MEDICAL JOURNAL IMJ HEALTH

VOLUME-11, ISSUE-7, JULY 2025

DOWNLOAD NOW

www.imjhealth.org

Preface

We would like to present, with great pleasure, the inaugural volume-11, Issue-7, July 2025, of a scholarly journal, *International Multispeciality Journal of Health*. This journal is part of the AD Publications series *in the field of Medical, Health and Pharmaceutical Research Development*, and is devoted to the gamut of Medical, Health and Pharmaceutical issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Medical, Health and Pharmaceutical as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical statistics investigations. Its mission is to become a voice of the Medical, Health and Pharmaceutical community, addressing researchers and practitioners in below areas

Clinical Specialty and Super-specialty Medical Science:

It includes articles related to General Medicine, General Surgery, Gynecology & Obstetrics, Pediatrics, Anesthesia, Ophthalmology, Orthopedics, Otorhinolaryngology (ENT), Physical Medicine & Rehabilitation, Dermatology & Venereology, Psychiatry, Radio Diagnosis, Cardiology Medicine, Cardiothoracic Surgery, Neurology Medicine, Neurosurgery, Pediatric Surgery, Plastic Surgery, Gastroentrology, Gastrointestinal Surgery, Pulmonary Medicine, Immunology & Immunogenetics, Transfusion Medicine (Blood Bank), Hematology, Biomedical Engineering, Biophysics, Biostatistics, Biotechnology, Health Administration, Health Planning and Management, Hospital Management, Nephrology, Urology, Endocrinology, Reproductive Biology, Radiotherapy, Oncology and Geriatric Medicine.

Para-clinical Medical Science:

It includes articles related to Pathology, Microbiology, Forensic Medicine and Toxicology, Community Medicine and Pharmacology.

Basic Medical Science:

It includes articles related to Anatomy, Physiology and Biochemistry.

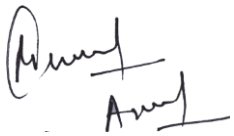
Spiritual Health Science:

It includes articles related to Yoga, Meditation, Pranayam and Chakra-healing.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within

that community who supported the idea of creating a new Research with *IMJ Health*. We are certain that this issue will be followed by many others, reporting new developments in the Medical, Health and Pharmaceutical Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IMJ Health* readers and will stimulate further research into the vibrant area of Medical, Health and Pharmaceutical Research.

Dr. Kusum Gaur
(Chief Editor)



Mr. Mukesh Arora
(Managing Editor)

Board Members

Dr. Kusum Gaur (Editor-in-chief)

Dr. Kusum Gaur working as professor Community Medicine and member of Research Review Board of Sawai Man Singh Medical College, Jaipur (Raj) India.

She has awarded with WHO Fellowship for IEC at Bangkok. She has done management course from NIHFW. She has published and present many research paper in India as well as abroad in the field of community medicine and medical education. She has developed Socio-economic Status Scale (Gaur's SES) and Spiritual Health Assessment Scale (SHAS). She is 1st author of a book entitled " Community Medicine: Practical Guide and Logbook.

Research Area: Community Medicine, Biostatics, Epidemiology, Health and Hospital Management and Spiritual Health.

Mukesh Arora (Managing Editor)

BE (Electronics & Communication), M.Tech (Digital Communication), currently serving as Assistant Professor in the Department of ECE.

Dr. AMER A. TAQA

Dr. AMER A. TAQA is Professor and Head in Dental Basic Science Mosul University, Mosul, IRAQ. He has been registrar of department of Dental Basic Science Mosul University, Mosul, IRAQ. He has published about 100 of research papers and out of that 50 were of international level. He has awarded many times for scientific researches by Government. He has been member of many examination committees and also is a Member in Iraqi Scientific Staff. He has been working as Editor - reviewer in many journals.

Research Area: Dental Science.

Dr. I.D. Gupta

Dr. I. D. Gupta is Professor Psychiatry and working as additional Principal and Dean of student welfare in SMS Medical College, Jaipur.

He is recipient of Prof. Shiv Gautam oration award by Indian Psychiatric Society. He has done training in YMRS at Monte Carlo and BPRS at Singapore. He has been President Indian Psychiatric Society, Rajasthan State Branch. He is author of "Psycho Somatic Disorder" chapter in 1st edition post graduate text book of Psychiatry by Vyas and Ahuja. He has also worked with National Mental Health Programme and has a lot of publication.

Research Area: Community Mental Health, Psycho somatic and liaison Psychiatry.

Dr. Lokendra Sharma

Dr. Lokendra Sharma is Associate Professor Pharmacology and working as Nodal officer of SMS Medical College, Jaipur.

He is recipient of WHO Fellowship award on Poison Patient Management at Vietnam. He is resource faculty for Experimental Toxicology and Basic Course for Medical Education. He is presented and published a lot of research articles at national and international level.

Research Area: PHARMACOLOGY

Dr. Anuradha Yadav

Dr. Anuradha Yadav is working as Professor Physiology, SMS Medical College, Jaipur (Rajsthan) India. She is a popular medical teacher and research scholar who had many publications in indexed journals. **Research Area:** CVS & CNS physiology, Medical Education and Spiritual Health.

Dr. Rajeev Yadav

Dr. Rajeev Yadav is working as Associate Professor Community Medicine, SMS Medical College, Jaipur (Rajsthan) India. He is member of Research Review Board of the Institute.

He has authored a book entitled "Community Medicine: Practcal Guide and Logbook".

Research Area: His area of Interest are Epidemiology, Biostatistics and Spiritual Health.

Prof. Dillip Kumar Parida

Professor and Head in the Department of Oncology, AIIMS, Bhubaneswar.

He has done the Professional Training in Japan (Oska University, NIBI, AHCC Research Association, Hyogo Ion Beam Center), ESTRO Fellowship in Denmark and India(AIIMS Delhi, BARC Mumbai, SCB Medical College-Cuttak, MKCG Medical College-Berhampur).

Research Area: Brachytherapy, Total Skin Electron Irradiation, Palliative Radiotherapy, Stereotactic & Conformal radiotherapy, Radiation Cell Biology, Cancer Genetics.

Dr. Praveen Mathur

Dr. Praveen Mathur is working as Professor- Pediatric Surgery and is recipient of Commonwealth Fellowship in Pediatric Laparoscopy from Uk and fellowship award in minimal access Surgery (FMAS). He has done Clinical observer ship in the Department of Pediatric Surgery, Johns Hopkins University, Baltimore, USA. (2008). He has presented and published a number of research articles at national and international level. He is reviewer of prestigious Journal of Pediatric Surgery (JPS) and World Journal of Gastroenterology, Journal of neonatal Surgery (JNS).

Research Area: Pediatric Surgery & Laparoscopy.

Dr. Lokendra Sharma

Dr. Lokendra Sharma is Associate Professor Pharmacology and working as Nodal officer of SMS Medical College, Jaipur.

He is recipient of WHO Fellowship award on Poison Patient Management at Vietnam. He is resource faculty for Experimental Toxicology and Basic Course for Medical Education. He is presented and published a lot of research articles at national and international level.

Research Area: PHARMACOLOGY.

Dr Rajeev Sharma (MS; FMAS; FIMSA;FCLS)

He is working as Professor, Department of Surgery, Government Medical College, Chandigarh, India. He has done FMAS, FIMSA and FCLS along with MS (Gen Surgery).

He has about 50 international and national publications to his credit. He has held various positions in the Association of Minimal Access Surgeons of India (AMASI) from time to time. He has also acted as instructor of various AMASI skill courses held at different places in India. He has established Surgical Technique learning centre at GMCH Chandigarh for imparting training to the budding surgeons in the field of minimal access surgery. He is also the reviewer in the subject in various journals.

Research Area: Minimal Access Surgery.

Dr Anshu Sharma (MS ANATOMY)

She is Presently working as assistant professor in the department of Anatomy, GMCH, Chandigarh. She has many publications in various national and international journals. She is executive member of Anatomical Society of India (ASI) and North Chapter of ASI. She is also a member of editorial board of Journal of Medical College Chandigarh.

Research Area: Congenital Malformation, Developmental Anatomy.

Dr. Rajeev Yadav

Dr. Rajeev Yadav is working as Associate Professor Community Medicine, SMS Medical College, Jaipur (Rajsthan) India. He is member of Research Review Board of the Institute.

He has authored a book entitled "Community Medicine: Practical Guide and Logbook".

Research Area: His areas of Interest are Epidemiology, Biostatistics and Spiritual Health.

Dr. Dilip Ramlakhyani

Dr. Dilip Ramlakhyani working as Associate professor Pathology and member of IT Committee of Sawai Man Singh Medical College, Jaipur (Raj) India. He has published many articles in indexed journals.

Dr. Virendra Singh

Dr. Virendra Singh worked as Supernatant and head of department of Pulmonary Medicine, SMS Medical College, Jaipur (Rajsthan) India.

He has gone abroad for many training courses and to present research papers. He had been chairman of Research Review Board of SMS Medical College, Jaipur. He is a great research scholar and had published book related to his faculty and had many publications in indexed journals.

Dr. Mahesh Sharma

Dr. Mahesh Sharma is a Principle specialist General Surgery in Rajasthan State Government, India. He has been PMO of district hospitals for more than 15 years. He has gone abroad as observer of many of training related to his speciality. He has published and present many research paper in India as well as abroad.
He has developed Spiritual Health Assessment Scale (SHAS) with Dr. Kusum Gaur.

Research Area: General Surgery, Health and Hospital management and Spiritual Health.

Dr. Ravindra Manohar

Professor Community Medicine, working as head of department of PSM,SMS Medical College, Jaipur (Rajsthan) India.

Previously he has worked in BP Kiorala Institute of Medical Sciences, Nepal. He has visited CDC Atlántica for a Statistical workshop. He has been convener of MBBS and PG exams. He is a research scholar and had many publications in indexed journals.

Dr. Praveen Mathur

Dr. Praveen Mathur is working as Professor- Pediatric Surgery and is recipient of Commonwealth Fellowship in Pediatric Laparoscopy from Uk and fellowship award in minimal access Surgery (FMAS). He has done Clinical observer ship in the Department of Pediatric Surgery, Johns Hopkins University, Baltimore, USA. (2008). He has presented and published a number of research articles at national and international level. He is reviewer of prestigious Journal of Pediatric Surgery (JPS) and World Journal of Gastroenterology, Journal of neonatal Surgery (JNS).

Research Area: Pediatric Surgery & Laparoscopy.

Table of Content

Volume-11, Issue-7, July 2025

INK-4a Downregulated in *P53* and *P21* Mutation Induce BAT and VSMCs Proliferation: Obesity and Hypertension

Peni K. Samsuria Mutalib

Department of Medical Physics, Faculty of Medicine University of Indonesia/ Cipto Mangunkusumo National General Centrum Hospital, Jakarta 10430, Indonesia

Received:-02 July 2025/ Revised:- 10 July 2025/ Accepted: 21 July 2025/ Published: 31-07-2025

Abstract—

Background: *Epidemiology of obesity and hypertension are in high prevalence on low- and middle-neighborhood socioeconomic status (nSES) area. Later, the subjects become Diabetes and Chronic Kidney Diseases (CKDs) 1-5, and cancer due to p53, p21 and p16 mutation in AFB1 exposure population. Neglected p16/INK4a upregulated in early years which induce stunting, later induce proliferation of BAT (Uncoupling Protein UCPs132) or central Obesity, and vascular smooth muscle cells (VSMCs)/Hypertension.*

Aims: *p16 first upregulated in p21 and p53 mutation due to AFB1 exposure cause stunting, then when p16 downregulated in the older age induce cancer cells proliferation. This study recorded more the proliferation, reveal proliferation of BAT and VSMCs in healthy young age, in the downregulated p16 stage.*

Method: *Review article of Systematic Review and Meta-Analysis references of p16/INK-4a decreasing induced proliferation, also p16 Knock Out (KO) in p21/ p53 mutation population (AFB1 exposure).*

Result: *Mutation of p21/p53 in AFB1 exposure, induce p16/INK-4a downregulated in central Obese & VSMCs patients. Silencing/ downregulated p16/INK-4a induced proliferation, but still controversial with the upregulated in p53/p21 mutation (before).*

Discussion: *Proliferation of UCPs132 BAT cells, proliferation of SMCs, SGLT-2/GLP-1 therapy.*

Conclusion: *proliferation of BAT UCPs132 (central obesity) or VSMCs (hypertension) is due to downregulated p16 under p21/p53 mutation due to AFB1 exposure in low- and middle-nSES area population.*

Keywords— AFB1 exposure population, Hypertension, INK-4a, Obesity, p21/p53.

