

ISSN - 2070-0284
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VOLUME 11 | NUMBER 09 | 2022

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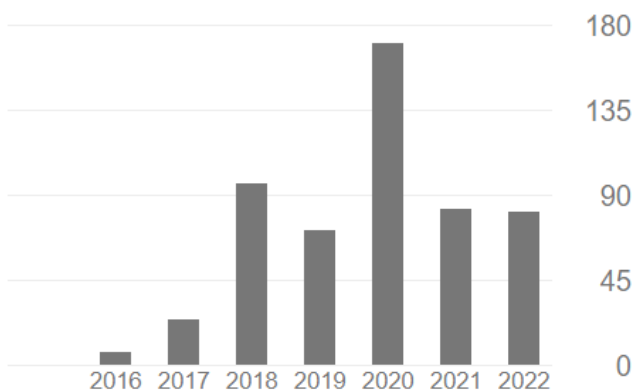
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 Yosephina Elizabeth Sumartini Gunawan, Cicilia Ambu Njurumana, Umbu Nggiku Njakatara, Leni Landudjama, Wanti Wanti


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Review of the implementation of malaria elimination program in East Indonesia

 Yosephina Elizabeth Sumartini Gunawan, Imelda Sariyanti Lalo, Uumbu Nggiku Njakatara, Leni Landudjama, Yurissetiowati Yurissetiowati


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A randomized prospective study

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N-Acetyl Cysteine in combination with vancomycin or linezolid for biofilm eradication of methicillin-resistant Staphylococcus aureus (MRSA)

 Eny Purwoningsih, M. Vitanata Arfijanto, Agung Dwi Wahyu Widodo

 803-810

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A case study on the Mawar Emas Program of West Nusa Tenggara


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Risk factors of platelet refractoriness after thrombocyte concentrate transfusion in pediatric acute leukemia

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Case report

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Effect of education level, income level, and tax policy on taxpayer compliance during the COVID-19 pandemic

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A case report

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PCR detection of Gene Stmpr1, Stmpr2 and Stmpr3 encoding protease of Stenotrophomonas malltophilia isolated from different infection

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The negative effect of COVID-19 pandemic on the different undergraduate student during 2020-2021

 Mohammed Al-Mousaw, Mohsin Abdulhussein Hasan, Hujran Abed Al-Raheem Abed, Hasan Ayad Kareem, Fahad Najd Obaid

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Role of organic nutrient (Tecnokel) and application methods on some productive traits of two cauliflower hybrids (Brassica Oleracea var. botrytis)

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Utilization of Egyptian mulberry fruits to improvement the physicochemical and nutritional properties of ice milk

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Biological activity against breast cancer cells of iron(II), copper(II) & nickel(II) complexes with Di-m-bromobenzalcylohexanone & dibenzalcylohexanone - thiosemicarbazide schiff bases

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 Eka Wilda Faida, Stefanus Supriyanto, Setya Haksama, Wihasto Suryaningtyas, Widi Astuti, Bambang Nudji, Siti Nur Hasina

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The existence of a healthy environmental protection law and state responsibilities in Indonesia

 Budi Sulistiyo Nugroho, Ahmad Wahyu Hidayat, B. M. A. S. Anaconda Bangkara, Irma Rachmawati Maruf, Puji Hastuti

 1206-1218


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A cross sectional study

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 1219-1225

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A systematic review

 Rekha Kaushal, Shiny Charles Rose, Sunita Sehrawat, Rachna Sharma, Parvesh Lata, Igona Gorakhnath

 1236-1247

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Spectrophotometric method for the determination of chlordiazepoxide, diazepam and alprazolam drugs by using oxidation coupling reaction

 Esraa Muhammad Mohsen, Abdulbari Mahdi Mahood, Aula M. Al Hindawi

 1260-1276

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Stability of dental implants placed in healed bony sites of hyperlipidemic patients

A case series


 Maie Ismaiel Mohammed Fathy, Marwa Amr Al-Nawawy, Amr Fouad Zahran

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Evaluation of salivary parameters (flow rate, pH, amylase and total protein) in periodontitis patients based on CPITN criteria


 Farah Ambreen, Dur E Shehwar Ali, Sundus Faiz, Inayat Shah, Bazilina Majid, Zaid Khan

 1286-1295

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Virtual reality versus whole body vibration on quadriceps muscle strength in children with thigh burn


 Mohamed Ali, Hazem Nour, Naglaa A Zaky, Manal Abdel Wahab

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A cross sectional study on cytomorphological diagnosis and clinical presentation of various thyroid diseases in Tribal areas of Odisha


