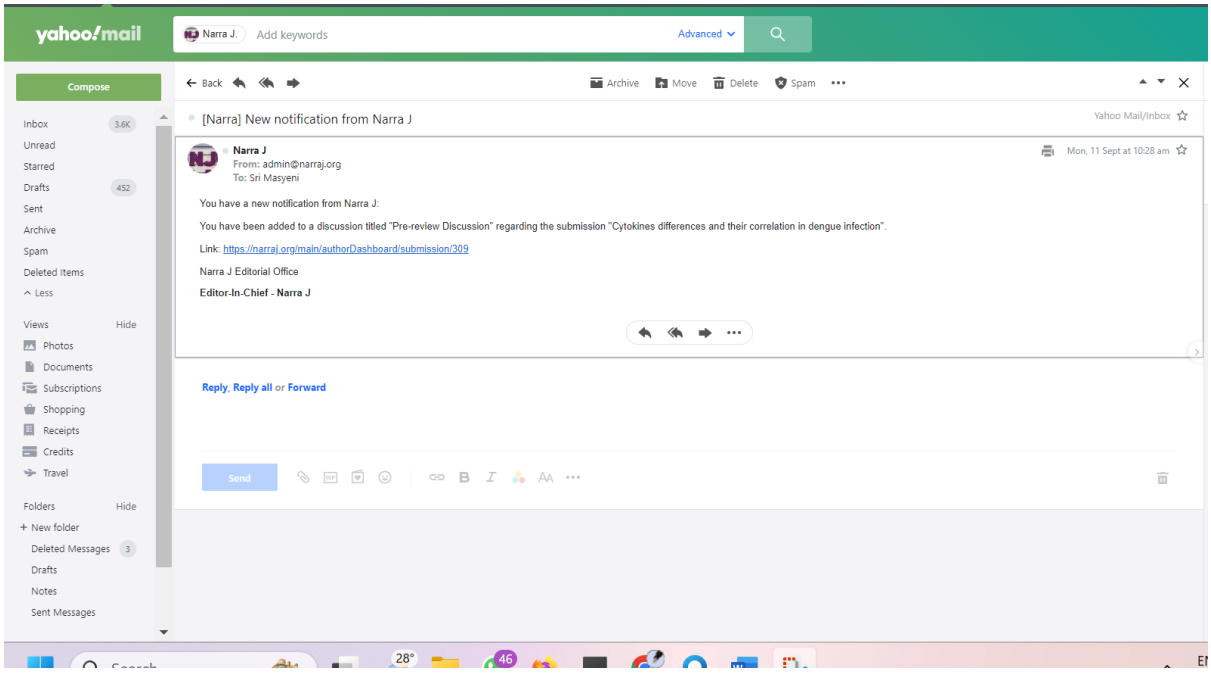

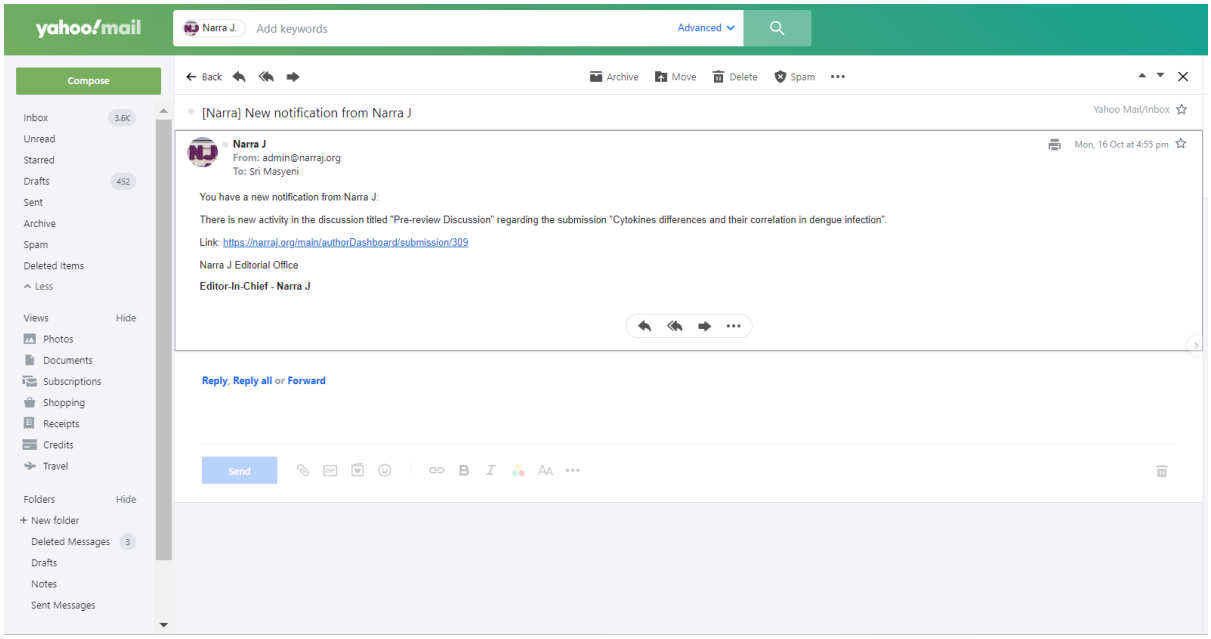

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E. **PERAN MITRA:** Tuliskan realisasi kerjasama dan kontribusi Mitra baik *in-kind* maupun *in-cash* (untuk Penelitian Terapan, Penelitian Pengembangan, PTUPT, PPUPT serta KRUPT). Bukti pendukung realisasi kerjasama dan realisasi kontribusi mitra dilaporkan sesuai dengan kondisi yang sebenarnya. Bukti dokumen realisasi kerjasama dengan Mitra diunggah melalui BIMA.

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Dear sir or madam,
I am writing to confirm that the Walter and Eliza Hall Institute in Melbourne, Australia has successfully attracted funding through philanthropic donations to support the research Dr Sri Masyeni and her team at the Faculty of Medicine and Health Sciences, University of Warmadewa, Bali, Indonesia, for the discovery of biomarker of severe dengue fever, research that she is conducting in collaboration with Dr Tedjo Sasmono and my team in Melbourne. The funding covers the cost of a new centrifuge and a liquid nitrogen tank, to support the collection of

blood mononuclear cells from patients infected with dengue virus that are recruited in our study. My laboratory remains strongly connected to this research and I will provide my expertise in all areas of the research project, including study design, cell preparation and storage, data analysis and interpretation, as well as preparation of manuscripts arising from the results of this research for publication in competitive virology journals.

Yours sincerely,

Professor Diana Hansen

Laboratory Head

Infectious Diseases and Immune Defense Division


BIOBASE
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dr. Masyeni

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F. **KENDALA PELAKSANAAN PENELITIAN**: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Tidak ada

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G. **RENCANA TAHAPAN SELANJUTNYA:** Tuliskan dan uraikan rencana penelitian di tahun berikutnya berdasarkan indikator luaran yang telah dicapai, rencana realisasi luaran wajib yang dijanjikan dan tambahan (jika ada) di tahun berikutnya serta *roadmap* penelitian keseluruhan. Pada bagian ini diperbolehkan untuk melengkapi penjelasan dari setiap tahapan dalam metoda yang akan direncanakan termasuk jadwal berkaitan dengan strategi untuk mencapai luaran seperti yang telah dijanjikan dalam proposal. Jika diperlukan, penjelasan dapat juga dilengkapi dengan gambar, tabel, diagram, serta pustaka yang relevan. Pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai.

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H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan akhir yang dicantumkan dalam Daftar Pustaka.

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Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. **HASIL PELAKSANAAN PENELITIAN:** Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian meliputi data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

Hasil penelitian:

Short communication

Cytokines differences and their correlation in dengue infection

(preliminary study)

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Abstract

Background: Dengue infection was one of several reemerging neglected tropical diseases with the highest-burden worldwide. This study aims to determine the differences and correlations of various cytokines in dengue infection.

Methods: This study was conducted in Bali, at the second semester of 2022 and gathered more than the minimum number of patients required. Laboratory examinations were used to find out several biomarkers such as Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels. Serological tests and RT-PCR were used to confirm dengue infection.

Results: The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in these patients were 208.36 ± 186.73 pg/mL, 184 ± 101.51 pg/mL, 130.12 ± 74.12 pg/mL, and 100.45 ± 72.58 pg/mL, respectively. Dengue patients were then grouped into several categories. The first category was based on severity which consists of DF and DHF, and the second category was based on infection status which consists of primary and secondary infection. There were differences in cytokines levels between these groups with p <0.05 and moderate to strong correlations with r values spanning 0.37 to 0.71. There were also significant correlations between these cytokines and symptoms of dengue infection.

Conclusions: PAF, Syndecan-1, hyaluronan, and MMP-9 levels were found to vary and associated with severity or infection status in dengue infection. These cytokines were also tied with dengue infection symptoms.
Keywords: biomarker, dengue, severity, infection status

Introduction

Dengue continues to be one of numerous neglected diseases in tropical countries, with cases increasing nearly 30-fold in the previous decade.[1] Dengue fever is endemic in Southeast Asia, but it has spread to non-endemic countries in Europe and the United States. Dengue virus (DENV) infection puts 3.9 billion people at risk across the world, with Asia having the highest incidence.[2] The worldwide economic impact of dengue infection is unknown. However, it is believed to be roughly $8.9 billion USD each year.[3]

Dengue infection is caused by one of four serotypes of DENV (DENV 1-4), each of which has a unique immunoreactivity.[4] There are no distinguishing clinical characteristics for each serotype because they can all present with symptoms that range from mild dengue fever (DF) to more severe dengue haemorrhagic fever (DHF), which can include dengue shock syndrome (DSS).[5] Primary DENV infection often results in a moderate to asymptomatic disease. However, secondary infection may result in a more severe condition.[6] This condition is well explained by antibody-dependent enhancement (ADE) theory and alteration of pro- and anti-inflammatory mediators known as cytokines storm.[7] Several studies showed that increased dysregulations of cytokines have an important role in the pathogenesis of DHF and DSS.[8] Cytokines that have major implications in the severity of dengue infection are Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels.[9] Pro-inflammatory cytokines group consists of Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels, while the anti-inflammatory cytokine only consists of IL-10. IL-6 has a unique property where it can be both pro- and anti-inflammatory cytokine accordingly.[10]

The study aims to determine the differences and correlations of several cytokine levels Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels with severity and infection status, as well as symptoms of dengue infection.