I. INTRODUCTION

There are so many controversies in Obesity and Diabetes, especially in nature or nurture,[1] geographical or eating habit,[2] also in p53-p21-p16 upregulated or downregulated in this high prevalence area. p16 is a protein that slows cell division, by slowing the progression of the cell cycle from the G1 phase to the S phase, thereby acting as a tumor suppressor. It is encoded by the P16 gene, the name of p16 is derived from its molecular weight 16 kD, and the similar name INK4 refers to its role in inhibiting CKD4 (cyclin-dependent kinase) involved in regulation of the cell cycle, act as tumor suppressor cells and stunting. Down regulated of p16/INK-4a (by p53 mutation) in UCPs 132,[3] VSMCs,[4] and later proliferation of cancer cells in AFB1 exposure population.[5] Uncoupling proteins are abundant in mitochondria of Brown Adipose Tissue (BAT) cells, function as thermogenesis metabolism, low of ATP synthesis, like the metabolism of hibernate bears.

The down regulation of p16 is controversial with upregulated p16Ink4, is as a suppressor protein that considered a tumor suppressor protein because both are high in p53 and p21 mutation in cancer patients and senescence.[5,6] The Paradoxical downregulated[7] of p16 mRNA with advancing age in BRAF-mutated polyps/adenomas indicates a senescence barrier in the serrated route to colon cancer,[8] unlike healthy cells. P16 upregulated as a compensate of the failure of p53 and p21 tumor suppressor cells.[6,7] The p16 pathway is a key regulator of the cell cycle, which controls the passage of cells from G, to S phase. P16 targets CDK4 and prevents Rb phosphorylation. Similar to p16 protein overexpression, is cause by viral E7 protein

(indirect marker of HPV-induced squamous cell carcinoma in head and neck (SCCHN).[9] The p16 gene is often mutated or epigenetically-silenced in SCCHN.[9] This made the survival rate low, and also the prognosis. P16 inhibits cancer cell growth by downregulating eEF1A2 through a direct interaction.[10] So, in p53 and p21 mutation in AFB1 exposure, p16 become the last defense, p16 +/+ or p16 -/- which induced senescence, also depends on Estrogen (year of age), to become Breast cancer after menopause years.[11,12] Guo et al, 2017, demonstrate that P16 may be associated with the Cigarette smoke extract (CSE)-induced proliferation of vascular smooth muscle cells (VSMCs),[13] suggesting that P16 serves a role in the development of CS-associated vascular diseases. Cell proliferation and cell cycle distribution were evaluate by flow cytometry, Western blotting for examine protein expression, and bisulfite genomic sequencing polymerase chain reaction was used to determine the hypermethylation of P16 promotor CpG island (repeat CGG).[14] Concentration- and time-dependent exposure induced a downregulation in P16 (all $P<0.05$).[13] Significant decrease in gene assay transcriptional activity reduced P16 protein expression in human aortic smooth muscle cells (HAOSMCs) (both $P<0.01$). Hypermethylation (silencing), mutation, or deletion leads to downregulation of the P16 gene.[14]

II. METHOD

Review article of p16 downregulated that induced proliferation, which supported UCPs132/ Brown Adipose Tissue (BAT) and VSMCs proliferation in stunting, obesity, and Diabetes Mellitus high prevalence area/ populations.[5] Using my Library, and academic search engine mainly ScienceDirect and EBSCOhost. Keywords of Bayesian network of p16 and downregulated were used, Systematic Review and Meta-Analysis references are preferable. P53-p21-p16 downregulated in AFB1 exposure food depends on keywords urine AFM1 in the population area of obesity and hypertension high prevalence. This epidemiology record is long before p16 downregulation is associated with AFB1 exposure in food, but it will be interesting that p16INK-4a downregulated induced UCPs132 cells and VSMCs proliferation in low- and middle-nSES population area.

III. RESULT

The UCPs132 BAT and VSMCs proliferation in AFB1 exposure population, should be supported by down-regulation of p16 gen or epigenetically. P53-p21 mutation due to AFB1 exposure, induced up-regulation of p16.[6] Paradoxical down-regulation of p16 mRNA with advancing age in AML[7] and GISTs[15] is similar to aging.[6] Table 1. describes p16 downregulation that induce proliferation, and the argumentation are supported as follows:

3.1 Epidemiology of p16 downregulation depends on estrogen/senescence:

The upregulation p16 induce stunting[16] but become downregulation after estro ER-/-/ senescence.[12] Childhood stunting is an important and intractable public health problem that cause about 20% of deaths among children age below 5 y in low- and middle-nSES population.[16] The evaluate interventions to limit exposure and reduce childhood stunting should be promoted. Senescence's after menopause happens because Estrogen promotes estrogen receptor negative BRCA1-deficient tumor initiation and progression in breast cancer.[12] Possible Down Regulation of the p16 gene promoter in individuals with carcinoma,[17] and give poor prognosis.[18] Hepato cellular carcinoma (HCC)[17] and breast cancer (BC) caused by AFB1 exposure has been broadly known[5]

3.2 Epidemiology meet Knock Out p16, induce proliferation:

Down-Regulated of the p16 Gene Promoter in Individuals with negative regulator of the cell cycle. In act of p16, especially promotor,[17] down-reg p16 expression predicts poor prognosis in patients with extrahepatic biliary tract carcinomas patients.[18] The immunohistochemically evaluated down-regulation of p16 in tumor specimen surgical has reported.[18] Over expressed p16, which normally inhibits cell proliferation, induces G1 cell cycle arrest in cervical cancer cells and precancerous lession.[19] The downregulation of p16 in SiHa and HeLa cells inhibited their proliferation, migration and invasion, also in cervical cancer.[19] p16 maybe a useful strategy in the diagnostic and treatment of cervical cancer.[19] P16 deficiency promotes tumor formation in various tissues.[20] Also induced leanness especially in old age, lower body weight as a protection against cerebellar senescence.[20]

3.3 p16 +/+ vs. +/- vs -/- and Estrogen Reseptor +/+ vs -/-:

p16 deficiency does not alter homeostasis in WAT, so induces leanness especially in old age.[20] Expression in estrogen receptor beta (ERB) also increase in deep cerebellar nuclei, implying cross talk between p16 and ERB. Protection against cerebellar senescence by promoting neuronal proliferation and homeostasis via ERB (in response to estrogen).[20]

p16 mRNA expression in T cells, a marker of cellular senescence, with BC are significant with the increase risk.[11] It is differed by age, race, family history of cancer, marital status, annual income, and smoking status,[11] which is represented to low- and middle-nSES.[5]

Wang[12] describe the distinctive features of senescent cancer cells and how these changes in proliferation and senolysis.[11,12] Also the deletion of cell cycle inhibitors p16 is required for development of Brca1-deficient basal-like mammary tumors in ER-positive vs. ER-negative, which estrogen stimulate proliferation and inisiating in both ER positive and ER-negative mammary tumor initiation and metastasis (independent of ER).[12]

3.4 Viral E7 protein to p16 down-regulation:

Viral E7 protein Loss of 9p, leads to p16 down-regulation and enables O-6-methylguanine-DNA methyltransferase (MGMT) promotes the anti-proliferative and pro-apoptotic when cervical cancer cells stimulated with 5-Aza-dC.[21] Methylation of p16 and MGMT was reversed. 5-Aza-dC inhibited E6 and E7expression and up-regulated p53, p21, and Rb expression.

HPV E6/E7 mRNA tests determine the oncogenic activity of the virus and represent a good clinical biomarker for predicting the risk of developing cervical cancer.[22] Also in Epstein Bar Virus (EBV) have shown increase cell proliferation.[23] Which is also paralleled liver Hepatitis B virus (HBV) DNA values.[24]

TABLE 1
DOWN-REGULATION P16 INDUCES PROLIFERATION

Reference/year	Type of p16 down-regulation	Cases	Effect	pathway
[7]de Jonge, 2009	P16 mRNA	AML	Aging: p16-p53→ proliferation	P16 up and then downregulation
[8]Kriegl, 2011	P16 expression	Polyp adenoma (senescence barrier)	Colon Cancer	
[15]Haller, 2008	P16INK4A and P16 mRNA	GISTS	Inhibits the CDK4 from phosphorylating RB	P16 located at 9p21
[6]Mijit, 2020	P53→p16	Aging	Upregulation p16	p53 mutation-AFB1exposure
[16]Smith, 2012	P16	Stunting	Upregulation-Estro-/-→downregulation	Food chain AFB1 exposure
[17]Shiraz, 2011	KO p16 (gene promoter)	HCC	Poor prognosis	P16 downregulation
[18]Ichikawa, 2002	P16 down-regulated	Tumor specimen surgical	Extra biliary tract cancer Prognosis	P16 downregulation
[5]Samsuria, 2018	P53 mutation	Ob-DM-HCC/BC	High prevalence	AFB1: P53-p21
[19]Zhang, 2014	P16 gene downregulation	Cervical Ca	Dx/Rx cervical ca	P16 downregulation
[26]Zhang 2015	P16 expression	Breast cancer	Effect of hypoxia	Fibroblast
[27]Zhang, 2021	LATS1: Large tumor suppressor kinase 1	VSCMs	Effect of CSE: Cigarette Some Extract	P16-G1 arrest
[20]Kim, 2019	P16 deficiency P16-Estrogen Receptor Beta	Various tissue Non WAT	Tumor formation Leanness in old age	P16 downregulated-Estrogen
[11]Shen, 2020	P16 mRNA	T cell senescence	Increase BC risk	Age, black, fam history of ca, nSES, smoking status
[12]Wang, 2022	P16 deletion	BC-after menopause	Senescence: change to proliferation	Estrogen Receptor independent
[21]Chen G-d, 2017	Methylation p16	Cervical cancer	Rx/ 5-Aza-dC inhibit E6/E7 exp	P53, p21, p16, Rb
[22]Sharma, 2022	HPV DNA and mRNA	Cervical cancer	proliferation	E6/E7
[23]Uehara, 2021	EBV and HPV double infection	Oral ca tissue samples	proliferation	Reduce P53 induction
[24]Pan, 2004	HBV	HPV mRNA and HBV	proliferation	Therapy
[25]Kumari, 2021	Upregulation p16	Through ROS, DNA damage, senescence	Aging cells process	P53/p21WAF1CIP1 and p16INK4A/pRB play a central role in regulating senescence.
[13]Guo, 2017	P16	CSE	VSMCs proliferation	
[14]Breuer, 2005	P16 promoter Bisulfite Genomic PCR	Hypermethylation	Concentration and time associated	CpG island (repeat CGG)

Activation of p16 (upregulated p16) through ROS, DNA damage, or senescence leads to the p16 in tissues, and is implicated in aging of cells,[25] BAT,[3] SMCs,[4] and Cancer cells.[18,19,20,21,30] This is about earlier upregulation of P16 gene-stunting then through senescence barrier, change to downregulation which induce proliferation. Conversely, p16 hypermethylation (silencing/KO), mutation, or deletion leads to downregulation of the gene and can lead to cancer through the dysregulation of cell cycle progression.

IV. DISCUSSION

CDKN (inhibitor)2A gene as the main tumor suppressor gene with p53 and p21, has another name P16/INK-4a and act as inhibiting CKD4 (cyclin-dependent kinase), with the help of hormone, antioxidant, antiviral. P16 induced by hypoxia are downregulated has been recorded. Table 2. Urine AFM1 (AFB1 exposure) in low- and middle-nSES home area describe the location of p16 downregulated high prevalence area. The mechanism are as followed:

4.1 Estrogen factors anti inflammation reaction:

Waist Circumference and Breast Cancer are associated with menstrual status[5] and Estrogen factors act as anti-inflammation protect to P16 down-regulation, inhibit proliferation.

4.2 HBV, EBV, HPV:

HBV. EBV, HPV infection without p16 down-regulation, suppressed tumor cells to proliferated.