 Shree Kumar Chinmayananda Mishra, Rabinarayan Rout, Debasis Debadatta Behera, Binod Kumar Sahu

 1305-1311

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Minimum relevant features to obtain AI explainable system for predicting breast cancer in WDBC


 Agarwal Rashi, Revanth Madamala

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Study of infiltration patterns of lymphomas in bone marrow trephine biopsy and immunohistochemical analysis in a tertiary care institute


 Mustafa Ali, Anil Meena, Fatima Bhopalwala, Neha Banseria


 1327-1334

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Physiological and psychological variables among elite female athletes from three categories of sports and non-athletes

 Jyotsna Aggarwala, Rinku Garg, Subhra Chatterjee

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Sexual education intervention for the prevention of premarried sexual behavior in early adolescents

A literature review

 Indra Fajarwati Ibnu, Chatarina Umbul Wahyuni, Shrimarti Rukmini Devy, Hari Basuki Notobroto


 1348-1354

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Correlation between C-Reactive Protein (CRP) and lymphocyte levels in the acute phase with spirometry test in severe Coronavirus Disease 2019 (COVID-19) survivors after twelve weeks

 Agustina Dwiningtyas, Ida Bagus Ngurah Rai, Ketut Suryana, I Gede Ketut Sajinadiyasa, Ida Ayu Jasminarti, Ni Wayan Candrawati, Eka Arisanti

 1355-1372

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Effects of moderate intensity treadmill exercise with gradual increase in speed and inclination on serum C-reactive protein in male with type 2 diabetes mellitus

 Dimas Bagus Respati, Damayanti Tinduh, Indrayuni Lukitra Wardhani, Sony Wibisono Mudjanarko


 1373-1382


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Implementation of education to batik workers about contact dermatitis in Tanjung Bumi, Bangkalan, Madura, Indonesia

 Cita Rosita Sigit Prakoeswa, Damayanti, Sylvia Anggraeni, Menul Ayu Umborowati, Sri Awalia Febriana, Katharina Oginawati, Ikeu Tanziha

 1383-1394


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Effectiveness of selenium supplementation in children with autoimmune thyroiditis

A systematic review and meta-analysis

 Yuni Hisbiyah, Anang Endaryanto, Bagoes Setyo-boedi, Nur Rochmah, Muhammad Faizi, Citrawati Dyah Kencono Wungu, Qurrota Ayuni Novia Putri

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A case study in Java-Bali Indonesia

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A case-control study


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To evaluate effect of three different reducing agents in recovery of bond strength to sodium hypochlorite treated dentin with composite resin

An in vitro study

 Abhishek Shelke, Aniket Jadhav, Anupam Sharma, Abhijit Jadhav, Shivani Chavan, Damini Bhagwat

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An in vitro study


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What makes a sustained development and participation of Triathlon in Southern China?

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An original research

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An original research

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 1733-1744

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A rare case report

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Purple sweet extract activates autophagy through enhanced mTOR expression in D-galactose-induced dementia rats

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Perspectives and reality

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A comparative study

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The impact of ESG strategies integration on the operational and reputational risks

Literature review for the listed insurance companies at Amman Stock Exchange

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Diagnostic Value of Volumetric Histogram- Based Diffusion Analysis in the Differentiation between Benign & Malignant Gynecologic Lesions

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Prevalence of salivary gland neoplasms in the head and neck in educational hospitals in Cairo Governorate

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 1840-1852

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 1866-1877

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 1894-1907

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 1918-1926

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 Nur Rochmah, Muhammad Faizi, Yuni Hisbiyah, Rayi Kurnia Perwitasari, Tyas Maslakhatien Nuzula, Anang Endaryanto, Soetjipto Soetjipto


 1927-1936

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A systematic review

 Shrikanth Muralidharan, Swapna Patankar, Yashodhara Shah, Prashant Rao, Prasad Karande

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The impact of family-based nutrition education intervention program on eating habit among obese school age children in Rania city / Iraq

 Mahmood Arif Mahmood, Norhan Zeki Shaker, Sherzad Ali Ismael

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An evaluative study

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A quasi-experimental

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Macronutrient intake pattern among type 2 diabetic adults visiting a tertiary care hospital of rural Vadodara

A cross sectional study

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 B. M. A. S. Anaconda Bangkara, Irma Chmawati Maruf, Abdul Latif, Hasna Hashibah, Ikhsan


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Knowledge, attitude and acceptance of amputation among amputee patients admitted in National Orthopaedic Hospital Enugu - Nigeria

 Justin Agorye Ingwu, Happiness Ogadi Anikwe, Chinenye Anehkhatai, Hope Opara, Patricia Okpala, Mathias Agba