Materials and methods

Study design

This was a prospective cross-sectional study that comprised confirmed dengue infection patients in Bali. Written informed consent was obtained from all subjects. Patients admitted to the hospital were asked to participate in the study, and recruitment took place from June 2022 to December 2022. Patients above 18 years old who were admitted to the emergency room with a fever > 38^0C accompanied by at least one other symptom like headache, myalgia, retroorbital pain, or vomiting were enrolled. Patients with known malignancy, autoimmune disease, coinfections or other inflammatory disorders were excluded from this study.

$$n = \left\{ \frac{Z\alpha + Z\beta}{0{,}5 \ln \left[\frac{1+r}{1-r}\right]} \right\}^2 + 3$$

The number of patients required for this study was calculated using the above equation with Zα or standard deviation α (1.64), Zβ or standard deviation β (1.28), and r as the minimum accepted correlation coefficient (0.4). Based on these calculations, we determined the minimum number of patients was 51.

Patients were then grouped into two categories based on severity and infection status. The first category consists of dengue fever (DF) and dengue haemorrhagic fever (DHF) were based on the severity at the time of admission depending on the presentation of haemoconcentration (haematocrits level more ≥ than 50% or any decrease of haematocrits level ≥ 20% after fluid administration). The second category was based on infection status which consists of primary and secondary infection determined by serology and RT-PCR findings.

Laboratory analysis

Serum samples were tested at admission using a dengue NS1 antigen rapid test, Dengue Duo IgM/IgG Capture Elisa (Standard Diagnostics/SD, Bio line, Korea) and RT-PCR to confirm dengue infection. Dengue viral RNA was extracted from acute serum samples using the QI Amp Viral RNA mini kit (Qiagen, Hilden, Germany), according to the Qiagen instructions. To determine the level of cytokines, samples were tested using enzyme-linked immunosorbent assay using reagents from Elabscience® for Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels, according to the manufacturer's instructions. Complete blood counts (CBC) such as haemoglobin, haematocrit, erythrocytes, platelets and leucocytes were also documented on the day of admission. To determine the

infection status, the study combined the results of serological and RT-PCR tests. Patients with negative serological and positive RT-PCR tests were considered to have a primary infection, while those with positive serological and positive RT-PCR were considered to have a secondary infection.

Statistical analysis

SPSS Version 25 (SPSS Inc., Chicago, IL) was used to statistically analyse the data that were collected. A probability value of $p \leq 0.05$ was considered statistically significant. Numerical data was compared using the Independent-Samples or Mann-Whitney's test according to its distribution, and Chi-square was used for categorical data. As for correlation, Pearson's test or Spearman's test was used to find the relationship between this data and their associated correlation coefficient.

Result

Dengue patients characteristics

The study successfully enrolled 64 patients within six months. Most patients were female patients with a median age of 26 years. The median day of fever onset was on day four. There were four different dengue virus serotypes present in the patients, with DENV-3 having the highest prevalence. More than half of dengue patients had symptoms like headaches, retroorbital pain and nausea. For further analysis, patients are then grouped into 2 categories according to severity and infection status. Based on severity, there were no differences between DF and DHF in terms of sex, age, fever onset, and serotypes. DHF patients had longer lengths of stay in the hospital and more severe symptoms than DF. There were more patients in secondary infection than primary infection with most of them being female. Based on infection status, secondary infection had longer lengths of stay and more severe symptoms than primary infection. Detailed information on patients characteristics was shown in table 1.

Table 1. Dengue patients characteristics

	Total (N =64)	DF (N=32)	DHF (N=32)	*p*	1st (N=22)	2nd (N=42)	*p*
Sex (%)				0.8			0.048*
Male	27 (42.2)	13 (48.1)	14 (51.9)		13 (48.1)	14 (51.9)	
Female	37 (57.8)	19 (51.4)	18 (48.6)		9 (24.3)	28 (75.7)	
Age (year)	26 (18-84)[a]	31 (18-84)[a]	25 (18-49)[a]	0.12[b]	30 (18-52)[a]	25 (18-84)[a]	0.63[b]
Fever onset (day)	4 (2-6)[a]	4 (2-5)[a]	4 (2-6)[a]	0.22[b]	4 (3-5)[a]	4 (2-6)[a]	0.11[b]
Length of stay (days)	6 (1-10)[a]	5 (1-8)[a]	7 (3-10)[a]	0.001[b]*	4 (1-6)[a]	7 (3-10)[a]	<0.001[b]*
Serotype (%)				0.66			0.21
DENV-1	6 (9.4)	4 (66.7)	2 (33.3)		4 (66.7)	2 (33.3)	
DENV-2	10 (15.6)	4 (40)	6 (60)		4 (40)	6 (60)	
DENV-3	44 (68.8)	22 (50)	22 (50)		12 (27.3)	32 (72.7)	
DENV-4	1 (1.6)	1 (100)	0 (0)		1 (100)	0 (0)	
NA	3 (4.7)	1 (33.3)	2 (66.7)		1 (33.3)	2 (66.7)	
Symptoms (%)							
Headache	56 (87.5)	26 (46.4)	30 (53.6)	0.13	16 (28.6)	40 (71.4)	0.01*
Retroorbital pain	30 (46.9)	9 (30)	21 (70)	0.004*	6 (20)	24 (80)	0.045*
Nausea	43 (67.2)	14 (32.6)	29 (67.4)	<0.001*	6 (14)	37 (86)	<0.001*
Vomiting	23 (35.9)	3 (13)	20 (87)	<0.001*	0 (0)	23 (100)	<0.001*
Bleeding	16 (25)	0 (0)	16 (100)	<0.001*	0 (0)	16 (100)	0.001*

Note: 1st = Primary Infection, 2nd = Secondary Infection

[a] Presented as the median (minimum-maximum)

[b] The Mann-Whitney test was used to compare the group

* Significant value at $p \leq 0.05$

DHF and secondary infection had higher cytokine level

Cytokine levels in patients with DF and DHF differed markedly. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in DF patients were 126.41 ± 52.69 pg/mL, 129.79 ± 50.06 pg/mL, 173.14 ± 83.37 pg/mL, and 58.62 ± 36.48 pg/mL, respectively. PAF, Syndecan-1, and hyaluronan levels were higher in DHF patients compared to DF patients, whereas MMP-9 levels were lower. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in DHF patients were 282.62 ± 230.22 pg/mL, 233.69 ± 111.27 pg/mL, 91.15 ± 32.49 pg/mL, and138.37 ± 76.64 pg/mL, respectively.

The study also discovered that secondary infection had notable cytokine differences from primary infection. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in primary infection were 126.08 ± 62.43 pg/mL, 119.60 ± 42.09 pg/mL, 172.43 ± 68.17 pg/mL, and 50.74 ± 19.27 pg/mL, respectively. Meanwhile, the cytokines levels in secondary infection were 245.58 ± 211.56 pg/mL, 213.57 ± 213.57 pg/mL, 110.99 ± 69.24 pg/mL, and 122.94 ± 76.70 pg/mL, respectively. This result shown in Table 2 confirmed secondary infection had higher PAF, Syndecan-1, hyaluronan, levels, while the level of MMP-9 was lower.

Table 2. Complete blood count and cytokines result of the dengue patients

	Total (N=64)	DF (N=32)	DHF (N=32)	*p*	1st (N=22)	2nd (N=42)	*p*
CBC at admission							
Haemoglobin (g/dL)	14.27 ± 1.57	13.58 ± 1.29	14.89 ± 1.55	0.001*	13.58 ± 1.17	14.58 ± 1.64	0.01*
Haematocrit (%)	43.11 ± 5.26	40.58 ± 4.32	45.41 ± 5.03	<0.001*	40.25 ± 4.12	44.40 ± 5.25	0.002*
Leucocyte (x10^3/mL)	3.05 ± 1.14	2.90 ± 1.04	3.18 ± 1.23	0.61	3.30 ± 1.10	2.93 ± 1.15	0.09
Thrombocyte	93.66 ± 44.61	99.07 ± 42.42	88.75 ± 46.62	0.16	119.11 ±	82.14 ± 41.26	<0.001*

($x10^3$/mL)	4.96 ± 0.65	4.72 ± 0.59	5.17 ± 0.64	0.008*	41.98	5.02 ± 0.67	0.45
RBC ($x10^6$/mL)					4.83 ± 0.61		
PAF (pg/mL)	208.36 ± 186.73	126.41 ± 52.69	282.62 ± 230.22	0.001*	126.08 ± 62.43	245.58 ± 211.56	0.003*
Synde-1 (pg/mL)	184 ± 101.51	129.79 ± 50.06	233.69 ± 111.27	<0.001*	119.60 ± 42.09	213.57 ± 213.57	<0.001*
Hyaluronan (pg/mL)	130.12 ± 74.12	173.14 ± 83.37	91.15 ± 32.49	<0.001*	172.43 ± 68.17	110.99 ± 69.24	<0.001*
MMP-9 (pg/mL)	100.45 ± 72.58	58.62 ± 36.48	138.37 ± 76.64	<0.001*	50.74 ± 19.27	122.94 ± 76.70	<0.001*

Note: 1st = Primary Infection, 2nd = Secondary Infection
* Significant values at $p \leq 0.05$

Cytokines correlations with symptoms, severity, and infection status

The level of cytokines was found to be significantly related to many symptoms such as nausea, vomiting, and bleeding. The correlation coefficients varied from weak to strong. These symptoms were correlated with higher PAF, Syndecan-1, hyaluronan, but not with MMP-9 levels.

Cytokines also had a notable association with the severity of infection. Higher levels of PAF, Syndecan-1, and hyaluronan, correlated with more severe disease. MMP-9 levels were higher in mild conditions. The correlation coefficient in this category was varied from moderate to strong. There was a marked relationship between PAF, Syndecan-1, hyaluronan, and MMP-9 with infection status. The correlation coefficient in this category varied from weak to moderate. These findings were shown in Table 3.

Table 3. Correlation between cytokines and several variables in dengue infection

	Headache		Retroorbital pain		Nausea		Vomiting		Bleeding		Severity of Infection		Infection Status	
	p	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r
PAF	0.12	0.19	0.46	0.09	0.01*	0.31	0.01*	0.32	<0.001*	0.45	<0.001*	0.43	0.003*	0.37
Synde-1	0.08	0.22	0.13	0.19	<0.001*	0.47	0.001*	0.41	0.003*	0.36	<0.001*	0.60	<0.001*	0.55
Hyalu	0.95	-0.01	0.03*	-0.27	0.02*	-0.29	<0.001*	-0.43	0.011*	-0.32	<0.001*	-0.58	<0.001*	-0.47
MMP-9	0.26	0.14	0.01*	0,33	<0.001*	0.47	<0.001*	0.62	<0.001*	0.64	<0.001*	0.71	<0.001*	0.59

Note: * Significant values at $p \leq 0.05$

Discussion

This cross-sectional study, which included 64 patients from multiple hospitals in Bali, added further evidence of the important role cytokines played in dengue infection. The main cytokines that have been examined in this study were PAF, Syndecan-1, hyaluronan, and MMP-9. These cytokine levels were evaluated based on the severity and status of dengue infection. The study also identified a connection between cytokine levels and dengue symptoms.