Kras activation and p16 inactivation are required to develop pancreatic ductal adenocarcinoma (PDAC). Mutant Kras- and p16-regulated NOX4 activation overcomes metabolic checkpoints in development of PDAC.[28] Similar expression profile of KRAS and p16 reported in periampullary cancer.[30] In precancerous lession,[23]reveal the co-expression of low-risk HPV E6/E7 and EBV LMP-1 does not induce malignant transformation, but it allows accumulation of somatic mutations secondary to increase DNA damage and suppression of DNA DDR genes (DNA damage response and repair).[23]

4.3 Hypoxia induced proliferation:

Cigarette smoke extract (CSE) exposure, similar to hypoxis, down-regulated p16[13] and induce Vascular Smooth Muscle Cells (VSMCs) proliferation. The role of P16 in CSE-induced VSMCs proliferation and the underlying mechanism. also has been reported in the CKD1 in low- and middle-nSES AFB1 exposure population/ p53 mutation.[3,4,5]

4.4 Time-dependent exposure:

Time-dependent exposure induced p16 downregulation-proliferation of precancer cells, BAT cells mitochondrial rich UCPs132 and SMCs proliferation supported.[13] Not only time-exposure, but the level concentration of exposure are also recorded.[13]

4.5 Reduce p16 expression in HAOSMCs:

Activation of p16 (upregulated p16) through ROS, DNA damage, or senescence leads to the buildup of p16 in tissues and is implicated in aging of cells.[25] P53/p21WAF1CIP1 and p16INK4A/pRB play a central role in regulating senescence.[25] Boys and Girls in Sierra Leone, West Africa, frequently and constantly exposed by AFB1.[32] Birth weight of infant is discussed, and the cord blood aflatoxin found in 58% pregnant woman.[32] After the senescence reveal by stunting due to upregulated p16, reduce genetic or epigenic p16 expression induced proliferation in specific tissue e.g. human aortic smooth muscle cells (HAOSMCs),[13] UCP132 (BAT),[3] and fibroblast.[26] The loss of p16 expression due to p16 promotor hypermethylation occurs late in carcinogenic process at the level of severe dysplasia.[14]

4.6 Fibroblast migration and invasion:

Fibroblast migration and invasion in 83% breast CAFs compared primary cells and also in breast cancer tissues in hypoxia is reported.[26]

Up and downregulation of p16 expression in BRAF-mutated polyps/ adenomas indicates a senescence barrier in the Colorectal carcinoma (CRC) process.[8] P16 hypermethylation, mutation, or deletion leads to downregulation of the gene and can lead to cancer through the dysregulation of cell cycle progression. The liberates E2F1 (Transcription Factor) from its bound state in the cytoplasm and allows it to enter the nucleus. Once in the nucleus, E2F1 promotes the transcription of target genes that are essential for transition from G1 to S phase.[15] This pathway connects the processes of tumor oncogenesis and senescence, fixing them on opposite ends of a spectrum. All Obesity and Hypertension high prevalence populations, are in the high prevalence of

stunting with AFB1 exposure-p53 mutation.[5 and 16] Urine AFM1 describe AFB1 exposure in low- and middle-nSES home area had high Obesity (BAT proliferation) and Hypertension (SMCs proliferation) prevalence.

TABLE 2
IDENTIFIED LITERATURES ON URINE AFM1 (AFB1 EXPOSURE) IN LOW- AND MIDDLE-nSES HOME AREA

Study, year	Area of interest	Adjustment of interest	Comparative urine AFM1	Comorbidities influence	Influence of LC-MS/MS
[33]Ali, 2016	Hot and Humid climate	Bangladesh	Rural vs. Urban	Adult, children	Urine AFM1 as biomarker of AFB1 exposure
[34]Gerding, 2015	Germany	23 mycotoxins	Bangladesh Germany Haiti	Prevention of harmful health	Bangladesh and Haiti only, not Germany
[35]Jager 2016	UPLC-MS/MS	Brazil	HPLC with fluorescence detection	Prevention of harmful health	Confirmed urine AFM1 very sensitive for AFB1 exp
[36]Ezekiel, 2014	Rural North Nigeria	LC-MS/MS multi biomarker	Children Adolescents Adults	Major Public Health Challenge	Call for urgent intervention
[37]Warth, 2014	Bangkok	Max AFB1	LC-MS/MS urine AFM1	4 urine biomarkers	First in SEA
[38]Mitchell, 2013	AFB1 is a persistent public health issue in Ghana	Urine AFM1	UPSN vs. placebo	intervention	UPSN reduced AFM1 biomarker
[39]Solfrizo, 2011	1st time reported	High pressure LC-MS/MS	Urinary biomarkers	Aflatoxin exposure could be measured	Urine AFM1 can be used
[40]Solfrizo, 2014	AFB1 exp	Urine AFM1	UPLC MS/MS in pg/mL	Southern Italy ZEA 100%, AFM1 6%	Urine AFM1 detected
[41]Ahn, 2010	Quantitative	Urine AFM1 in pg/ml	LC-MS/MS	Using immunoaffinity collumn	Korean population 1/12 detected
[42]De Cassia, 2009	Brazillian population	Urine AFM1	UPLC low pressure with fluorescence detection	Food contamination	AFB1 exposure
[31]Jonsyn, 2001	Seasonal observational	Urine AFM1 from children in Sierra Leone	Dry and Rainy season	Boys and girls	Frequently and constantly exposed
[43]Shephard, 2013	1st void morning urine	Urinary multi-mycotoxin	LC-MS/MS	Esophageal cancer region	Maize-based evening meal
[44]Warth, 2012	Quantitative in sub-ppb	AFM1 in Cameroon	LC/ESI-MS/MS	In human urine	Key metabolite
[45]Chen, 2017	Aptamer AFB1	Fluorescence enhancement	LOD 1.6 ng/ml	DNA strand containing a quencher moiety	+
[46]Jonsyn, 1999	Aflatoxin	HPLC specimen of infants	High contamination rate	AFB1 G2, OTA, OTB	Urine sample were 100% contaminated
[47]Jonsyn, 2007	Aflatoxin	Urine sample of school children	High concentration level	57% serum samples + aflatoxin	Low compared to urine
[32]Jonsyn, 1995	Cord blood sample from pregnant	OTA	OTA in 25% Aflatoxin in 58%	No urine AFM1	Birth weight of infant

V. LIMITATION:

Many study didn't specify proliferation after stunting (aging) but report proliferation due to $p16^{INK4a}$downregulation due loss of 9p chromosome of p21.[15] p16(INK4a) upregulated up to p16 downregulation by epimutation/ hypermethylation should be known by the researchers.[14]

This study also didn't specify age-dependent p16 epimutation, but proposes as the therapeutic target for colorectal cancer.[48] And KRAS, p53 are on mutation sequence before DCC gene,[49] similar to what KRAS activation and p16 downregulation in other precancer.[28,30] Mutant Kras- and p16-regulated NOX4 activation overcomes metabolic checkpoints in development of Pancreatic ductal adenocarcinoma.

This study also didn't specify that p16 downregulation depends on senescence, while Wang reported exploiting senescence for the treatment of cancer,[50] where 5-Aza-dC is reveal for the treatment of cancer based on hypermethylation of p16 promotor.[21] Stunting/ senescence due to p53 mutation.[16] 5-Aza-dC is also reported to other broad range detrimental health cause by hypermethylation other than cancer.[51]

This study also didn't characterize Sodium-Glucose Cotransporter 2 (SGLT2) Inhibitors as therapy of afferent contraction due to VSMCs proliferation on afferent glomerulus.[52, 53, 54] The area of AFB1 exposure could be found in low- and middle nSES (income) in Table 2,[56] especially in children and infant and moreover since pregnant women.[57,58,59,60,61] which have associated to growth impairment.[60,62,63,64] In children, adolescence and adults, is associated with major public health challenge, incl. HIV.[36] It is emphasize that AFB1 especially in utero, associated to DNA methylation in white blood cells of infants in the Gambia.[64]

Mild, moderate, severe stunting & underweight on Systematic Analysis in 141 developing countries,[61,65,66,67,] is completed by Sousa reported stunting and overweight/obese high prevalence in Brazilian children (Systematic Review and Meta-Analysis),[68] and Romero report Urine AFM1 in Brazilian population associated with food consumptions parallel with the highest Diabetes Melitus (IDF), CVD (WHO).[42] Stern 2001 reported HBV and AFB1 exposure due to p53-codon 249 mutation in China with Meta-Analysis.[69] It is supported by chronic hepatomegali,[70] aflatoxin cause stunting in Benin,[71] Aflatoxin exposure in young children Benin and Togo, West Africa,[71] complete this finding of AFB1 exposure induce the proliferation by downregulation of p16INK4a after stunting.[5,16] p53-p21-p16 axis,[73] p16 deletion,[50] p16 methylatioon,[21] which VSMCs function in atherosclerosis-DNA methylation.[55]

VI. CONCLUSION

Proliferation of BAT UCP132 (obesity) or SMCs (hypertension) is due to downregulated p16 under p21/p53 mutation due to AFB1 exposure in low- and middle nSES area population with high childhood stunting (cell senescence) prevalence.

ACKNOWLEDGEMENT

Thank you for CODHY and CODI, who always support the author to the research on cellular BAT (UCP132) and Diabetes Nephropathy which ended that Obesity and CKD1 in the population depends on p16 downregulation which induce proliferation.

CONFLICT OF INTEREST

Nothing

REFERENCES

[1] Kmietowicz Z. Nurture is more important than nature in childhood obesity, study finds. BMJ 2015:350h817. https://doi.org/10.1136/bmj.h817

[2] Andreadou I, Schulz R, Badimon L, Adameova A, Klienbongard P, Lecour S, et al. Hyperlipidaemia and cardioprotection: Animal models for translational studies. Br J Pharmacol 2020;177(23):5287-311. https://doi.org/10.1111/bph.14931

[3] Czech MP. Mechanisms of insulin resistance related to white, bdige, and brown adipocytes. Mol Metab 2020;34:27-42. https://doi.org/10.1016/j.molmet.2019.12.014

[4] Cao RY, Eves R, Jia L, Funk CD, Jia Z, and Mak AS. Effect of p53-knockout in vascular smooth muscle cells on atherosclerosis in mice. PLoS One 2017;12(3):e0175061. https://doi.org/10.1371/journal.pone.0175061

[5] Marchese S, Polo A, Ariano A, Velotto S, Costantini S, and Severino L. Aflatoxin B1 and M1: Biological Properties and Their Involvement in Cancer Development. Toxina (Basel) 2018;10(6):214. https://doi.org/10.3390/toxins10060214

[6] Mijit M, Caracciolo V, Melillo A, Amicarelli F, and Giordano A. Role of p53 in the regulation of cellular senescence. Biomolecules 2020; 10(3):420.

[7] deJonge HJM, Woolthuis CM, de Bont ESJM, Huls G. Paradoxical down-regulation of p16INK4a mRNA with advancing age in Acute Myeloid Leukemia. Aging 2009;1(11):949-53.

[8] Kriegl L, Neumann J, Vieth M, Greten FR, Reu S, Jung A, Kirchner T. Up and downregulation of p16 Ink4a expression in BRAF-mutated polyps/adenomas indicates a senescence barrier in the serrated route to colon cancer. Mol Pathol 2011; 24(7):1015-22.

[9] Aggarwal N, Yadav J, Thakur K, Bibban R, Chhokar A, Tripathi T, et al. Human Papillomavirus infection in Head and Neck squamous cell carcinomas: Transcriptional triggers and changed disease patterns. Front Cell Infect Microbiol 2020;10:537650.

[10] Lee MH, Choi BY, Cho Y-Y, Lee S-Y, Huang Z, Kundu JK, et al. Tumor suppressor p16INK4a inhibits cancer cell growth by downregulating eEF1A2 through a direct interaction. J Cell Sc 2013;126(8):1744-52.

[11] Shen, Song R, Fuemmeler BF, McGuire KP, Chow W-H, and Zhao H. Biological Aging Marker $p16^{INK4a}$ in T Cells and Breast Cancer Risk. Cancer (Basel) 2020;12(11):3122.

[12] Wang C, Bai D, Zhang L-h, Scott A, Enxiao L, Pei X-H. Estrogen promotes estrogen receptor negative BRCA1-deficient tumor initiation and progression. Breast Cancer Res 2018;20:74.

[13] Guo T, Chai X, Liu Q, Peng W, Peng Z, Cai Y. Downregulation of P16 promotes cigarette smoke extract-induced vascular smooth muscle cell proliferation via preventing G1/S phase transition. Exp Ther Med 2017;14(1):214-220.

[14] Breuer RH, Snijders PJ, Sutedja GT, Sewalt RG, Otte AP, Postmus PE, Meijer CJ, Raaphorst FM, Smit EF. Expression of p16(INK4a) gene product, methylation of the p16(INK4a) promoter region and expression of the polycomb-group gene BMI-1 in squamous cell lung carcinoma and premalignant endobronchial lesions. Lung cancer (Amsterdam, Netherlands) 2005;48(3):299-306.