Clinical classifications of dengue infection severity comprise DF, DHF, and DSS.[1] The severity in this study was classified into DF and DHF, and the study found that there were significant differences in cytokine levels between this group. PAF, Syndecan-1, and hyaluronan levels

were higher in DHF patients compared to DF patients, although MMP-9 level was lower. This corresponds to higher pro-inflammatory along with lower anti-inflammatory cytokines activity in DHF patients. PAF, Syndecan-1, hyaluronan, and MMP-9 were endothelial activation products formed by CD4+ T helper 2 cells.[11,12] Although previous studies indicated that PAF, Syndecan-1, hyaluronan, and MMP-9 were associated with plasma leakage in dengue infection, they frequently fell short of explaining the severity of the disease.[13,14] These findings were in accordance with our results and the study was able to establish a significant correlation between each cytokine and severity. In contrast to other research where severe dengue was associated with increased MMP-9 levels, our DHF patients had lower levels of MMP-9. As the study was unable to identify the source of MMP-9 in these patients, several sources of MMP-9, including T helper cells, monocytes, as well as a wide variety of immune effector cell types, including B cells, cytotoxic T cells, and granulocytes, such as neutrophils, could explain this finding.[15] PAF, Syndecan-1, and hyaluronan, and MMP-9 had moderate correlations with severity, whereas IL-17 had strong correlations. This indicates that these cytokines significantly contribute to the severity of the dengue infection.

Infection status provided the basis for the second classification in this study. The study classified dengue infections as either primary or secondary. Cytokine levels differ markedly between primary and secondary infections. The secondary infection had higher PAF, Syndecan-1, hyaluronan, and MMP-9. These findings were linked to two well-known concepts in dengue infection: the antibody-dependent enhancement (ADE) phenomena and the cytokine storm.[6,7] ADE increases dengue virus protein synthesis, viral RNA generation and release. Although PAF and gamma interferon were not generated in the presence of dengue virus, Syndecan-1 and hyaluronan were produced at elevated titers, indicating a signalling component of ADE.[16] Cytokine storm was a condition in which cytokine levels shifted, causing endothelial cells to break down and subsequent plasma leakage.[7] PAF showed a weak correlation, while Syndecan-1, hyaluronan, and MMP-9 had moderate correlations with infection status. This result also supported the previously mentioned concepts.

Dengue infection has several unspecific symptoms like headache, retroorbital pain, nausea, vomiting, and bleeding.[1] The study found that some of these symptoms were associated with cytokine levels. The most notable finding was the strong correlation between MMP-9 with bleeding. MMP-9 functions as a protective mediator in barrier immunity through a variety of methods.[17] MMP-9 supports epithelial integrity by modulating tight junction proteins, which link and stabilize epithelial cell connections to maintain the barrier and keep out gut luminal contents and commensal microbes.[18] The findings vary from those of a recent study, which

found that IL-8 levels were considerably greater in DF with bleeding than in DF without bleeding during the acute phase of infection. IL-8 features contribute to platelet activation and endothelial permeability, resulting in thrombocytopenia and worsening bleeding in dengue infection.[20] Other notable findings in this study were weak to strong correlation between PAF, Syndecan-1, hyaluronan, and MMP-9 with retroorbital pain, nausea and vomiting.

This study has several limitations. This study had restricted resources and funding, which limited the cytokines that were planned to be included, such as IL-8, interferon, and other cytokines. Following that, the study was unable to determine the serotype of three dengue-infected individuals. We suspected that it was a simple human error. Also, the study was only able to include symptomatic individuals who were seeking treatment at a hospital. Finally, because this study was conducted during the COVID-19 pandemic, numerous patients withdrew for various reasons such as coinfections and personal concerns. Despite these constraints, the study yielded substantial results.

Conclusions

The study found considerable differences and correlations between cytokine levels in dengue infection based on severity and infection status. PAF, Syndecan-1, and MMP-9 levels were considerably greater in DHF and secondary infection, but hyaluronan levels were substantially lower. The study additionally found a link between cytokines and dengue symptoms, with particular emphasis on bleeding.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Source

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Ethical Approval Statement

The study protocol was approved by the Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Bali (document number 46/Unwar/FKIK/EC-KEPK/VI/2022).

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D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta mengunggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui BIMA.

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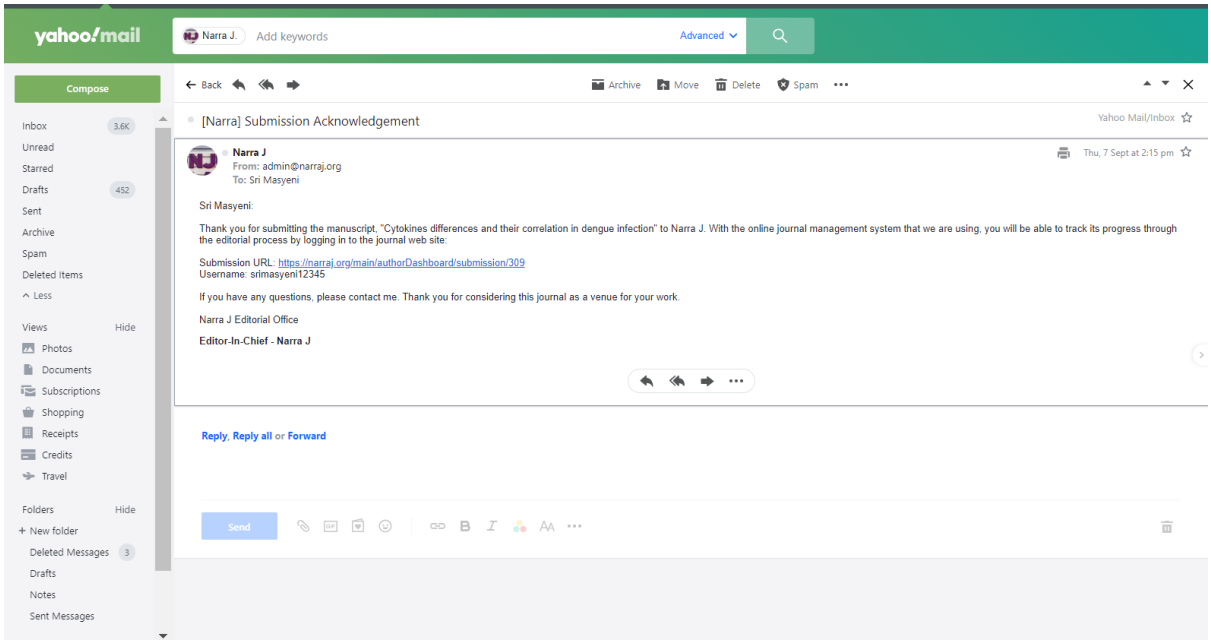

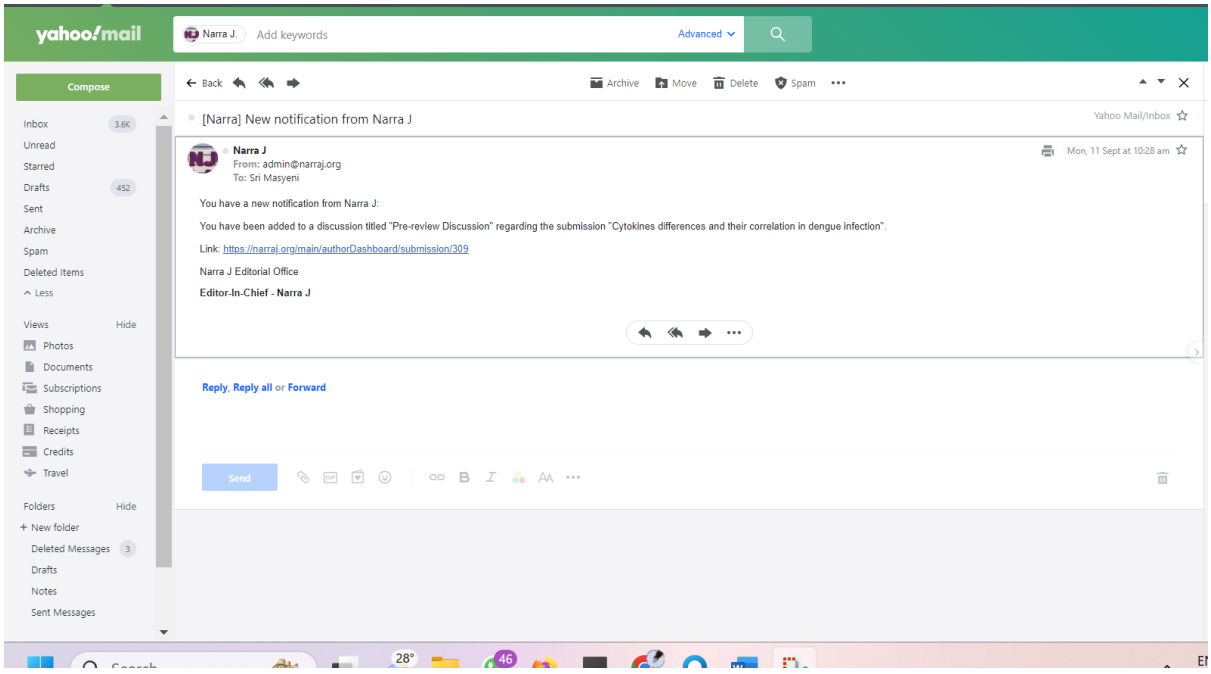

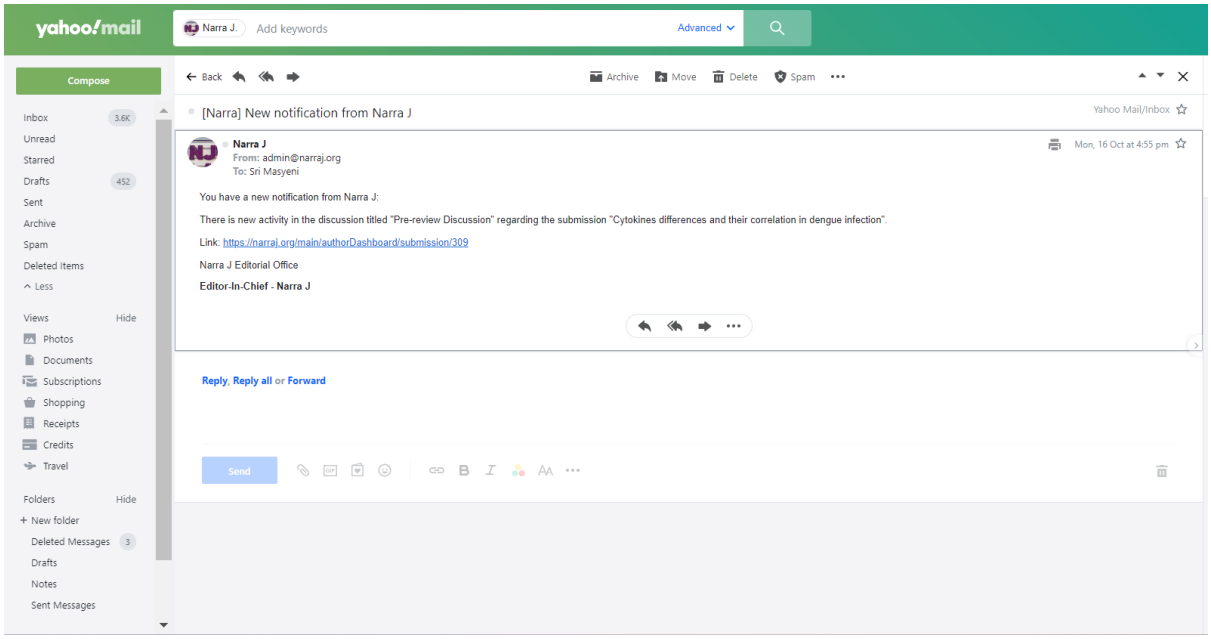

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E. **PERAN MITRA:** Tuliskan realisasi kerjasama dan kontribusi Mitra baik *in-kind* maupun *in-cash* (untuk Penelitian Terapan, Penelitian Pengembangan, PTUPT, PPUPT serta KRUPT). Bukti pendukung realisasi kerjasama dan realisasi kontribusi mitra dilaporkan sesuai dengan kondisi yang sebenarnya. Bukti dokumen realisasi kerjasama dengan Mitra diunggah melalui BIMA.

Mitra menyumbangkan 1 buah alat sentrifuge dan 1 tabung nitrogen cair.

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Dear sir or madam,
I am writing to confirm that the Walter and Eliza Hall Institute in Melbourne, Australia has successfully attracted funding through philanthropic donations to support the research Dr Sri Masyeni and her team at the Faculty of Medicine and Health Sciences, University of Warmadewa, Bali, Indonesia, for the discovery of biomarker of severe dengue fever, research that she is conducting in collaboration with Dr Tedjo Sasmono and my team in Melbourne. The funding covers the cost of a new centrifuge and a liquid nitrogen tank, to support the collection of

blood mononuclear cells from patients infected with dengue virus that are recruited in our study. My laboratory remains strongly connected to this research and I will provide my expertise in all areas of the research project, including study design, cell preparation and storage, data analysis and interpretation, as well as preparation of manuscripts arising from the results of this research for publication in competitive virology journals.

Yours sincerely,

Professor Diana Hansen

Laboratory Head

Infectious Diseases and Immune Defense Division


BIOBASE
SET
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PENELITIAN
dr. Masyeni

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F. **KENDALA PELAKSANAAN PENELITIAN**: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Tidak ada

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G. **RENCANA TAHAPAN SELANJUTNYA:** Tuliskan dan uraikan rencana penelitian di tahun berikutnya berdasarkan indikator luaran yang telah dicapai, rencana realisasi luaran wajib yang dijanjikan dan tambahan (jika ada) di tahun berikutnya serta *roadmap* penelitian keseluruhan. Pada bagian ini diperbolehkan untuk melengkapi penjelasan dari setiap tahapan dalam metoda yang akan direncanakan termasuk jadwal berkaitan dengan strategi untuk mencapai luaran seperti yang telah dijanjikan dalam proposal. Jika diperlukan, penjelasan dapat juga dilengkapi dengan gambar, tabel, diagram, serta pustaka yang relevan. Pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai.

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H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan akhir yang dicantumkan dalam Daftar Pustaka.

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Cytokines differences and their correlation in dengue infection

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Abstract

Background: Dengue infection was one of several reemerging neglected tropical diseases with the highest-burden worldwide. This study aims to determine the differences and correlations of various cytokines in dengue infection.

Methods: This study was conducted in Bali, at the second semester of 2022 and gathered more than the minimum number of patients required. Laboratory examinations were used to find out several biomarkers such as Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels. Serological tests and RT-PCR were used to confirm dengue infection.

Results: The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in these patients were 208.36 ± 186.73 pg/mL, 184 ± 101.51 pg/mL, 130.12 ± 74.12 pg/mL, and 100.45 ± 72.58 pg/mL, respectively. Dengue patients were then grouped into several categories. The first category was based on severity which consists of DF and DHF, and the second category was based on infection status which consists of primary and secondary infection. There were differences in cytokines levels between these groups with p <0.05 and moderate to strong correlations with r values spanning 0.37 to 0.71. There were also significant correlations between these cytokines and symptoms of dengue infection.

Conclusions: PAF, Syndecan-1, hyaluronan, and MMP-9 levels were found to vary and associated with severity or infection status in dengue infection. These cytokines were also tied with dengue infection symptoms.

Keywords: biomarker, dengue, severity, infection status

Introduction

Dengue continues to be one of numerous neglected diseases in tropical countries, with cases increasing nearly 30-fold in the previous decade.[1] Dengue fever is endemic in Southeast Asia, but it has spread to non-endemic countries in Europe and the United States. Dengue virus (DENV) infection puts 3.9 billion people at risk across the world, with Asia having the highest incidence.[2] The worldwide economic impact of dengue infection is unknown. However, it is believed to be roughly $8.9 billion USD each year.[3]

Dengue infection is caused by one of four serotypes of DENV (DENV 1-4), each of which has a unique immunoreactivity.[4] There are no distinguishing clinical characteristics for each serotype because they can all present with symptoms that range from mild dengue fever (DF) to more severe dengue haemorrhagic fever (DHF), which can include dengue shock syndrome (DSS).[5] Primary DENV infection often results in a moderate to asymptomatic disease. However, secondary infection may result in a more severe condition.[6] This condition is well explained by antibody-dependent enhancement (ADE) theory and alteration of pro- and anti-inflammatory mediators known as cytokines storm.[7] Several studies showed that increased dysregulations of cytokines have an important role in the pathogenesis of DHF and DSS.[8] Cytokines that have major implications in the severity of dengue infection are Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels. [9] Pro-inflammatory cytokines group consists of Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels, while the anti-inflammatory cytokine only consists of IL-10. IL-6 has a unique property where it can be both pro- and anti-inflammatory cytokine accordingly.[10]

The study aims to determine the differences and correlations of several cytokine levels Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels with severity and infection status, as well as symptoms of dengue infection.

Materials and methods

Study design

This was a prospective cross-sectional study that comprised confirmed dengue infection patients in Bali. Written informed consent was obtained from all subjects. Patients admitted to the hospital were asked to participate in the study, and recruitment took place from June 2022 to December 2022. Patients above 18 years old who were admitted to the emergency room with a fever > 38^0C accompanied by at least one other symptom like headache, myalgia, retroorbital

pain, or vomiting were enrolled. Patients with known malignancy, autoimmune disease, coinfections or other inflammatory disorders were excluded from this study.

$$n = \left\{ \frac{Z\alpha + Z\beta}{0{,}5 \ln \left[\frac{1+r}{1-r}\right]} \right\}^2 + 3$$

The number of patients required for this study was calculated using the above equation with $Z\alpha$ or standard deviation α (1.64), $Z\beta$ or standard deviation β (1.28), and r as the minimum accepted correlation coefficient (0.4). Based on these calculations, we determined the minimum number of patients was 51.

Patients were then grouped into two categories based on severity and infection status. The first category consists of dengue fever (DF) and dengue haemorrhagic fever (DHF) were based on the severity at the time of admission depending on the presentation of haemoconcentration (haematocrits level more ≥ than 50% or any decrease of haematocrits level ≥ 20% after fluid administration). The second category was based on infection status which consists of primary and secondary infection determined by serology and RT-PCR findings.

Laboratory analysis

Serum samples were tested at admission using a dengue NS1 antigen rapid test, Dengue Duo IgM/IgG Capture Elisa (Standard Diagnostics/SD, Bio line, Korea) and RT-PCR to confirm dengue infection. Dengue viral RNA was extracted from acute serum samples using the QI Amp Viral RNA mini kit (Qiagen, Hilden, Germany), according to the Qiagen instructions. To determine the level of cytokines, samples were tested using enzyme-linked immunosorbent assay using reagents from Elabscience® for Platelet activating factor (PAF), Syndecan-1, hyaluronan, and matrix metalloprotein-9 (MMP-9) levels, according to the manufacturer's instructions. Complete blood counts (CBC) such as haemoglobin, haematocrit, erythrocytes, platelets and leucocytes were also documented on the day of admission. To determine the infection status, the study combined the results of serological and RT-PCR tests. Patients with negative serological and positive RT-PCR tests were considered to have a primary infection, while those with positive serological and positive RT-PCR were considered to have a secondary infection.

Statistical analysis

SPSS Version 25 (SPSS Inc., Chicago, IL) was used to statistically analyse the data that were collected. A probability value of $p \leq 0.05$ was considered statistically significant. Numerical data was compared using the Independent-Samples or Mann-Whitney's test according to its distribution, and Chi-square was used for categorical data. As for correlation, Pearson's test or

Spearman's test was used to find the relationship between this data and their associated correlation coefficient.

Result

Dengue patients characteristics

The study successfully enrolled 64 patients within six months. Most patients were female patients with a median age of 26 years. The median day of fever onset was on day four. There were four different dengue virus serotypes present in the patients, with DENV-3 having the highest prevalence. More than half of dengue patients had symptoms like headaches, retroorbital pain and nausea. For further analysis, patients are then grouped into 2 categories according to severity and infection status. Based on severity, there were no differences between DF and DHF in terms of sex, age, fever onset, and serotypes. DHF patients had longer lengths of stay in the hospital and more severe symptoms than DF. There were more patients in secondary infection than primary infection with most of them being female. Based on infection status, secondary infection had longer lengths of stay and more severe symptoms than primary infection. Detailed information on patients characteristics was shown in table 1.