[15] Haller F, Lobke C, Ruschhaupt M, Cameron S, Schulten HJ, Schwager S et al. Loss of 9p leads to p16INK4A down-regulation and enables RB/E2F1-dependent cell cycle promotion in gastrointestinal stromal tumours (GISTs). J Pathol 2008;215(3):254-62.

[16] Smith LE, Stoltzfus RJ, Prendergast A. Food chain mycotoxin exposure, gut health, and impaired growth: a conceptual framework. Adv Nutr 2012; 3(4):526-31.

[17] Shiraz OB, Galehdari H, Yavarian M, Geramizadeh B. Possible Down Regulation of the p16 Gene Promoter in Individuals with Hepatocellular Carcinoma. Hepat Mon 2011;11(9):719-23.

[18] Ichikawa K, Imura J, Kawamata H, Takedia J, Fujimori T, Down-regulated p16 expression predicts poor prognosis in patients with extrahepatic biliary tract carcinomas. Int J Oncol 2002;20(3): 453-61.

[19] Zhang CV, Bao W, Wang LH. Downregulation of p16(Ink4a) inhibits cell proliferation and induces G1 cell cycle arrest in cervical cancer cells. Int J Mol Med 2014;33(6):1577-85.

[20] Kim KH, Cho Y, Lee J, Jeong H, Lee Y, Kim SI, Kim CH, Lee HW, Nam KT. Sexually dimorphic leanness and hypermobility in $p16^{Ink4a}$/CDKN2A-deficient mice coincides with phenotypic changes in the cerebellum. Sci Rep 2019;9(1):11167.

[21] Chen G-d, Qian D-Y, Li Z-G, Fan Y-G, You K-L, Wu Y-L. 2017 Wiley. Down-regulation of p16 and MGMT promotes the anti-proliferative and pro-apoptotic effects of 5-Aza-dC and radiation on cervical cancer cells. Cell biochemistry and function 2017;35(8):488-496.

[22] Sharma B, Lakhanpal V, Singh K, Oberoi L, Bedi PK, Devi P. Evaluation of HPV E6/E7 mRNA Detection in Clinically Suspected Cases of Cervical Cancer with Abnormal Cytology: Time to Upgrade the Screening Protocols. J Lab Physicians 2022;14(3):336-42.

[23] Uehara K, Tanabe Y, Hirota S, Higa S, Toyoda Z, Kurima K, Kina S, et al. Co-expression of low-risk HPV E6/E7 and EBV LMP-1 leads to precancerous lesions by DNA damage. BMC Cancer 2021; 21:688.

[24] Pan W-H, Xin P, Morrey JD, Clowson GA. A self-Processing Ribozyme Cassette: Utility against Human Papillomavirus 11 E6/E7 mRNA and Hepatitis B Virus. Mol Ther 2004; 9(4):596-606.

[25] Kumari R, Jat P. Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype. Front Cell Dev Biol 2021;9.

[26] Zhang J, Li L, Lu Y. Effect of Hypoxia, surrounding fibroblasts, and p16expression on breast cancer cell migration and invasion. J Cancer 2015; 6(5):430-37.

[27] Zhang W, He S, Qian Y, Wang D. Cigarette smoke extract induces migration of rat vascular smooth muscle cells through regulation of expressions of MOB2 and LATS1. Tropical Journal of Pharmaceutical Research 2021;20(3):551-7.

[28] Ju H-Q, Ying H, Tian T, Ling J, Fu J, Lu Y, et al. Mutant Kras- and p16-regulated NOX4 activation overcomes metabolic checkpoints in development of Pancreatic ductal adenocarcinoma. Nature Communications 2017;8:14437.

[29] Wang L, Lankhorst L, Bernards R. Exploiting senescence for the treatment of cancer. Nat Rev Cancer 2022;22(6):340-355.

[30] Tewari M, Swain J, Mishra RR, Dixit VK, Shukla HS. Expression Profile of KRAS and p16 in periampullary cancer. Indian J Surg Oncol 2023.

[31] Jonsyn-Ellis FE. Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls. Mycopathologia 2001;152(1):35-40. 2001

[32] Jonsyn FE, Maxwell SM, Hendricks RG. Ochratoxin A and aflatoxins in breast milk samples from Sierra Leone. Mycopathologia 1995;1(2):121-6.

[33] Ali N, Hossain K, Blaszkewicz M, Rahman M, Mohanto NC, Alim A, et al. Occurrence of aflatoxin M1 in urines from rural and urban adult cohorts in Bangladesh. Arch Toxicol 2016;90(7):1749-55.

[34] Gerding J, Ali N, SchwartzbordJ, Cramer B, Brown DL, Degen GH, et al. A comparative study of the human urinary mycotoxin excretion patterns in Bangladesh, Germany, and Haiti using a rapid and sensitive LC-MS/MS approach. Mycotoxin Res 2015;31(3):127-36.

[35] Jager AV, Tonin FG, Baptista GZ, Souto PCMC, Oliveira CAF. Assessment of aflatoxin exposure using serum and urinary biomarkers in Sao Paulo, Brazil: A pilot study. Int J Hyg Environ Heal 2016;219(3):294-300.

[36] Ezekiel CN, Warth B, Ogara IM, Abia WA, Ezekiel VC, Atehnkeng J et al. Mycotoxin exposure in rural residents in northern Nigeria: a pilot study using multi-urinary biomarkers. Environ Int 2014;66:138-45.

[37] Warth B. Petchkongkaew A, Sulyok M, Krska R. Utilising an LC-MS/MS-based multi biomarker approach to assess mycotoxin. Exposure in the Bangkok metropolitan area and surrounding provinces. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2014;31(12):2040-6.

[38] Mitchell NJ, Kumi J, Johnson NM, Dotse E, Marroquin-Cardona A, Wng J-S et al. Reduction in the urinary aflatoxin M1 biomarker as an early indicator of the efficacy of dietary interventions to reduce exposure to aflatoxins. Biomarkers Biochem Indic Expo Response, Susceptibility To Chem 2013;18(5):391-8.

[39] Solfrizo M, Gambacorta L, Lattanzio VMT, Powers S, Visconti A. Simultaneous LC-MS/MS detemination of aflatoxin M1, ochratoxin A, deoxynivalenol, de-epoxydeoxynivalenol, α and β-zearalenols and fumonisin B1 in urine as a multi-biomarker method to assess exposure to mycotoxins. Anal Bioanal Chem 2011;401(9):2831-41.

[40] Solfrizzo M, Gambacorta L, Visconti A. Assessment of multi-mycotoxin exposure in southern Italy by urinary multi-biomarker determination. Toxin (Basel) 2014;6(2):523-38.

[41] Ahn J, Kim D, Kim H, Jahng K-Y. Quantitative determination of mycotoxins in urine by LC-MS/MS. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2010;27(12):1674-82.

[42] De Cassia A, Raquel T, Ferreiira B, Tadeu C, Calori-domingues MA, Micotti E. Occurrence of AFM1 in urine samples of a Brazillian population and association with food consumption. J food control 2009:6-10.

[43] Shepard GS, Burger H-M, Gambacorta L, Gong YY, Krska R, Rheeder JP et al. Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the former Transkei, South Africa. Food Chem Toxicol An Int J Publ Br Ind Biol Res Assoc 2013;62:217-25.

[44] Warth B, Sulyok M, Fruhmann P, Mikula H, Berthiller F, Schuhmacher R et al. Development and validation of a rapid multi-biomarker liquid chromatography/tandem mass spectrometry method to assess human exposure to mycotoxins. Rapid Commun Mass Spectrom 2012;26:1533-40.

[45] Chen L, Wen F, Li M, Guo X, Li S, Zheng N et al. A simple aptamer-based fluorescent assay for the detection of Aflatoxin B1 in infant rice cereal. Food Chem 2017;215:377-82

[46] Jonsyn FE. Intake of aflatoxins and ochratoxins by infants in Sierra Leone: possible effects on the general health of these children. J Nutr Environ Med 1999;9(1):15-22.

[47] Jonsyn-E FE. Aflatoxins and ochratoxins in serum samples of school children. J Nutr Environ Med 2007;16(1):52-8.

[48] Yang L, Chen X, Lee C, Shi J, Lawrence EB, Zhang L, et al. Functional characterization of age- Functional characterization of age-dependent p16 epimutation reveals biological drivers and therapeutic targets for colorectal cancer. J Exp Clin Cancer Res 2023;42,113.

[49] Mutalib PKS, Ibrahim AS, Dardjat MT. What is the firelighter of colorectal carcinoma's (CRC's) fircracker/fishbone: U-238. Are we missing this point? Cytometry Res 2010;20(1):27-30.

[50] Wang L, Lankhorst L, Bernards R. Exploiting senscence for the treatment of cancer. Nature Reviews Cancer 2022; 22:340-55.

[51] Samsuria PK, Samsuria IK, Samsuria WD. Decitabine Self Monitoring in Unstable Methylation of DNMT Patients: A Quasi Systematic Review. IJOEAR 2019;5(9):29-35.

[52] Durante W, Effect of Sodium-Glucose Co-Transporter 2 Inhibitor on Vascular Smooth Muscle Cells. Int J Mol Sci 2021;16:8786.

[53] Soares RN. Ramirez-Perez FI, Cabral-Amador FJ, Morales-Quinones M, Foote CA, Ghiarone T. et al, SGLT2 inhibition attenuates arterial dysfunction and decreases vascular F-actin content and expression of. Proteins associated with oxidative stress in aged mice. GeroScience 2022;44:1657-75.

[54] Adam CA, Anghel R, Marcu DTM, Mitu Q, Roca M, and Mitu F. Impact of Sodium-Glucose Cotransporter 2 (SGLT2 Inhibitors on Arterial Stiffness and Vascular Aging-What Do we know so far? (A narative review). Life (Basel) 2022; 12(6):803.

[55] Chen Y, Liang L, Wu C, Cao Z, Xia L, Meng J, and Wang Z. Epigenetic Control of VSMC function in atherosclerosis: A role for DNA Methylation. DNA and Cell Biol 2022;41:9:834-837.

[56] Miller AC, Murray MB, Thomson DR, Arbour MC. How consistent are associations between stunting and child development? Evidence from a meta-analysis of associations between stunting and multidimensional child development in fifteen low- and middle-income countries. Public Health Nutr 2016;19(8):1339-47.

[57] Castelino JM, Routledge MN, Wilson S, Dunne DW, Mwatha JK, Gachuhi K et al. Aflatoxin exposure is inversely associated with IGF1 and IGFBP3 levels in vitro and in Kenyan schoolchildren. Mol Nutr Food Res 2015;59(3):574-81.

[58] Castelino JM, Domininguez-Salas P, Routledge MN, Prentice AM, Moore SE, Hening BJ et al. Seasonal and gestation stage associated differences in aflatoxin exposure in pregnant Gambian women. Trop Med Int Heal TMIH 2014;19(3):348-54.

[59] Chittmittrapap S, Chieochansin T, Chaiteerakij R, Treeprasertsuk S, Klaikaew N, Tangkijvanich P et al. Prevalence of Aflatoxin induced p53 mutation at Codon 249 (R249s) in Hepatocellular Carcinoma patients with and without Hepatitis B surface antigen (HBsAg). Asian Pacific J Cancer Prev 2013;14(12):7675-9.

[60] Lombard MJ. Mycotoxin exposure and infant and young child growth in Africa: what do we know? Ann Nutr Metab 2014;64 Suppl 2:42-52.

[61] Wild CP, Miller JD, Groopman JD. Mycotoxin Control in Low and Middle-Income Countries. IARC Working Group Report 2015;9. International Agency for Research on Cancer, Lyon, France.

[62] Khlangwiset P, Shephard GS, Wu F. Aflatoxins and growth impairment: a review. Crit Rev Toxicol 2011;41(9):740-55.

[63] Smith LE, Prendergast A, Turner PC, Mbuya MNN, Mutasa K, Kembo G et al. The potential Role of Mycotoxins as a Contributor to Stunting in the SHINE Trial. Clin Infect Dis An Off Publ Infect Dis Soc: Am 2015;61 Suppl 7:S733-7.

[64] Hernandez-Vargaz H, Castelino J, Silver MJ, Dominguez-Salas P, Cros M-P, Durand G et al. Exposure to aflatoxin B1 in utero is associated with DNA methylation in white blood cells of infants in The Gambia. Int J Epidemiol 2015;44(4):1238-48.