Table 1. Dengue patients characteristics

	Total (N =64)	DF (N=32)	DHF (N=32)	*p*	1st (N=22)	2nd (N=42)	*p*
Sex (%)				0.8			0.048*
Male	27 (42.2)	13 (48.1)	14 (51.9)		13 (48.1)	14 (51.9)	
Female	37 (57.8)	19 (51.4)	18 (48.6)		9 (24.3)	28 (75.7)	
Age (year)	26 (18-84)[a]	31 (18-84)[a]	25 (18-49)[a]	0.12[b]	30 (18-52)[a]	25 (18-84)[a]	0.63[b]
Fever onset (day)	4 (2-6)[a]	4 (2-5)[a]	4 (2-6)[a]	0.22[b]	4 (3-5)[a]	4 (2-6)[a]	0.11[b]
Length of stay (days)	6 (1-10)[a]	5 (1-8)[a]	7 (3-10)[a]	0.001[b]*	4 (1-6)[a]	7 (3-10)[a]	<0.001[b]*
Serotype (%)				0.66			0.21
DENV-1	6 (9.4)	4 (66.7)	2 (33.3)		4 (66.7)	2 (33.3)	
DENV-2	10 (15.6)	4 (40)	6 (60)		4 (40)	6 (60)	
DENV-3	44 (68.8)	22 (50)	22 (50)		12 (27.3)	32 (72.7)	
DENV-4	1 (1.6)	1 (100)	0 (0)		1 (100)	0 (0)	
NA	3 (4.7)	1 (33.3)	2 (66.7)		1 (33.3)	2 (66.7)	
Symptoms (%)							
Headache	56 (87.5)	26 (46.4)	30 (53.6)	0.13	16 (28.6)	40 (71.4)	0.01*
Retroorbital pain	30 (46.9)	9 (30)	21 (70)	0.004*	6 (20)	24 (80)	0.045*
Nausea	43 (67.2)	14 (32.6)	29 (67.4)	<0.001*	6 (14)	37 (86)	<0.001*
Vomiting	23 (35.9)	3 (13)	20 (87)	<0.001*	0 (0)	23 (100)	<0.001*
Bleeding	16 (25)	0 (0)	16 (100)	<0.001*	0 (0)	16 (100)	0.001*

Note: 1st = Primary Infection, 2nd = Secondary Infection

[a] Presented as the median (minimum-maximum)

[b] The Mann-Whitney test was used to compare the group

* Significant value at $p \leq 0.05$

DHF and secondary infection had higher cytokine level

Cytokine levels in patients with DF and DHF differed markedly. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in DF patients were 126.41 ± 52.69 pg/mL, 129.79 ± 50.06 pg/mL, 173.14 ± 83.37 pg/mL, and 58.62 ± 36.48 pg/mL, respectively. PAF, Syndecan-

1, and hyaluronan levels were higher in DHF patients compared to DF patients, whereas MMP-9 levels were lower. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in DHF patients were 282.62 ± 230.22 pg/mL, 233.69 ± 111.27 pg/mL, 91.15 ± 32.49 pg/mL, and138.37 ± 76.64 pg/mL, respectively.

The study also discovered that secondary infection had notable cytokine differences from primary infection. The mean levels of PAF, Syndecan-1, hyaluronan, and MMP-9 in primary infection were 126.08 ± 62.43 pg/mL, 119.60 ± 42.09 pg/mL, 172.43 ± 68.17 pg/mL, and 50.74 ± 19.27 pg/mL, respectively. Meanwhile, the cytokines levels in secondary infection were 245.58 ± 211.56 pg/mL, 213.57 ± 213.57 pg/mL, 110.99 ± 69.24 pg/mL, and 122.94 ± 76.70 pg/mL, respectively. This result shown in Table 2 confirmed secondary infection had higher PAF, Syndecan-1, hyaluronan, levels, while the level of MMP-9 was lower.

Table 2. Complete blood count and cytokines result of the dengue patients

	Total (N=64)	DF (N=32)	DHF (N=32)	*p*	1st (N=22)	2nd (N=42)	*p*
CBC at admission							
Haemoglobin (g/dL)	14.27 ± 1.57	13.58 ± 1.29	14.89 ± 1.55	0.001*	13.58 ± 1.17	14.58 ± 1.64	0.01*
Haematocrit (%)	43.11 ± 5.26	40.58 ± 4.32	45.41 ± 5.03	<0.001*	40.25 ± 4.12	44.40 ± 5.25	0.002*
Leucocyte ($x10^3$/mL)	3.05 ± 1.14	2.90 ± 1.04	3.18 ± 1.23	0.61	3.30 ± 1.10	2.93 ± 1.15	0.09
Thrombocyte ($x10^3$/mL)	93.66 ± 44.61	99.07 ± 42.42	88.75 ± 46.62	0.16	119.11 ± 41.98	82.14 ± 41.26	<0.001*
RBC ($x10^6$/mL)	4.96 ± 0.65	4.72 ± 0.59	5.17 ± 0.64	0.008*	4.83 ± 0.61	5.02 ± 0.67	0.45
PAF (pg/mL)	208.36 ± 186.73	126.41 ± 52.69	282.62 ± 230.22	0.001*	126.08 ± 62.43	245.58 ± 211.56	0.003*
Synde-1 (pg/mL)	184 ± 101.51	129.79 ± 50.06	233.69 ± 111.27	<0.001*	119.60 ± 42.09	213.57 ± 213.57	<0.001*
Hyaluronan (pg/mL)	130.12 ± 74.12	173.14 ± 83.37	91.15 ± 32.49	<0.001*	172.43 ± 68.17	110.99 ± 69.24	<0.001*
MMP-9 (pg/mL)	100.45 ± 72.58	58.62 ± 36.48	138.37 ± 76.64	<0.001*	50.74 ± 19.27	122.94 ± 76.70	<0.001*

Note: 1st = Primary Infection, 2nd = Secondary Infection

* Significant values at $p \leq 0.05$

Cytokines correlations with symptoms, severity, and infection status

The level of cytokines was found to be significantly related to many symptoms such as nausea, vomiting, and bleeding. The correlation coefficients varied from weak to strong. These symptoms were correlated with higher PAF, Syndecan-1, hyaluronan, but not with MMP-9 levels.

Cytokines also had a notable association with the severity of infection. Higher levels of PAF, Syndecan-1, and hyaluronan, correlated with more severe disease. MMP-9 levels were higher in mild conditions. The correlation coefficient in this category was varied from moderate to strong. There was a marked relationship between PAF, Syndecan-1, hyaluronan, and MMP-9 with infection status. The correlation coefficient in this category varied from weak to moderate. These findings were shown in Table 3.

Table 3. Correlation between cytokines and several variables in dengue infection

	Headache		Retroorbital pain		Nausea		Vomiting		Bleeding		Severity of Infection		Infection Status	
	p	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r
PAF	0.12	0.19	0.46	0.09	0.01*	0.31	0.01*	0.32	<0.001*	0.45	<0.001*	0.43	0.003*	0.37
Synde-1	0.08	0.22	0.13	0.19	<0.001*	0.47	0.001*	0.41	0.003*	0.36	<0.001*	0.60	<0.001*	0.55
Hyalu	0.95	-0.01	0.03*	-0.27	0.02*	-0.29	<0.001*	-0.43	0.011*	-0.32	<0.001*	-0.58	<0.001*	-0.47
MMP-9	0.26	0.14	0.01*	0,33	<0.001*	0.47	<0.001*	0.62	<0.001*	0.64	<0.001*	0.71	<0.001*	0.59

Note: * Significant values at $p \leq 0.05$

Discussion

This cross-sectional study, which included 64 patients from multiple hospitals in Bali, added further evidence of the important role cytokines played in dengue infection. The main cytokines that have been examined in this study were PAF, Syndecan-1, hyaluronan, and MMP-9. These cytokine levels were evaluated based on the severity and status of dengue infection. The study also identified a connection between cytokine levels and dengue symptoms.

Clinical classifications of dengue infection severity comprise DF, DHF, and DSS.[1] The severity in this study was classified into DF and DHF, and the study found that there were significant differences in cytokine levels between this group. PAF, Syndecan-1, and hyaluronan levels were higher in DHF patients compared to DF patients, although MMP-9 level was lower. This corresponds to higher pro-inflammatory along with lower anti-inflammatory cytokines activity in DHF patients. PAF, Syndecan-1, hyaluronan, and MMP-9 were endothelial activation products formed by CD4+ T helper 2 cells.[11,12] Although previous studies indicated that PAF, Syndecan-1, hyaluronan, and MMP-9 were associated with plasma leakage in dengue infection, they frequently fell short of explaining the severity of the disease.[13,14] These findings were in accordance with our results and the study was able to establish a significant correlation between each cytokine and severity. In contrast to other research where severe dengue was associated with increased MMP-9 levels, our DHF patients had lower levels of MMP-9. As the study was

unable to identify the source of MMP-9 in these patients, several sources of MMP-9, including T helper cells, monocytes, as well as a wide variety of immune effector cell types, including B cells, cytotoxic T cells, and granulocytes, such as neutrophils, could explain this finding.[15] PAF, Syndecan-1, and hyaluronan, and MMP-9 had moderate correlations with severity, whereas IL-17 had strong correlations. This indicates that these cytokines significantly contribute to the severity of the dengue infection.

Infection status provided the basis for the second classification in this study. The study classified dengue infections as either primary or secondary. Cytokine levels differ markedly between primary and secondary infections. The secondary infection had higher PAF, Syndecan-1, hyaluronan, and MMP-9. These findings were linked to two well-known concepts in dengue infection: the antibody-dependent enhancement (ADE) phenomena and the cytokine storm.[6,7] ADE increases dengue virus protein synthesis, viral RNA generation and release. Although PAF and gamma interferon were not generated in the presence of dengue virus, Syndecan-1 and hyaluronan were produced at elevated titers, indicating a signalling component of ADE.[16] Cytokine storm was a condition in which cytokine levels shifted, causing endothelial cells to break down and subsequent plasma leakage.[7] PAF showed a weak correlation, while Syndecan-1, hyaluronan, and MMP-9 had moderate correlations with infection status. This result also supported the previously mentioned concepts.

Dengue infection has several unspecific symptoms like headache, retroorbital pain, nausea, vomiting, and bleeding.[1] The study found that some of these symptoms were associated with cytokine levels. The most notable finding was the strong correlation between MMP-9 with bleeding. MMP-9 functions as a protective mediator in barrier immunity through a variety of methods.[17] MMP-9 supports epithelial integrity by modulating tight junction proteins, which link and stabilize epithelial cell connections to maintain the barrier and keep out gut luminal contents and commensal microbes.[18] The findings vary from those of a recent study, which found that IL-8 levels were considerably greater in DF with bleeding than in DF without bleeding during the acute phase of infection. IL-8 features contribute to platelet activation and endothelial permeability, resulting in thrombocytopenia and worsening bleeding in dengue infection.[20] Other notable findings in this study were weak to strong correlation between PAF, Syndecan-1, hyaluronan, and MMP-9 with retroorbital pain, nausea and vomiting.

This study has several limitations. This study had restricted resources and funding, which limited the cytokines that were planned to be included, such as IL-8, interferon, and other cytokines. Following that, the study was unable to determine the serotype of three dengue-infected individuals. We suspected that it was a simple human error. Also, the study was only

able to include symptomatic individuals who were seeking treatment at a hospital. Finally, because this study was conducted during the COVID-19 pandemic, numerous patients withdrew for various reasons such as coinfections and personal concerns. Despite these constraints, the study yielded substantial results.

Conclusions

The study found considerable differences and correlations between cytokine levels in dengue infection based on severity and infection status. PAF, Syndecan-1, and MMP-9 levels were considerably greater in DHF and secondary infection, but hyaluronan levels were substantially lower. The study additionally found a link between cytokines and dengue symptoms, with particular emphasis on bleeding.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Source

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Ethical Approval Statement

The study protocol was approved by the Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Bali (document number 46/Unwar/FKIK/EC-KEPK/VI/2022).

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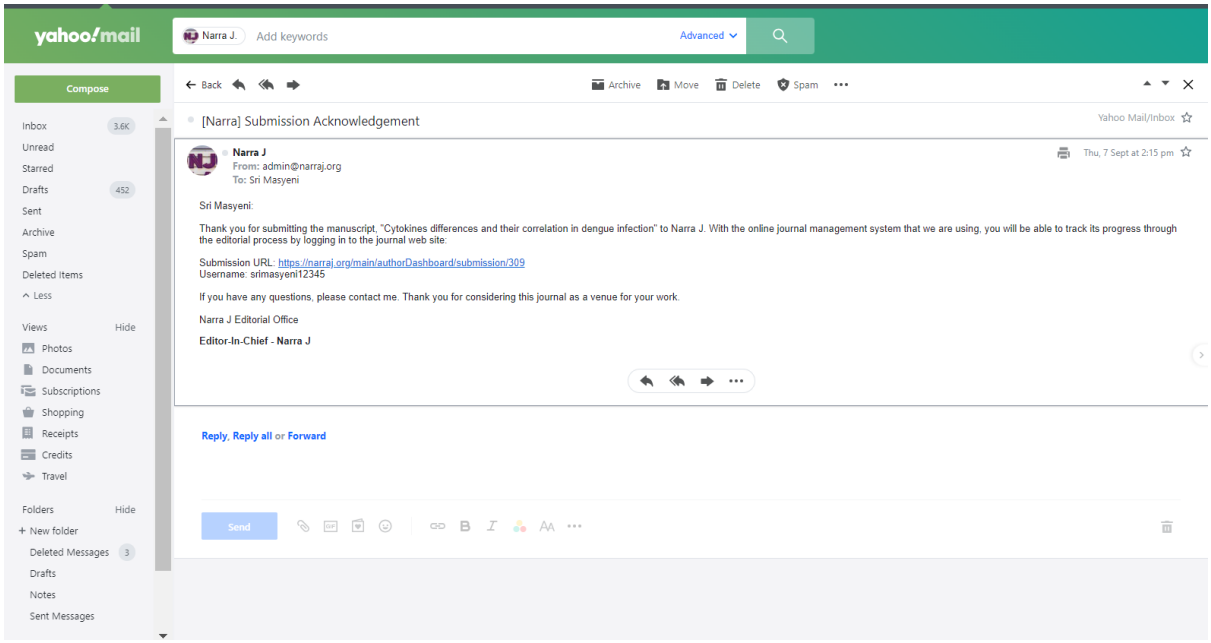
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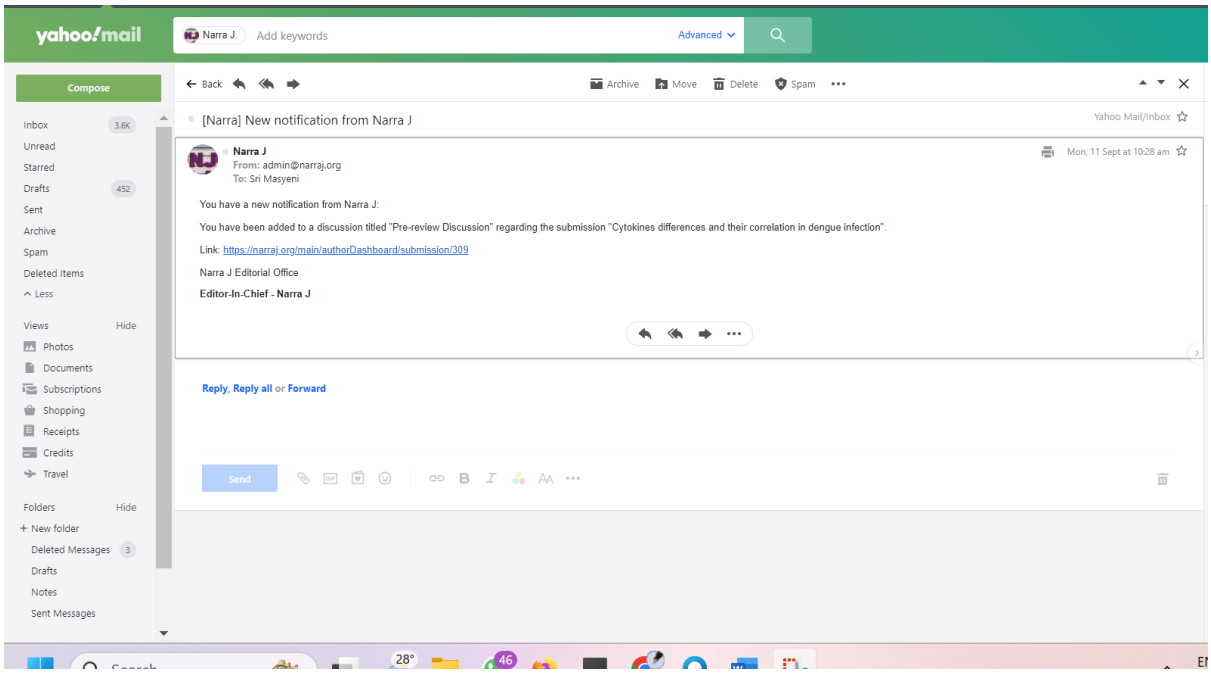
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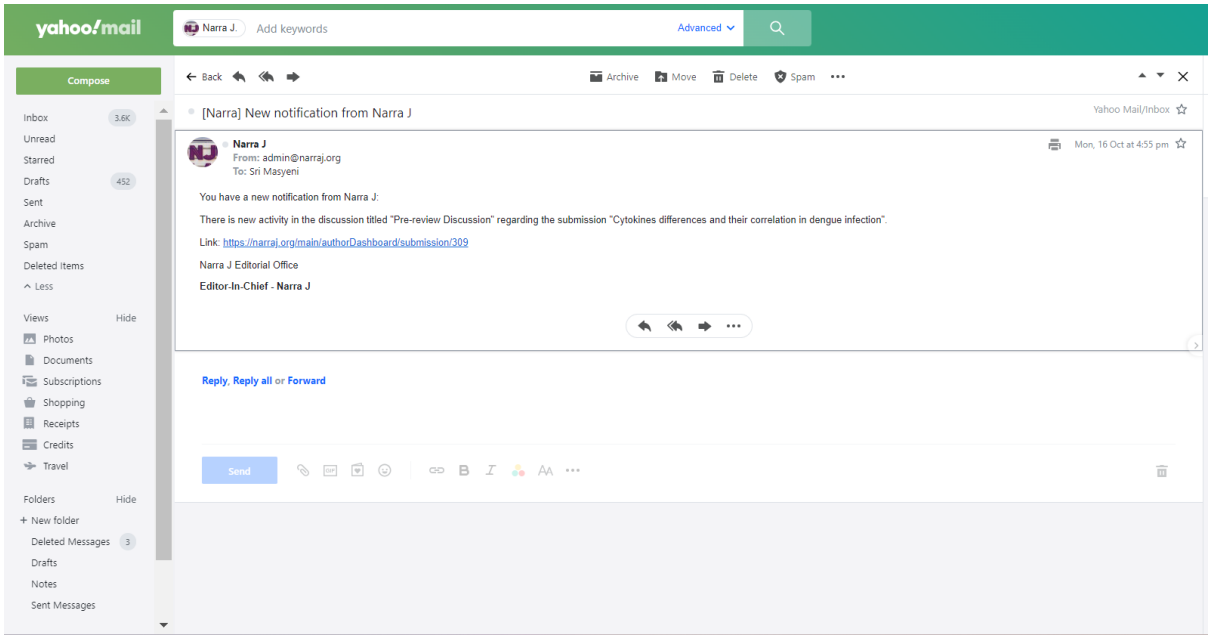
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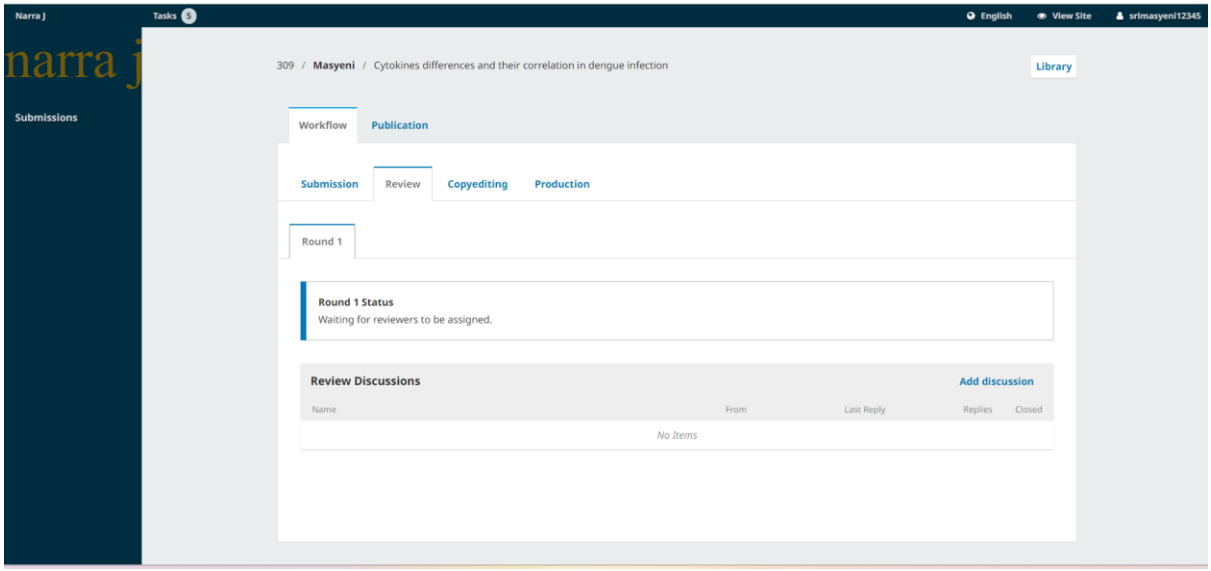
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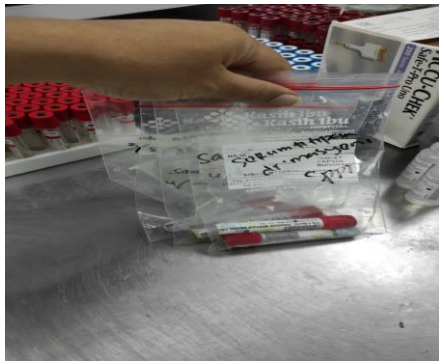
LAPORAN KEMAJUAN HIBAH DIKTI 2023