[65] Stevens GA, Finucane MM, Paciorek CJ, Flaxman SR, White RA, Donner AJ et al. Trends in mild, moderate, and severe stunting and underweight, and progress towards MDG1 in 141 developing countries: a systematic analysis of population representative data. Lancet (London, England) 2012;380(9844):824-34.

[66] Wild CP. Aflatoxin exposure in developing countries: the critical interface of agriculture and health. Food Nutr Bull 2007;28(2) Suppl):S372-80.

[67] Olivero J, Fa JE, Farfan MA, Lewis J, Hewlett B, Breuer T et al. Distribution and Numbers of Pygmies in Central African Forests. PLoS One 2016; 11(1):e01444499

[68] Sousa CP da Cunha, Olinda R, Pedraza DF. Prevalence of stunting and overweight/obesity among Brazilian children according to different epidemiological scenario: systematic review and meta-analysis. Sao Paolo Med J 2016;134(3):351-62.

[69] Stern MC, Umbach DM, Yu MC, London SJ, Zhang Z, Taylor JA. Hepatitis B, Aflatoxin B1, and p53 Codon 249 mutation in Hepatocellular Carcinoma from Guangxi, People's Republic of China, and a Meta-analysis of Existing Studies. Cancer Epidemiol Biomarkers Prev 2001;10(6):617-25.

[70] Gong YY, Wilson S, Mwatha JK, Routledge MN, Castelino JM, Zhao B et al. Aflatoxin Exposure May Contribute to Chronic Hepatomegaly in Kenyan School Children Environ Health Perspect 2012;120(6):893-6.

[71] Gong Y, Hounsa A, Egal S, Turner PC, Sutcliffe AE, Hall AJ et al. Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. Environ Health Perspect 2004;112(13):1334-8.

[72] Gong YY, Egal S, Hounsa A, Turner PC, Hall AJ, Cardwell KF et al. Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. Int J Epidemiol 2003;32(4):556-62.

[73] Leong WF, Chau JF, Li B. p53 Deficiency leads to compensatory up-regulation of p16INK4a. Mol Cancer Res 2009;7(3):354-60.

Severe Thrombocytopenia in DLMF/Lymphoma Malignant/ITP: Review and Case report: A New WHO Dengue Fever classification beyond DHF

Peni K. Samsuria Mutalib[1*]; Indranila Kustarini Samsuria[2]; Arindra Adi Rahardja[3]

[*1]Medical Physics, Faculty of Medicine, University of Indonesia/RSUPN Cipto Mangunkusumo, Jakarta
[2]Clinical Pathology Ret. Department, Medical Faculty, Diponegoro University, Semarang 50244
[2]Central Laboratory of Medical Faculty, Diponegoro University-Dr.Kariadi Hospital, Semarang 50244
[3]Primary Care UPTD Puskesmas Tlogosari-Kulon, Semarang, Central Java, Indonesia
*Corresponding Author

Received:-05 July 2025/ Revised:- 11 July 2025/ Accepted: 18 July 2025/ Published: 31-07-2025

Abstract—

Introduction: *Nowadays, incidence of Lymphoma Malignant with mortality is high. Peoples and former health ministry of Indonesia, FS, suspect Wolbachia-Aedes aegypti (DHF), but pediatrician thought to DENV-3/-4 secondary heterogeneity infection in endemic DENV -1/-2 in tropical rainforest area.*

Method: *Review the pathogenesis of the two Lymphoma Malignant dead cases report, in association with DENV-3 or -4 as secondary heterogeneity infection in tropical rainforest area, Indonesia.*

Result: *Severity Grade of DHF associated with secondary heterogenous DENV-3 or -4 infections. Conclusion: DENV 1-4 is ss RNA in association with emergency pandemic booster of SARS-CoV-2 which is also ss-RNA virus coated, secondary heterogeneity infection, is in one group with virus which could associated as the caused to Leukemia revealed Immune Thrombocytopenia and other tumor incl. Lymphoma Malignant-non-Hodgkin.*

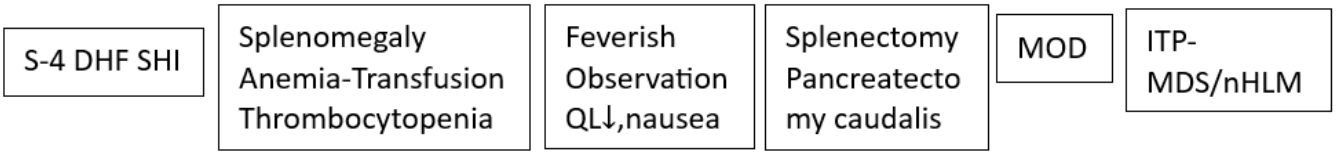
Conclusions: *Ss RNA incl. DENV-4 infection as secondary heterogenous DENV-1/-2 or vice versa, which induce anemia and thrombocytopenia ITP with splenomegaly/Lymphoma Malignant, should be classified to DLMF (grade-4) of DHF classification.*

Keywords— ITP-MDS, Large Granular Chronic Leukemia, Neutrophil Lymphocyte Ratio (NLR), Secondary heterogeneity infection DENV-4, Sepsis, Splenectomy.

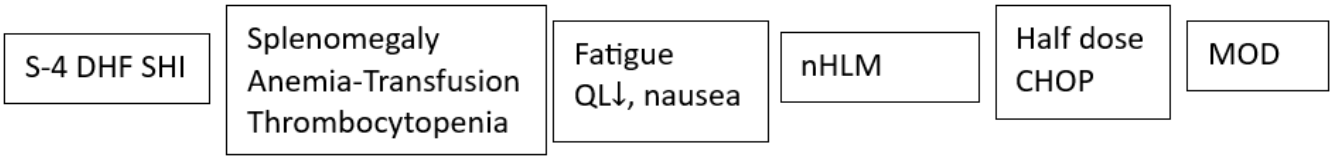
I. INTRODUCTION

Two cases of Acute Lymphoma Malignant mortality in the beginning, and the end of the rainy season in tropical rainforest area (DHF) endemic has shocked peoples in 2024. In Indonesia Aedes aegypti-Wolbachia is spreading in big cities incl. West Jakarta, Surabaya, Semarang and Bali. These two cases are diagnosed in one family. Besides, in the other family, there are also such cases. Number of cases on DENV-3/-4 secondary heterogeneity infection (SHI) 2023 and 2024, and the need of tetravalent DHF vaccine (and no booster COVID-19 AZ due to the recollection from circulation) has been reported in this period, is emphasized not because thrombosis and thrombocytopenia syndrome). AZ pull back the ss RNA vaccine circulation of

COVID-19 all over the world on May 7, 2024 due the asking on March 5, 2024, and loose in court on April 2024 caused of Thrombosis with Thrombocytopenia Syndrome (TTS) as a side effect. SS RNA AZ COVID-19 vaccine, and Serotype-3 or -4 DHF secondary heterogeneity infection, both caused TTS. Most COVID-19 vaccine development efforts aim to activate the immune system against the spike protein (glycoprotein spike on a viral capsid or viral envelope). Pfizer and Moderna have already stopped their mRNA vaccine production since the pandemic COVID-19 is over, which is no longer a pandemic-level threat declared by the WHO May 17, 2023 (while global pandemic was revealed on March 11, 2020). Exactly, the permission to use is only on emergency pandemic conditions. So, there are no causes of Wolbachia and booster COVID-19 vaccine in association with thrombocytopenia and these two Lymphoma Malignant non-Hodgkin cases. The aims of this study are no surgery, and no chemotherapy on severe thrombocytopenia. Many nodules in splenomegaly at the beginning, but the spleen is clear post-op (Fig.1). The liver has supple contour, no hepatomegaly and cirrhosis.



a) Case 1: Healthy Man, 73 y with BPJS (government health insurance) limited therapy



b) Case 2: Healthy Man, 70 y with no limit therapy

FIGURE 1: Dx/ Case 1: Splenectomy & cauda pancreatectomy, 3 days after post-op with never conscious/ wake-up. Macroscopy: Clean of nodules, Microcopy/PA: Lymphoma Malignant; Dx/ Case 2: nHLM and Thyroid Cancer & MOD oedema, dead after 1 week chemotherapy.

1.1 Hypothesis: DLMF caused by secondary heterogeneity infection of DENV ssRNA:

The COVID-19 and DHF, both are caused by viral infection which is well known in Indonesia, SEA. SARS and COVID-19 which attack tractus respiratory, and could be fatal, emerge in China, SARS in 2002, while COVID-19 in 2019. In the early 2020 up to 2023, Indonesia has vaccinated due the COVID-19 pandemic. SARS-CoV-1 is SARS virus, which causes severe acute respiratory syndrome (SARS). SARS-CoV-2 is corona virus, which causes respiratory tract infection of COVID-19. This is the name of the virus, and the COVID-19 is the name of the disease. It is like HIV the virus, and AIDS (Acquired Immune Deficiency Syndrome) as the disease, has been well known. MERS-CoV is a different virus than SARS-CoV2 because of the difference vector, it's by camel, not bird or directly from human to human. DENV 1-4 causes Dengue Fever Classic (DFC), DHF, DHF Shock Syndrome, and DF Lymphoma Malignant (DFLM). DSS due to the endothelial cell's dysfunction (Shock Syndrome) AND Development coagulation disorders induced by thrombocyte/platelet-lymph nodes suppression, where monocytes and macrophages are recruited to replicated.[1] These pathogenesis has not yet associated to Lymphoma Malignant (LM) non- Hodgkin) as the diagnosis of 2 cases report.[1,2] Meanwhile CHOP and R-CHOP are reported successful for the therapy of non-Hodgkin LM in the most endemic area top referral hospital in East Java, Dr. Soetomo General Hospital Surabaya.[2] This inductive phenomena is supported by/ could be associated by Dengue Virus Serotype 4, which is responsible for the outbreak of Dengue in East Java City of Jember, Indonesia.[3] Lymphoma Malignant has been associated with DHF infection.[4,5,6,7].

1.2 DHF, DSS, thrombocytopenia and epigastric pain, and Wolbachia review:

1) Dengue Fever (DF) classic, usually in children have symptoms: fever, headache, nausea vomitus, epigastric pain, muscle joint, rash after the 4th day. On the 4th day is the most dangerous. Dengue symptoms appear 4 to 7 days after the bite of Aedes sp. mosquito.

   DD: typhoid or Upper Respiratory Tract Infection (common cold or staphylococcus).

2) Dengue Hemorrhagic Fever (DHF), DF classic + bruised violet color (arterial damage). Nose, gingiva, or under the skin (petechiae with or without Rumple Leed test)

3) Dengue Shock Syndrome (DSS), a child and adult with plasma is already extravascular due endothelial apoptosis/leakage, blood pressure: 0/0.

4) Lymphoma Malignant Non-Hodgkin (does not contain Reed-Steinberg cells) B in adult: Splenomegaly. Rx/ Cyclophosphamide (Cytoxan) (Endoxan, doxorubicin) are a group chemotherapy which decrease the growth of cancer cells, well known as CHOP regiment with the P for Prednisone.[2]

Thrombocytopenia cut-off: mild 101,000-140,000 per μL of blood, severe |< 25,000 very severe \< 20,000 per μL, moderate 50-100,000/ μL. Thrombocytopenia mild, moderate, severe, critical, and cause mortality is the grade to describe severity score (Table 1). Thrombocytopenia during chemotherapy (Lymphoma Malignant) is often accompanied by a reduction of other blood cell counts.

TABLE 1
CUT-OFF

		Grade I	75-99			**Normal**	2.58
Grade I	>500	**Grade II**	50-74	**Normal**	40-54 M 36-48 F	**Grade I**	>3.68
Grade II	2470	**Grade III**	25-49	**Grade I**	>54 M, >48 F, >44 C	**Grade II**	5.5
		Grade IV	\<25			**Grade III**	7.83
						Grade IV	10.84

a) D- dimer cut-off (ng/mL) N <500
Old age: 90x10

b) Thrombocytopenia cut-off 100,000/µL Successful 16.000 therapy with only NaCl 0,9% is usual in Indonesia

c) Hematocrit cut-off (%)Automated cell counter: Red cell number x MCV (Millions/mm^3 x femto L)

d) NLR cut-off (%)

TABLE 2
NLR CUT-OFF WAS 3.0. WHO CATEGORIES FOR COVID-19 SEVERITY (ASYMPTOMATIC 1.92, MILD 2.08, MODERATE 4.79 AND SEVERE 9.9 WERE USED

Normal	2.58	COVID-19
Grade I asymptomatic	>3.68	1.92
Grade II mild	5.5	2.08
Grade III moderate	7.83	4.79
Grade IV severe	10.84	9.9

Neutrophil Lymphocyte Ratio in COVID-19 is used for describing prognosis, in DHF for severity score diagnosis.[4]

II. METHOD

Review the pathogenesis of the 2 case reports Lymphoma Malignant, in association with DENV 1-4 secondary heterogeneity infection. First, using Chat-GPT, then Science Direct and other search engines preferred PubMed connection.