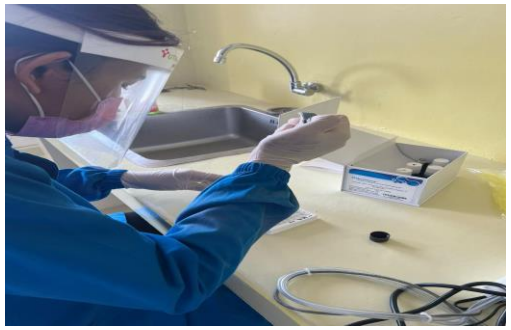
DAP SRI MASYENI
DIANA HANSEN
TEDJO SASMONO

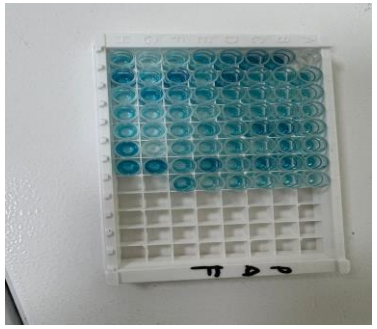
PENDAHULUAN

- JUDUL: IDENTIFICATION OF MOLECULAR AND CELLULARPATHWAYS PREDICTING SUSCEPTIBILITY OR RESISTANCE TO SEVERE DENGUE FEVER (Tahun ke-2 dari 2 tahun)
- TIM PENELITI: DAP SRI MASYENI, DIANA HANSEN (MONASH UNIVERSITY), TEDJO SASMONO (BRIN)
- MANFAAT PENELITIAN: MENEMUKAN BIOMARKER DENGUE BERAT
- RUMUSAN MASALAH: BAGAIMANAKAH PERBEDAAN SITOKIN PADA INFEKSI DENGUE

PROSES





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Human Syndecan 1,SDC1 BT-LAB Kit


BT LAB
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Human Matrix Metalloproteinase 9/Gelatinase B,MMP-9 BT-LAB Kit


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HASIL

Table 1. Dengue patients characteristics

	Total (N =64)	DF (N=32)	DHF (N=32)	*p*	1st (N=22)	2nd (N=42)	*p*
Sex (%)				0.8			0.048*
Male	27 (42.2)	13 (48.1)	14 (51.9)		13 (48.1)	14 (51.9)	
Female	37 (57.8)	19 (51.4)	18 (48.6)		9 (24.3)	28 (75.7)	
Age (year)	26 (18-84)[a]	31 (18-84)[a]	25 (18-49)[a]	0.12[b]	30 (18-52)[a]	25 (18-84)[a]	0.63[b]
Fever onset (day)	4 (2-6)[a]	4 (2-5)[a]	4 (2-6)[a]	0.22[b]	4 (3-5)[a]	4 (2-6)[a]	0.11[b]
Length of stay (days)	6 (1-10)[a]	5 (1-8)[a]	7 (3-10)[a]	0.001[b]*	4 (1-6)[a]	7 (3-10)[a]	<0.001[b]*
Serotype (%)				0.66			0.21
DENV-1	6 (9.4)	4 (66.7)	2 (33.3)		4 (66.7)	2 (33.3)	
DENV-2	10 (15.6)	4 (40)	6 (60)		4 (40)	6 (60)	
DENV-3	44 (68.8)	22 (50)	22 (50)		12 (27.3)	32 (72.7)	
DENV-4	1 (1.6)	1 (100)	0 (0)		1 (100)	0 (0)	
NA	3 (4.7)	1 (33.3)	2 (66.7)		1 (33.3)	2 (66.7)	
Symptoms (%)							
Headache	56 (87.5)	26 (46.4)	30 (53.6)	0.13	16 (28.6)	40 (71.4)	0.01*
Retroorbital pain	30 (46.9)	9 (30)	21 (70)	0.004*	6 (20)	24 (80)	0.045*
Nausea	43 (67.2)	14 (32.6)	29 (67.4)	<0.001*	6 (14)	37 (86)	<0.001*
Vomiting	23 (35.9)	3 (13)	20 (87)	<0.001*	0 (0)	23 (100)	<0.001*
Bleeding	16 (25)	0 (0)	16 (100)	<0.001*	0 (0)	16 (100)	0.001*

Note: 1st = Primary Infection, 2nd = Secondary Infection
[a] Presented as the median (minimum-maximum)
[b] The Mann-Whitney test was used to compare the group
* Significant value at $p \leq 0.05$

Table 2 Laboratory finding

	Total (N=64)	DF (N=32)	DHF (N=32)	*p*	1st (N=22)	2nd (N=42)	*p*
CBC at admission							
Haemoglobin (g/dL)	14.27 ± 1.57	13.58 ± 1.29	14.89 ± 1.55	0.001*	13.58 ± 1.17	14.58 ± 1.64	0.01*
Haematocrit (%)	43.11 ± 5.26	40.58 ± 4.32	45.41 ± 5.03	<0.001*	40.25 ± 4.12	44.40 ± 5.25	0.002*
Leucocyte (x10^3/mL)	3.05 ± 1.14	2.90 ± 1.04	3.18 ± 1.23	0.61	3.30 ± 1.10	2.93 ± 1.15	0.09
Thrombocyte	93.66 ± 44.61	99.07 ± 42.42	88.75 ± 46.62	0.16	119.11 ±	82.14 ± 41.26	<0.001*
(x10^3/mL)	4.96 ± 0.65	4.72 ± 0.59	5.17 ± 0.64	0.008*	41.98	5.02 ± 0.67	0.45
RBC (x10^6/mL)					4.83 ± 0.61		
PAF (pg/mL)	208.36 ± 186.73	126.41 ± 52.69	282.62 ± 230.22	0.001*	126.08 ± 62.43	245.58 ± 211.56	0.003*
Synde (pg/mL)	184 ± 101.51	129.79 ± 50.06	233.69 ± 111.27	<0.001*	119.60 ± 42.09	213.57 ± 213.57	<0.001*
Hyalu (pg/mL)	130.12 ± 74.12	173.14 ± 83.37	91.15 ± 32.49	<0.001*	172.43 ± 68.17	110.99 ± 69.24	<0.001*
MMP-9 (pg/mL)	100.45 ± 72.58	58.62 ± 36.48	138.37 ± 76.64	<0.001*	50.74 ± 19.27	122.94 ± 76.70	<0.001*

Table 3 Correlation of biomarker in dengue infection

	Headache		Retroorbital pain		Nausea		Vomiting		Bleeding		Severity of Infection		Infection Status	
	p	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r
PAF	0.12	0.19	0.46	0.09	0.01*	0.31	0.01*	0.32	<0.001*	0.45	<0.001*	0.43	0.003*	0.37
Synde	0.08	0.22	0.13	0.19	<0.001*	0.47	0.001*	0.41	0.003*	0.36	<0.001*	0.60	<0.001*	0.55
Hyalu	0.95	-	0.03*	-	0.02*	-	<0.001*	-	0.011*	-	<0.001*	-	<0.001*	-
		0.01		0.27		0.29		0.43		0.32		0.58		0.47
MMP-	0.26	0.14	0.01*	0,33	<0.001*	0.47	<0.001*	0.62	<0.001*	0.64	<0.001*	0.71	<0.001*	0.59

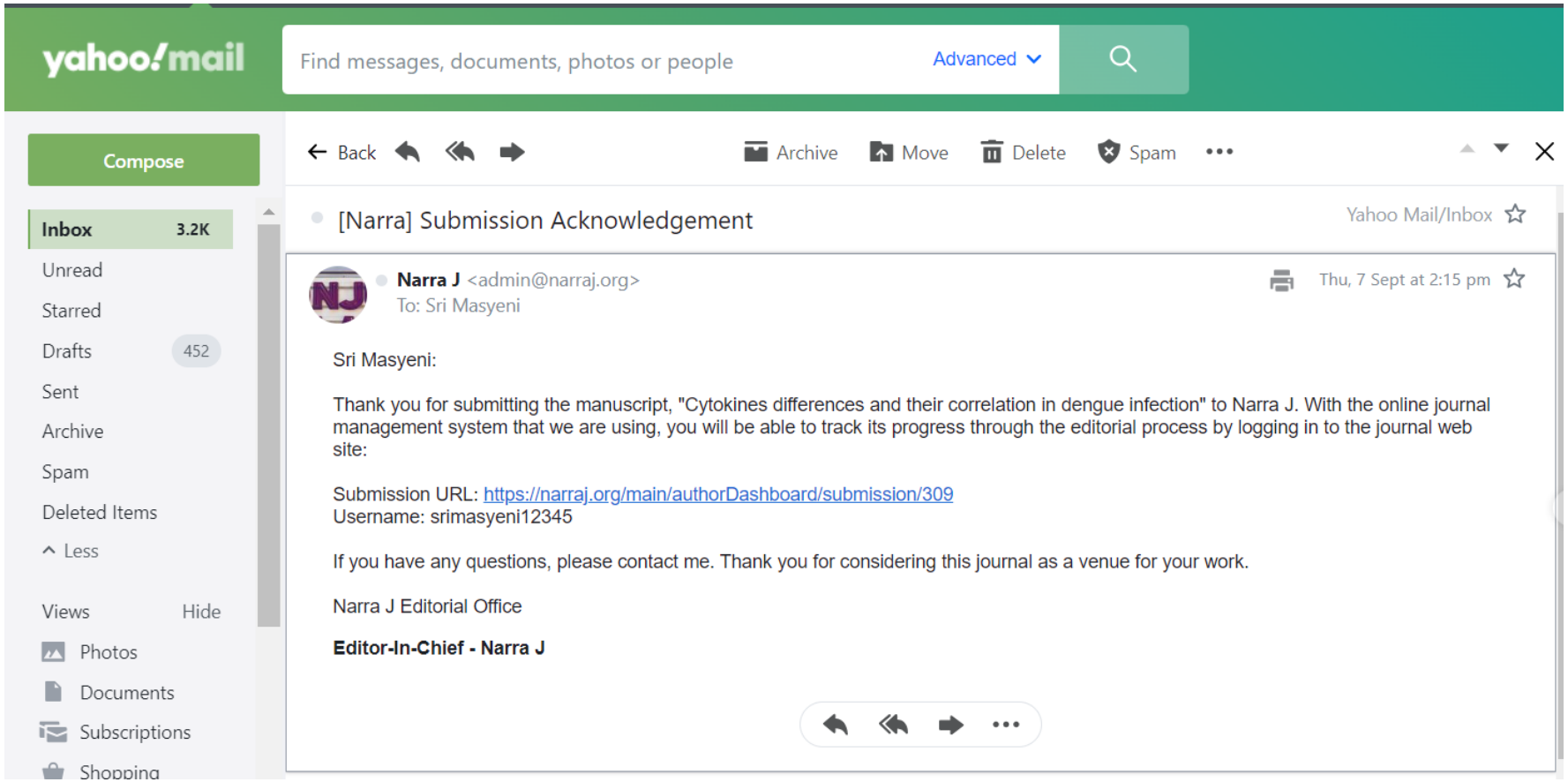
PENGISIAN BUKU HARIAN

1	07 September 2023	submit manuskript	97	1	
2	02 September 2023	Menulis manuskrip	95	0	
3	20 July 2023	Uji IgG IgM Dengue	80	1	
4	04 July 2023	Running PCR serotyping	75	1	
5	15 June 2023	Uji ELISA	65	1	
6	05 June 2023	Uji NS1	50	1	
7	15 May 2023	Sampling lanjutan	40	1	
8	06 May 2023	Rapat persiapan penelitian lanjutan	20	1	
9	02 May 2023	memesan bahan/BHP penelitian	10	1	