III. RESULT

Grade of DHF associated to secondary heterogenous DENV-3/-4 infection

3.1 Two Cases Report mortality of DLMF:

These two dead case reports came from fact and report of the case's subject, family and close friends, doctors, and the author meeting with the patients.

3.1.1 Cases 1 DLMF (HUS):

Man > 73 years, reported DHF secondary infection Aedes Aegypti-Wolbachia area DENV-3/-4, eggs outside the female mosquito doesn't yield (to decrease the population of Aedes Aegypti in his home area). SS DEN-3/-4 secondary heterogeneity induces the development of coagulation disorder[1] (marker: D-dimer). Abdominal pain or tenderness, persistent nausea and vomiting, clinical fluid accumulation: ascites and pleural effusion, mucosal bleed and rash on skin are dubious. Lethargy/faint/tired/apathetic/passive/become slow are dominant. Restlessness is not recorded. Liver enlargement > 2 cm. Laboratory finding of increasing Leukocyte, Monocyte, Lymphocyte and Granulocyte with rapid decrease in platelet count. In 1 month, the fever observed in and out of the hospital due to BPJS patients could not be more than 3 days of hospitalization. At 92 years old, at grandma's birthday celebration, the friendly, polite man steps aside and sleeps again and again (End of July 2023). 2 Sept 2023 HUS came to family lunch but didn't want to eat because of nausea (loss appetite). October still with fever observation with hepatosplenomegaly and cauda pancreas full of nodules by USG, and hospitalized again. His brother is also hospitalized for chronic fever observation but survives by taking Ivermectin. HUS died 6 days post splenectomy and cauda pancreatectomy, on Nov 19, 2023, after 3 months feverish observation with low RBC, thrombocytopenia, and high Leukocyte, hypoglycemia and Hb decreasing and drink plenty of fluids. Epigastric pain, postprandial fullness, upper abdominal bloating, early satiation, nausea, vomiting, lethargic. On Nov 11, 2024, 3 days before splenectomy, he asked for high protein milk low carbohydrate high fat which would make him fit. HUS was Dx/ Lymphoma Malignant by PA Post-op splenectomy and cauda pancreatectomy. Oct 2023, HUS decreased albumin levels were strongly associated with DHF (by meta-analysis of multiple studies, $p<0.05$, while elevated leukocyte Oct 14, 2023. IgG >/ 2.85 IV: Positive IgG antibody to DFV type- 2/4 detected which may indicate a current or past infection. IgG |< 1.64 IV: Negative-No significant level of detectable DFV.

3.1.2 Case 2 DLMF (EeS):

Man 69 years, came to the hospital for BHP medical check-up, go alone with the chauffeur and small luggage, then after 2 weeks Dx/ Lymphoma Malignant with thyroid Ca (T Lymphocyte high production): thymus Dx/ by USG, then were given half dose chemotherapy on 5 May 2024. April 29, 2024 a little bit of moon face. May 3, 2024, couldn't know it was his face. Dead on May 10, 2024 with MOD oedema anasarca.

These 2 cases, endothelial cells dysfunction caused by Imbalance profile of cytokine and other mediators is not reported. Replication phase in hepatocyte and macrophage in spleen, then apoptosis of both supports the diagnosis of Lymphoma Malignant.[1,2,3,5] Differential diagnosis cirrhosis hepatis due hepatomegaly but no varices esophagus, and the liver is supple and soft in case report 1. HUS. Both DLMF cases, never took a bone marrow biopsy because no indication of blast cells in the periphery blood. Spleen and Liver are working extra hard, but not the bone marrow. Suppressed of hemopoiesis especially erythrocyte and thrombocyte, are clinically shown.[1]

IV. DISCUSSION

The author and the whole family are rethinking DHFV-4 because HUS (case 1) loves to go to the garden, his house is at the edge of a rice field, where clean water flood is best habitual for larval mosquitoes of Aedes aegypti. There are so many mosquitos in his house, he got hemolysis, hepatomegaly and splenomegaly. Several times hospitalized, each and every hospitalized person got blood transfusion then the Hb drop again (more frequent transfusion).

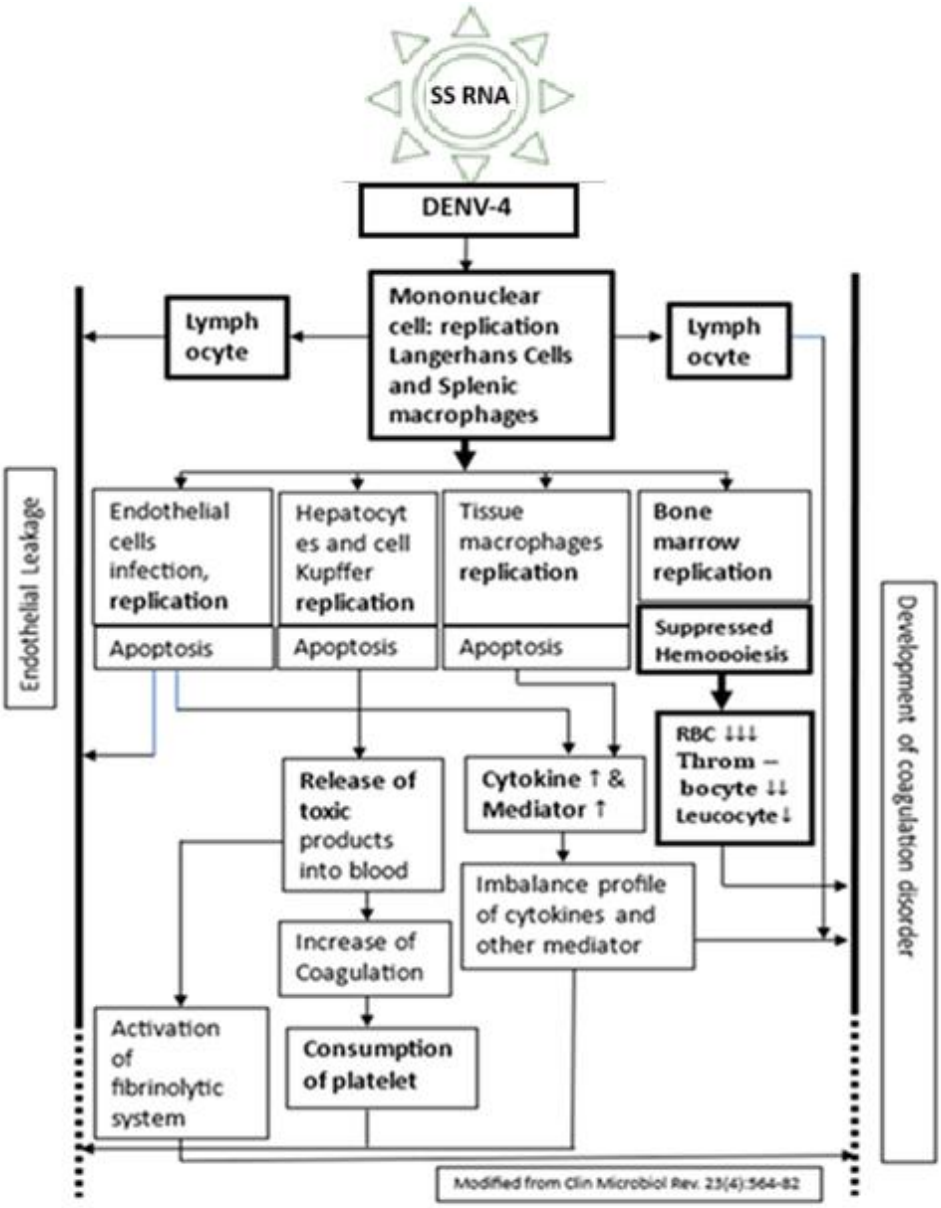


FIGURE 2: Pathogenesis of Lymphoma Malignant non-Hodgkin with drop of Hb and Thrombocyte

Pathogenesis of Development of coagulation disorder (marker: D-dimer) and the endothelial leakage caused by Imbalance profile of cytokine and other mediators in DF, DHF, DSS.[1]

Lymphoma malignant is the Pathologic Anatomy Diagnosis of the splenectomy and cauda pancreatectomy surgery in case 1. LM non-Hodgkin is the chemotherapy diagnosis of case 2. There are also Antibody cross react with platelets and plasmin due to mononuclear cells replications.[1]

4.1 Review cases:

4.1.1 Review Case report DHF, DSS and DEN 1-4:

Pathophysiology all types are the same, DEN Virus (DENV) has 4-5 serotypes. All types are ssRNA virus. So, if someone got DHF, it could be 3 times DHF again. Each person in at risk of dengue fever endemic area, could be 4 times in their lifetime.

Secondary heterogeneity infection is diagnosed by IgG, IgM, whereas **DENV-3** was the dominant serotype (75.9%).[5,6,7,8] Secondary infection with heterologous serotypes is more severe than primary DHF infection, could be explained by IgG for old infection usually DENV-1 or -2, IgM for new infection, usually DEN-3 or -4 in endemic area, tropical rainforest climate.

SS-DENV-3 or -4 secondary infection after -1 or -2, induced endothelial cells apoptosis, suppression hemopoiesis to development of coagulation disorder (increase D-dimer). Lever hepatocytes cells and Kupffer cells replication, necrosis and apoptosis in lever. Splenic and Tissue macrophage replication then apoptosis induced cytokine storm – imbalance profile of cytokine and other mediators. Stimulation B cells and T cells Lymphocytes production (Fig.2). Activation of fibrinolytic system (A) and Consumption of platelets (C). Both A & C induce the development of coagulation disorder (marker: D-dimer).[1] Endothelial cells dysfunction caused by Imbalance profile of cytokine and other mediators.[1]

Review Case report Grade 1: no evidence of bleeding, RL Tourniquet test Positive. Known as Dengue without warning signs.

Review Case report Grade 2: DHF (and Wolbachia). Adult female mosquitos are estimated to be 10 days old, and the lifespan is 14 days. Only the female mosquito could be a vector of DENV. The egg of a female mosquito with Wolbachia couldn't hatch. Known as Dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing hematocrit with decreasing platelets/thrombocytopenia). Evidence of bleeding episodes.

Review Case Report Grade 3: Dengue Shock Syndrome (DSS): presence of weak and rapid pulse rate, low blood pressure or narrow pulse pressure, severe dengue, dengue with severe plasma leakage, where Rx/Albumin is needed. DSS due to secondary heterogeneity DENV-3 infection. whereas DENV-4 occurs at a low prevalence World Wide[6] and spreads the least rapidly.[6] Den-2 has been the most common serotype over the last 50 years. Secondary infection and DENV-3 serotype is most common among dengue patients.[7]

DENV consists of 5 distinct genotypes (I-V). Genotype III is the most widespread and was associated with large outbreaks in Asia, Africa, and America:[8]

DENV-1 and DENV-3 are more pathogenic without immune priming from other serotypes.

Development of Dengue Infection Severity Score is due to thrombocytopenia and leakage of the endothelial cells, and blood pressure/ narrow pulse pressure/ rapid pulse rate, but not in Lymphoma.[4] The DENV also infect and replicates inside a specialized immune cell located in the skin, a type of dendritic cell called a Langerhans cells.[1]

Case report Grade 4 Deadly DLMF: DIC/sepsis (MOD) is when bacteria go inside the blood after post splenectomy or post chemotherapy. And the garden and forest lover only got it although back with antibiotics all the time while needed. Aims: To revised WHO dengue case classification: the system needs to be revised. DENV-4 severity during secondary infection to an antecedent primary DENV-1/-2 infection endemic area. Case DLMF 1 (HUS) only Dx/ by hematology abnormality pre-op, but Dx/ Lymphoma Malignant 3 weeks after Splenectomy and Cauda Pancreatectomy due the extreme drop of Hb and Thrombocyte. Case DLMF 2 (EeS, 70y) only Dx/ non-Hodgkin Lymphoma Malignant (nHLM) & Ca Thyroid by USG (Thymus) before half dose chemotherapy 1 week before mortality. DENV-4 was reported in non-Hodgkin type B with good differentiated (bad prognosis, high grade) vs. poor differentiated (good prognosis, low grade),[2] summons CHOP/ R-CHOP regiment.[2]

CD4+ CD8+ double positive (DP) T cells represent a heterogeneous population. One or more groups of clonal T-cells may be present in a person's lymphocyte population without being considered a lymphoma. Clonal selection of T cells taken place in the thymus and is in charge for producing a useful and functional collection of T cells. The DP thymocytes survive for approximately 3 days previous to undergoing apoptosis (programmed cell death). Splenomegaly and full of nodules, then the fact it is clear and supple post operative describe the process of the apoptosis was seen in case DLMF 1 (HUS, 73y).