SPTB 70%

No	Uraian	Jumlah
01	**Bahan** plester, plastik klip, blue tip, yellow tip, paraffin, sarung tangan nitril, masker sensi, sampel box, kapas alkohol, masker duckbill, BD vacutainer, ice box, sampel cup effendoff, masker sensi, BD vacuum tube red, cryovial, aguabidest,BD vacutainer flashback, antis, tissue, tabung gel/clot activator, dry ice, NS1 antigen, IgM, IgG dengue, PCR mix invitrogen superscript III,, primer DENV1-4, pipet 5 ml, pipet 10 ml, Reagen Syndecan-1, Hyaluronan, PAF, MMP-9	194,931,000
02	**Pengumpulan Data**	0
03	**Analisis Data(Termasuk Sewa Peralatan** Biaya sewa alat dan ruangan	16,000,000
04	**Pelaporan, Luaran Wajib dan Luaran Tambahan**	0
05	**Lain-lain**	0
	Jumlah	210,931,000

LUARAN: BUKTI SUBMIT JURNAL



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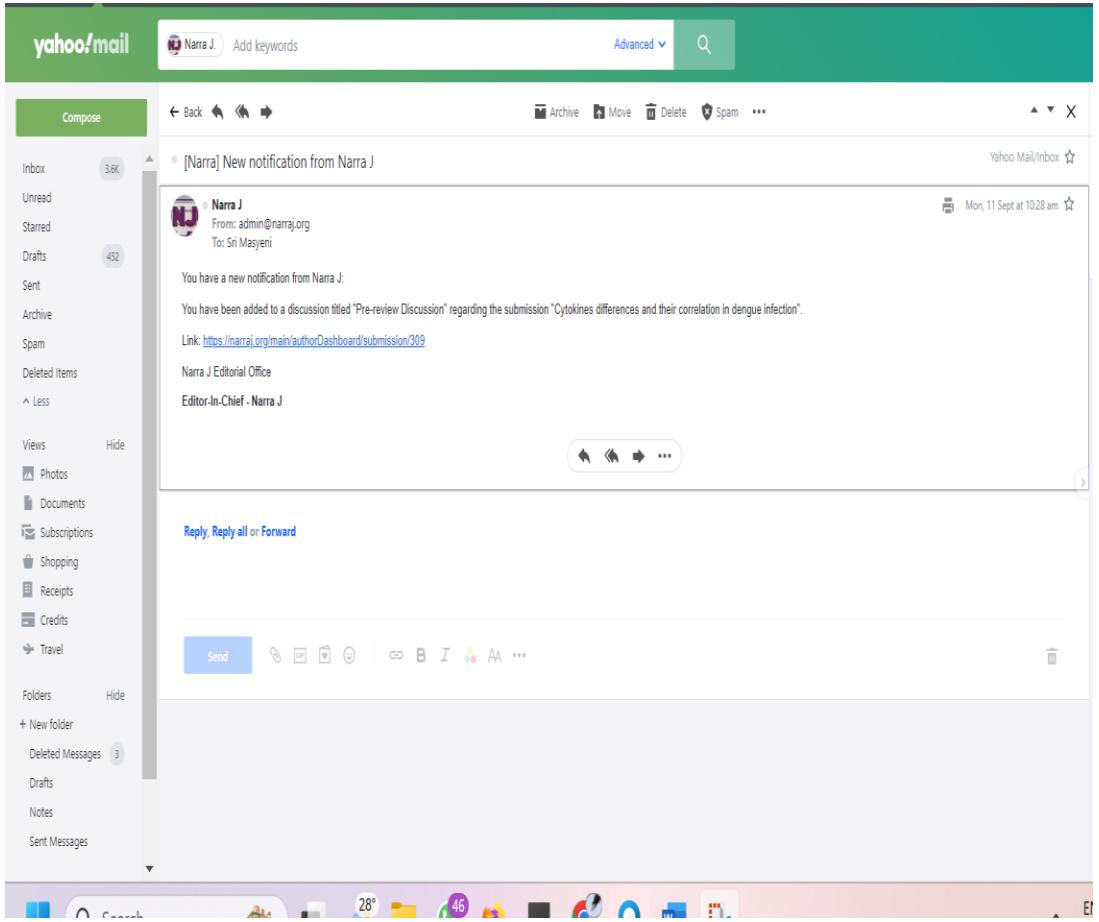

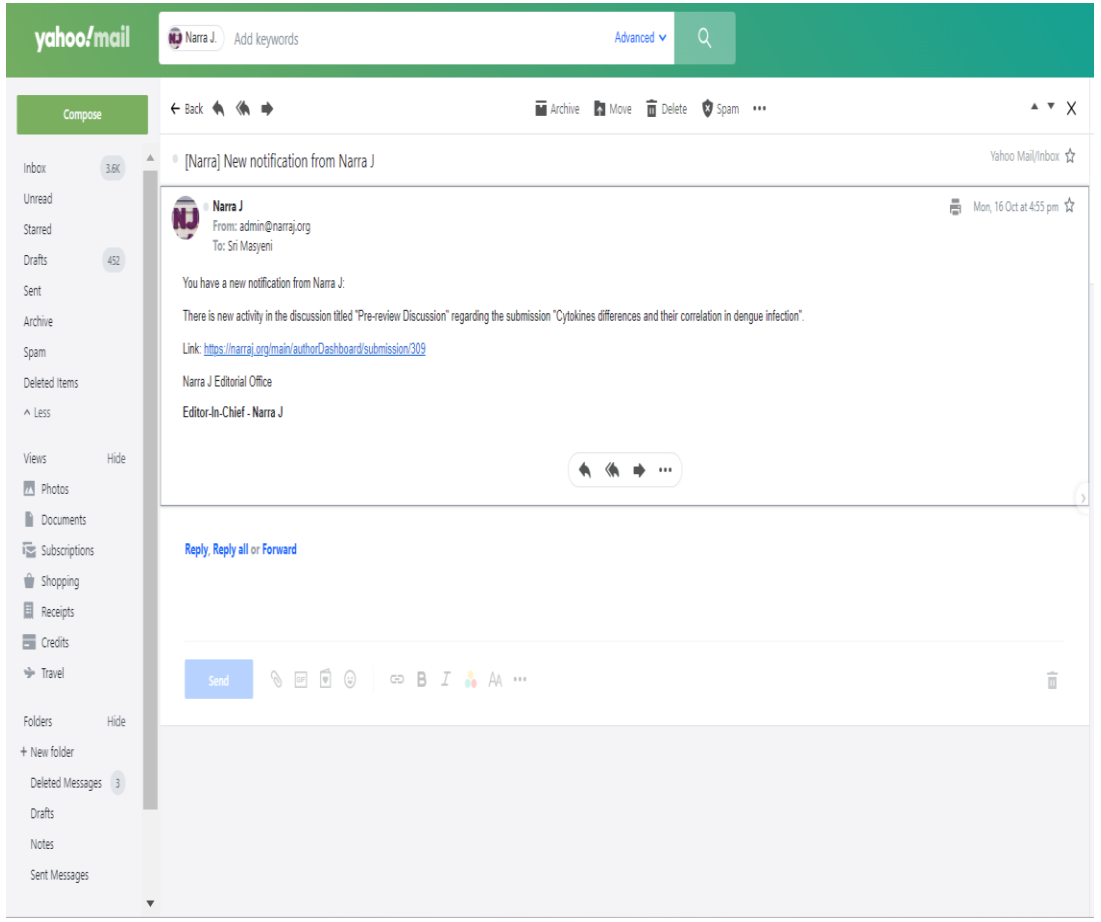

MATUR SUKSME

TERIMAKASIH

SURAT PERNYATAAN TANGGUNG JAWAB BELANJA

Yang bertanda tangan di bawah ini :

Nama : Dr DEWA AYU PUTRI SRI MASYENI Sp.P.D

Alamat : Jln Jayagiri X no 4 Denpasar Bali

berdasarkan Surat Keputusan Nomor 033/E5/PG.02.00.2022 dan Perjanjian / Kontrak Nomor 078/E5/PG.02.00.PL; 1757/LL8/AL.04/2023 mendapatkan Anggaran Penelitian Identification of molecular and cellular pathways predicting susceptibility or resistance to severe dengue fever Sebesar 301,330,000

Dengan ini menyatakan bahwa :

1. Biaya kegiatan Penelitian di bawah ini meliputi :

No	Uraian	Jumlah
01	**Bahan** plester, plastik, klip, blue tip, yellow tip, paraffin, sarung tangan nitril, masker sensi, sampel box, kapas alkohol, masker duckbill, BD vacutainer, ice box, sample cup effendoff, masker sensi, BD vacuum tube red, cryovial, aquabidest, BD vacutainer flashback, antis, tissue, tabung gel/clot activator, dry ice, NS1 antigen, IgM IgG dengue kit, PCR mix invitrogen, superscript III, primer DENV1-4, pipet 5 ml, pipet 10 ml, reagen Syndecan-1, Hyaluronan, PAF, MMP-9, FBS, DNeasy mini kit	214,931,000
02	**Pengumpulan Data** Honor sampling perawat, honor prosesing sample, honor pembantu lapangan, honor pembantu peneliti	28,000,000
03	**Analisis Data(Termasuk Sewa Peralatan** Analisa data, sewa ruangan, sewa peralatan	47,000,000
04	**Pelaporan, Luaran Wajib dan Luaran Tambahan** Proofread, biaya publikasi	11,399,000
05	**Lain-lain**	0
	Jumlah	301,330,000

2. Jumlah uang tersebut pada angka 1, benar-benar dikeluarkan untuk pelaksanaan kegiatan Penelitian dimaksud.

Demikian surat pernyataan ini dibuat dengan sebenarnya.

: 15-12-2023

Ketua,

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