In Hodgkin Lymphoma, DNA mutation at B Lymphocytes, and Non-Hodgkin Lymphoma, DNA mutation at B and T Lymphocytes. Case report 2 (EeS) was Dx/ Non-Hodgkin Lymphoma Malignant. He died on 10 Mei 2024.

B Lymphocytes are developed in bone marrow, detect intruder/ attacker and form antibodies to fight infection. After having been stimulated by antigen, B lymphocytes develop into cells producing and secreting antibodies (plasma cells, represent the end-stage of the differentiation of B lymphocytes after their stimulation by antigen). They are thus responsible for the antibody type in the immune response. During development, they are influenced by cytokines and growth factors. And can be characterized according to CD markers (CD19, CD20, CD24, CD72), and presence of immunoglobulins molecules on their outside. T Lymphocytes are produced in thymus. T lymphocytes damage cancer cells and cells which is infected. Th / Treg & effector functions (CD4+), Tkiller Tk and T cytotoxic Tc (CD8+) T suppressor: resulting in osmotic swelling (endothelial cells apoptosis) and following killing and lysis.[4] Th is one of lymphocyte types which help to harmonize immune response so as to approach infection and diseases. In AIDS patients, CD4/CD8 ratio > 1 with CD4 500-1500/mm^3 and CD8 Lymphocyte 150-1000/mm^3, means the immune system is strong and the patients may not have HIV. Case report DLMF 1 (HUS) has

Suppression hemopoiesis due to replication of stromal cells.[1] The author had enough time to think what it should be like, but no behavior representation give assistance to it. Both DLMF cases, the CD4 never deliver below 200 cells/mm^3.

The communication of CD4 with MHC class II greatly become less the number of antigenic peptides required for T cell activation and substantially increases cytokine production by helper T cells.[9] The interaction of CD8 with MHC I can climb up on to a response against pathogens by produce and discharge cytokines and defend from harm of tumors by directly killing transformed cells. Most Tk cells express T-cell receptors (TCRs) that can identify a specific antigen. CD4 cells lead the fight as protection to infections which help Tc/Tk to lyse infected cells. Tk recognizes any infected cell expressing MHC I. Th also helps B Lymphocyte cells to produce highly matured antibodies. Th also encouraging other immune cells, such as macrophages. Both, CD4 (Th) cells and CD8 (Tc/Tk) cells are growth or development different from the common lymphoid progenitor cells in the bone marrow and go on to full grown in the thymus. Low CD4/CD8 ratio reflects increased immune activation and is associated with an increased risk of severe non-AIDS events or cancers. High CD8 count means that the body is effectively controlling the infections. Development always generates more CD4 than CD8 T cells. During a CD8 T-cell reaction to virus infection, there are 3 characteristic phases: a period at the beginning activation and the action of becoming more extensive, a process of becoming smaller of the death phase, and the establishing and maintaining of memory. Memory CD8 T-cell differentiation throughout viral Infection.[1,10] It all needs energy, that's why hypoglycemia and lethargic happens**.**

Neutrophil increase, and Leukocyte decrease. Neutrophil normal 1,450-7,500/μL. In viral infection neutrophil can fall quite low and may stay low for many months. Neutrophil to Lymphocyte Ratio: Normal: 1-2. NLR >3 grade I; < 0.7 Low, mirroring a preserved immune balance. 2.3-3.0 early warning cancer, atherosclerosis, infection, inflammation, psychiatric disorders and stress. High grade NLR narrate bacteria that are already inside the blood vessels. Lymphocyte grow describes viral infection. In both DLMF cases high Lymphocytes have been reported. The NLR cut-off was 3.0. WHO categories for COVID-19 severity (asymptomatic 1.92, mild 2.08, moderate 4.79 and severe 9.9 were used.**[11]**

Hematocrit cut-off >54 Male, 48 Woman, and Rumple Leed and bleeding, none reported in both DLMF cases. Secondary infection and Den-3 serotype most common among dengue patients.[7,8]

4.1.2 SsRNA vs. DsRNA vs. DsDNA, and mRNA:

SsRNA DHF Virus, and also AZ vaccine which is March 2024 recollected from the circulation used for booster. ds RNA or RNAi synthesis to silencing DNA for plant, insect, agriculture and aquaculture. Ds-DNA Wolbachia outside the human body, Adenovirus is one of the DNA viruses.

Messenger RNA (mRNA) is an intermediate which brings genetic information from a gene in the nucleus to a ribosome in the cytoplasmic cell. Transcription is the synthesis of RNA from DNA template where the code in the DNA is converts to a complementary RNA code. Translation is the synthesis of a protein from an mRNA template, where the code in the mRNA is converted into an amino acid sequence in a protein, e.g. spike protein. So ssRNA could be from DHF virus,**[1]** but not from Wolbachia.

TABLE 3
SSRNAV, DSRNAV AND DNAV
1. VIRUS & DISEASE

Virus Name	Disease Name	Contains SS/DS/DNA
SARS-CoV-1 (SARS virus)	Severe Acute Respiratory Syndrome (SARS)	SS RNA
SARS-CoV-2 (corona virus)	breath canal infection of COVID-19	SS RNA
HIV	AIDS (Acquired Immune Deficiency syndrome)	Retrovirus that contains SS RNA
MERS-CoV	Difference than corona virus, Vector: Camel	SS RNA
DENV 1-4	Dengue Fever Classic, DHF, DHF Shock Syndrome, and DF Lymphoma Malignant	SS RNA

MERS (Middle East Respiratory Syndrome)

DENV-3/4 causes: Endothelial cells dysfunction/ leakage (Shock Syndrome) AND Development coagulation disorders (thrombocyte/platelet- LM non-Hodgkin)

2. VACCINE & MANUFACTURE:

Vaccine	Manufacture	Contains SS/DS/mRNA
SARS-CoV-2 (COVID-19)	AZ-Oxford, and J&J	SS RNA spike protein -> B cell used vector adenovirus chimpanzee
SARS-CoV-2 (COVID-19)	Pfizer-BioNTech	mRNA-BNT162b
SARS-CoV-2 (COVID-19)	Moderna	mRNA -1273
SARS-CoV-2 (COVID-19)	Sinovac	Deadly V
DHF Tetravalent (DENV1-4)	Takeda	SS RNA 1-4

4.2 MOD, Endothelial cells dysfunction AND Development coagulation disorders beyond DLMF:

Development coagulation disorders only after post-op (case 1, HUS) and after chemotherapy (case 2, EeS) due to the drop of Leukocytes and T Lymphocyte, Erythrocyte, Thrombocytes, but no report of endothelial cells and development coagulation disorders: Fig 2. Edema anasarca due to multi organ damages (MOD) has been reported in case 2 due to sepsis. Endothelial cells dysfunction AND development coagulation disorders is a good condition to the infection of bacteria into the blood (sepsis) and developed to the end stage known as MOD.

Although live in Wolbachia spreading area Semarang, and Jakarta Barat-Bali, both cases also haven't got ssRNA booster, but high-risk exposure to ssRNA DENV-4 with no IgG and IgM reported. EeS died from MOD post CHOP chemotherapy, also with no endothelial cell's dysfunction and no clinical coagulation disorders. While a long process of fever observation frequently hospitalized since before Sept 2023 and deadly operation of Splenectomy and Cauda Pancreatectomy with 3 days never to wake-up due the knock-down to pain anesthesiology till 19 November 2023. Case DLMF 2 (EeS, 70y) – ssRNA type-4 serologies IgG and IgM were also disregarded, although in Wolbachia spreading area West Jakarta, but no risk of DENV-4. Due to his BHP, fever, and lacking energy, faint and tiredness, EeS has done his demonstration of friendship, and getting in touch with the inheritance, reveals the chronicity of the illness.

Both patients got hepatomegaly and splenomegaly. Case 1 with full nodule in the cauda pancreas, could be due to extreme extra glucose per infusion and per oral. Hypoglycemic is due to replication phase and storm and imbalance cytokine and mediators.[1]

D-dimer cut-off before post op and chemotherapy, increases extremely after post-operation and chemotherapy due to sepsis and the end stage mainly MOD. Thrombocytopenia in the beginning, before operation/chemotherapy, drops at the last days. Decrease Hb in the beginning, before op/chemotherapy, drop at the last days. Hypoglycemia, infused dextrose 5%, extreme glucose per oral, and cauda pancreatomy in DLMF case 1 (HUS, 73y). Ca thyroid diagnosis in DLMF case 2 (EeS,70y), before chemotherapy, is due to the development in thymus,[12] which is normally atrophy in adolescence, which is located in the thyroid area.

Case 1 DLMF (HUS 73 y) splenectomy, and cauda pancreatectomy, 6 days post op Knock-Down due anesthesia pain killer, never wake-up again. When the author asked his sister (Clinical Pathologist) about the argument of hemolysis or for hematopoiesis as the cause of splenomegaly, she didn't give the description of the Blood Smear Morphology-Peripheral. BMP had never been done. And she commented in May 24, 2024 that she didn't know if hemolysis or strong hematopoiesis is the cause of the splenomegaly. He eats dirty street food randomly without any reason, maybe he got chronically poisoned. Sudden splenectomy was indicated by full nodules and quickly dropped the thrombocyte, and cauda pancreatomy by hypoglycemia for 3 months with full nodule on it (Dx/Ca pancreas). He swims 1x/ week, September still swimming, out of first hospitalization also still swimming. Mid November 2023 the last hospitalized, the condition is sleeping all the time, could not open the eyes,

sleepy continuously, difficult to open handphone, then could not open handphone, moreover could not grab the hp, could not self-drinking, could not walk. So, everything drops, Hb, glucose, thrombocyte, while splenomegaly and cauda pancreas increase the size and nodules. It is hemopoiesis due the splenomegaly and also destruction of blood cells, not hemolysis because no shivering was reported. September 29, 2023, he asked to go home, because September 30 he wants to celebrate his birthday. It is all to describe that no one becomes aware of the disease, except it is easy to sleep. Hospitalized not because of fever, or hypoglycemia, or low of Hb, but faint and don't want to eat. Abdominal Pain is a commonly reported symptom in DF.[4,13]

Severe hepatic damage is not common in DENV infections, but elevated liver enzymes suggest that the liver is affected.[1,4] The first diagnosis as cirrhosis has not ever been heard again post operation and post chemotherapy.

Case 2 DLMF (EeS 70 y) got chemotherapy for LGC disorder, 3 days post chemotherapy, lethargy, never talk again. Before the chemotherapy he can contact his cousin indistinctly. On a death case report to the family, a doctor said the thrombocyte become only 200, and could not be saved.

Case 1 (HUS, 73y) 3 months in and out the hospitalized for blood transfusion and hypoglycemic. After 3 months he said Leukemia and severe thrombocytopenia, but then hematology malignancy. PA 3 weeks after Splenectomy and cauda pancreatomy: Lymphoma Malignant.

Case 2 (EeS, 70y) never being hospitalized, come to the hospital himself with fever observation for BHP medical check-up, go out with Dx/ Lymphoma malignant, Ca thyroid with thrombocytopenia and edema anasarca caused by Multiple Organ Damages (MOD).

The thrombocytopenia in dengue is caused by the suppression of bone marrow, platelet destruction by antibodies to the dengue virus, excessive consumption of platelets, viral-replication-mediated destruction of platelets by complement-mediated lysis, and apoptosis.[1,14]

4.3 Comparison Serotype DEN 1-4:

The flavivirus genome consists of ± 11,000bps, which translates into three structural and 7 nonstructural proteins (See "Chimeric virus vaccines"), later. A successful extra valent dengue vaccine should concurrently protect against all 4 serotypes because each person could 4 times be infected by DENV. DENV1-4 have different 3 phases (fever, critical, and recovery), DENV-2 Hemorrhagic critical, DENV-3 Shock Syndrome critical, and DENV-4 have a long recovery phase which could be DLMF. Mutation by DsDNA could be ignoring the stop codon (i.e. UAA, UAG and UGA), which is a generated readthrough mechanism, continuing on that makes a longer string of amino acids. Sometimes this function is combined with the ribosomal frame-shifting to produce an even greater variety of viral proteins.[15] which induced Lymphoma Malignant,[16] which could be by STAT3 mutation,[17,18,19] The global trends in research on endothelial cells and sepsis 2002-2022 has been reported,[20] and the mechanism supported the mechanism in these 2 cases. Wolbachia 16S rRNA haplotypes detected in wild Anopheles stephensi has been reported in eastern Ethiopia,[21] whereas detection of Wolbachia genes in patient with non-Hodgkin's lymphoma already has been set up and reported before.[22] Clinical assessment of dengue and identification of risk factors for severe disease protocol for a multicenter study in 8 countries, incl. hematocrit, white blood cell count, lymphocyte count, thrombocyte count, etc. as well as SGOT/SGPT, plasma albumin etc.[23] Identification of early serological correlates of serious dengue are included, but not yet implicit Lymphoma malignant,[23] although splenomegaly 67% has reported caused by STAT3 -T-Cell Large Granular Lymphocytic Leukemia.[19] STAT3 mutations show the presence of subclinical T-cell clones in a subset of aplastic anemia (AA) and myelodysplastic syndromes (MDS) patients,[24] supported by Lymphotropic virus: chronic inflammation bringing rise of cancer.[25] It seems Adult T-Cell Leukemia/Lymphoma as new biologic insight, and new direction in treatment since 2017 by Mehta-shah, should not be neglected. Prevalence in Indonesia -1 (33.6%), -3 (28.4%); -2(20.5%), and -4(14.9%): 22Oct 2020.[2] Low carbohydrate, high fat, high protein should be given in this early biomarker for prediction of serious thrombocytopenia in DLMF new classification in tropical rainforest area (low- to middle- income countries during epidemics,[27] whereas decreased albumin level was strongly associated with DHF.[27] ITP associated SarCov2 has been revealed.[28]

LIMITATION

This study did not elaborate specifically severe score DENV-1 and DENV-3, which are more pathogenic without immune priming from other serotypes.[4]

It is also recorded unexplained cytopenia in MDS associated with excellence prognosis of Low-Risk MDS without detectable Myeloid-Related mutations.[29] The early detection of DLMF from DF to ITP/MDS/AML[30] and high protein therapy[31] support this study, early mass diagnosis and therapy, but not covered.

V. CONCLUSION

DENV 1-4 is Ss RNA incl. DENV-4 infection as secondary heterogenous DENV-1/-2 or visa versa, which induce anemia and thrombocytopenia ITP with splenomegaly/Lymphoma Malignant, should be classified to DLMF (grade-4) of DHF classification.

Ss RNA incl. DENV-4 infection as secondary heterogenous DENV-1/-2 or vice versa, which induce anemia and thrombocytopenia ITP, then splenomegaly/Lymphoma Malignant, should be classified to DLMF (grade-4) of Dengue Fever classification.

ACKNOWLEDGEMENT

AstraZeneca and detik.com, who exposed the withdrawal of SS-RNA all over the world in May 7, 2024.

CONFLICT OF INTEREST

The author declares No competing interests.

REFERENCES

[1] Martina BEE, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. Clin Microbiol Rev 2009;23(4):564-82. https://doi.org/10.1128/CMR.00035-09

[2] Salma RS, Sedana MP, Yudho SU. CHOP and R-CHOP Therapeutic Responses in Non-Hodgkin Lymphoma Patients in Dr. Soetomo General Hospital Surabaya. Biomol and Health Sc J 2018;1(2). https://doi.org/10.20473/bhsj.v1i2.9244

[3] Aryati A, Wrahatnala BJ, Yohan B, Fanny M, Hakim FKN, Sunari EP et al. Dengue Virus Serotype 4 is responsible for the outbreak of Dengue in East Java City of Jember, Indonesia. Viruses 2020; 12(9); 913. https://doi.org/10.3390/v12090913

[4] Bhandari S, Rankawat G, Goyal B, Lohmror A, Gupta V, Singh A. Establishment of SMS Dengue Severity Score. J Assoc Physicians India 2022 Sep;70(9):19-22. https://doi.org:10.5005/japi-11001-0095

[5] Zerfu B, Kassa T, and Leqesse M. Epidemiology, biology, pathogenesis, clinical manifestations, and diagnosis of dengue virus infection, and its trend in Ethiopia: a comprehensive literature review. Trop Med and Health 2023;51(11). https://tropmedhealth.biomedcentral.com/articles/10.1186/s41182-023-00504-0

[6] Kularatne SA and Dalugama C. Dengue infection: Global importance, immunopathology and management. Clin Med (Lond), 2022; 22(1):9-13. https://doi.org/10.7861/clinmed.2021-0791

[7] Ipa M, Astuti EP. Secondary infection and Den-3 serotype most common among dengue patients: a preliminary study. Health Science Indones 2010;1(1):14-19). https://media.neliti.com>55799-secondary-infection-and-den-3-serotype-m-a41e0739.pdf

[8] Naveca FG, Santiago GA, Maito RM, Meneses CAR, Do Nascimento VA, deSouza VC, do Nascimento FO, et al. Reemergence of Dengue Virus Serotype 3, Brazil, 2023. Emer Infec Dis 2023;29(7):1482-84. https://doi.org:10.3201/eid2907.230595

[9] Li Y, Yin Y, Mariuzza RA. Structural & Biophysical insight into the role of CD4 and CD8 in T cell activation. Front Immunol 2013;4. https://doi.org/10.3389/firmmu.2013.00206/full

[10] Wherry EJ, and Ahmed R. Memory CD8 T cell differention during Viral infection. J Virol 2004;78(11):5535-45. https://doi.org/10.1128/JVI.78.11.5535-5545.2004.

[11] Toori KU, Qureshi MA, Chaudhry, Safdar MF. Neutrophil to lymphocyte ratio (NLR) in COVID-19: A cheap prognostic marker in a resource constraint setting. Pak J Med Sc 2021;37(5):1435-39. https://doi.org/10.12669/pjms.37.5.4194

[12] Singer A, and Bosselut R. CD4/CD8 coreceptors in thymocyte development, selection, and lineage commitment analysis of the CD4/CD8 lineage decision. Adv Immunol, 2004;83:91-131. https://doi.org/10.1016/50065-2776(04)83003-7

[13] Kanna S, Vij JC, Kumar A, Singal D, and Tandon R. Etiology of Abdominal Pain in Dengue Fever. Dengue Bulletin 2005; 29(**..**): 1-5. https://www.academia.edu/70572269/Etiology_of_abdominal_pain_in_dengue_fever

[14] Sontakke RA, Aglave NR, Dua H. Correlation of Platelet Parameters with the Severity of Thrombocytopenia in DHF in Children Aged Less Than 18 years at a Tertiary Care Centre: A Cross-Sectional Study. Cureus, 2024. https://doi.org:10.7759/cureus.56829

[15] Bruslind L. 22: The Viruses, Viral Classification, readthrough mechanism. LibreTexts Biology 2024; June 4: 10.00 am. WIB https://bio.libretext.org/Bookshelves/Microbiology/Microbiology_(Bruslind)/22%3A_The_Viruses

[16] Freedman AS, Freidberg JW, Aster JC. Clinical presentation and initial evaluation of non-Hodgkin lymphoma. In: Post T, ed. Uptodate. UpToDate, 2022.:4pages Accessed 2023, Sept 13. https://www.cancer.org/camcer/types/non-hodgkin-lymphoma/about/what-is-non-hodgkin-lymphoma.html

[17] Morgan EA, Lee MN, DeAngelo DJ, Steensma DP, Stone RM, Kuo FC, Aster JC, et al. Systematic STAT3 Mutation Testing Identifies Patients with Unsuspected T-Cell Large Granular Lymphocytic Leukemia. Blood 2016;128(22):919.

https://doi.org/10.1182/blood.V128.22.919.919

[18] Munoz-Garcia N, Jara-Acevedo M, Caldas C, Barcena P, Lopez A, Puig N, et al. STAT3 and STAT5B Mutations in T/NK-Cell Chronic Lymphoproliferative Disorders of Large Granular Lymphocytes (LGL): Association with Disease Features. Cancers 2020;12(12)3508. https://doi.org/10.3390/cancers12123508

[19] Gorodetskiy V, Sidorova Y, Biderman B, Kupryshina N, Ryzhikova N, and Sudarikov A. STAT3 mutations in "gray-zone" cases of T-cell large granular lymphocytic leukemia associated with autoimmune rheumatic diseases. . Front Med (Lausanne), 2022;9:1000265. https://doi.org/10.3389/fmed.2022.1000265

[20] Shi Y, Ji S, Xu Y, Ji J, Yang X, Ye B, Lou J, Tao T. Global trends in research on endothelial cells and sepsis between 2002 and 2022: A systematic bibliometric analysis. Heliyon 2024;10(1): e23599. https://doi.org/10.1016/j.heliyon.2023.e23599

[21] Waymire E, Duddu S, Gebremeskel SY, Getachew D. Wolbachia 16S rRNA haplotypes detected in wild Anopheles stephensi in eastern Ethiopia. Parasites & Vectors 2022; 15(1). https://doi.org/10.1186/s13071-022-05293-9

[22] Chen X-P, Dong Y-J, Guo W-P, Wong W, Li M-H, Xu J, Dumler JS, Zhong Y-Z. Detection of Wolbachia genes in a patient with non-Hodgkin's lymphoma. J Clin Microb Infect 2015;21(2):182.e1-e4. https://doi.org/10.1016/j.cmi.2014.09.008

[23] Jaenisch T, Tam DTH, Kieu NTT, Ngoc TV, Nam NT, Kinh NV, et al. Clinical evaluation of dengue and identification of risk factors for severe disease protocol for a multicenter study in 8 countries. BMC Infec Dis 2016;16,120. https://doi.org/10.1186/s12879-016-1440-3

[24] Jerez A, Clemente MJ, Makishima H, Rajala H, Gomez-Segui I, Olson T, et al. STAT3 mutations indicate the presence of subclinical T-cell clones in a subset of aplastic anemia and myelodysplastic syndroms patients. Blood 2014;123(21):3364.
https://doi.org/10.1182/blood-2013-040494930

[25] Harhaj EW and Shembade N. Lymphotropic Viruses: Chronic Inflammation and Induction of Cancers. Biology 2020:9(11), 390. https://doi.org/10.3390/biology9110390

[26] Mehta-Shah N, Ratner L, and Horwitz SM. Adult T-Cell Leukemia/Lymphoma: Novel Biologic Insights and New Directions in Treatment. J Oncology Pract 2017;13:493-5 https://doi.org/10.1200/JOP.2017.021907

[27] Moallemi S, Lloyd AR, , and Rodrigo C. Early biomarkers for prediction of severe manifestations of dengue fever: a systematic review and a meta-analysis. Sci Rep 2023;13:17485. https://doi.org/10.1038/s41598-023-44559-9

[28] Gonzalez-Lopez TJ. Recommendations on the management of patients with immune thrombocytopenia (ITP) in the context of SARS-CoV-2 infection and vaccination: consensus guidelines from a Spanish ITP Expert Group. Infectious diseases and therapy 2023; 12(2):303-15. https://doi.org/10.1007/s40121-022-00745-2

[29] Polprasert N, Niparuck P, Rattanathammethee T, Chuncharunee S, Kobbuaklee S, Songserm K, et al. Excellent Prognosis of Low-Risk Myelodysplastic Syndromes (MDS)Without Detectable Myeloid-Related Mutations. Clin Lymphoma Myeloma Leuk 2022, May;22(5):e293-e299. https://doi.org/10.1016/j.clml.2021.10.015.

[30] Samsuria Mutalib PK, Samsuria IK, Rahardja AA. Early Laboratory Packet Diagnosis for Successful Fighting MDS and AML (ITP and LGC). International Journal of Environmental and Agriculture Research. 2024;10(12):31–5.
https://dx.doi.org/10.5281/zenodo.14575089

[31] Samsuria Mutalib PK, Samsuria IK, Rahardja AA. High Protein, Low Carbohydrate, High Non-Trans Fat, and Decitabine for Survival-ITP and LGC Leukemia. International Journal of Environmental and Agriculture Research. 2025;11(3):51–6.
https://doi.org/10.5281/zenodo.15106107

AD Publications

Sector-3, MP Colony, Bikaner, Rajasthan, INDIA

www.adpublications.org, www.imjhealth.org, info@imjhealth.org