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Study of endothelial cell loss in small incision cataract surgery (SICS)

 Sujata R. Charel, Hetaj K. Sheth, Astha Domadia, Jash Bavishi, Niyant N. Pandya


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Screening of catharanthus roseus stem extract for anti-ulcer potential in wistar rat

 Suraj Mandal, Sweta Goel, Monika Saxena, Polly Gupta, Jyoti Kumari, Prashant Kumar, Mukesh Kumar, Rahul Kumar, Km. Shiva


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The glycemc control among type 1 diabetes children pre and during COVID-19 pandemic

A systematic review and meta-analysis


 Wika Y. Deakandi, Farahdina Farahdina, Qurrota A. N. Putri, Tyas M. Nuzula, Katherine Fedora, Qorri Aina, Nur Rochmah, Muhammad Faizi

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Isolation, Screening and molecular characterization of bio surfactant producing microbes from hydrocarbon polluted soil


 Putha Deepika Sai Lakshmi, Beesa Hrushik Samyukth, Golamari Siva Reddy, Boddu Sumalatha

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Postoperative bone defects' reconstruction in patients with focal form of chronic post-traumatic osteomyelitis

 Aleksandr G. Sonis, Evgeniy A. Stolyarov, Jean Geringer, Denis G. Alekseev, Sergey V. Ladonin, Maria Yu. Sefedinova, Maria A. Bezrukova

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 Andrey V. Andreev, Mikhail V. Krasnov, Larisa P. Nikitina


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Effect of probiotic mouthwashes in comparison to chlorhexidine on periodontal health

Systematic review and meta-analysis


 Swetha Alahari, Ramasubbareddy Challa, Krishna Chaitanya, Lamea Afnan, Ankita Khandelwal, Sunnypriyatham Tirupathi

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A pilot trial

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A meta-analysis and systematic review

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A prospective observational study in a tertiary care teaching hospital Telangana India

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A systematic review and meta-analysis

 Sylvia Anggraeni, Damayanti, Menul Ayu Umborowati, Cintya Dipta Riswanto, Sholahuddin Rhatomy, Hari Basuki Notobroto, Anang Endaryanto, Isaak Effendy, Cita Rosita Sigit Prakoeswa


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A case report

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Correlation between alveolar-arterial oxygen tension difference gradient baseline with spirometry test in severe coronavirus disease 2019 (COVID-19)

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Mitral Para-valvular leakage following usage of non-pledgeted compared to Teflon-pledgeted sutures

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The effect of patient's characteristics on CYP2C19 polymorphisms based on weight and gender groups of epileptic seizure patients taking divalproex sodium

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Evaluation of the impact of successful percutaneous balloon mitral valvuloplasty on the left ventricular twist and untwist mechanics by the two dimensional speckle tracking imaging

Comparative study between Inoue and multi-track balloon mitral valvuloplasty techniques

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Kavinkumar P, Uma Maheswary, Aryamol K B, Arun Bharath U, R. Chandrasekar

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A literature review

Hiroj Bagde, Rachita Mustilwar, Supriya Mishra, Palak Upadhyay, Machireddy Bhavishyavani, Lalit Darade

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Lazem Najm Abdullah Ali Al-Jumaili, Sami Mahmoud Freih

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Predictive values of first trimester ultrasound screening for twin-to-twin transfusion syndrome and selective intrauterine growth restriction in monozygotic twin pregnancies

Abd El-Nasar Abd El-Gaber Ali, Mostafa Hussein Mohamed, Mostafa Mohamed Khodary, Altayeb Abd-alal Mostafa Mohamed

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Diagnostic & prognostic impact of 18F-NaF PET/CT versus 99mTc-MDP bone scan in detection of bone metastases

Initial and follow up assessment

 Salma AbdelAziz Badr, Hosna Moustafa, Walid Omar, Ibrahim Siddiq, Mai Amr Elahmadawy


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Selective approaches to synthesize a new series of fused 4-amino pyrimidine derivatives by using of 4- amino nicotino nitrile as an effective precursor

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 2832-2843

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 2844-2851

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Effect of empowerment program on parents' self competence regarding care of their children with cochlear implantation

 Hanan Mohamed Tork, Hanan Elsayed Metwally Mansour, Rawia Abd El-ghany Mohamed

 2852-2873


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A case study

 Atri Ghosh, Santosh Rahinj, Mayur Shiralkar, Anand Kale, Prashant Ganbote

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Dentigerous cyst enucleation

A conservative approach

 Sanika Gokhale, Sanket Kunte, Krishna Patil, Rohan Shah, Laxmi Lakade, Shweta Chaudhary

 2883-2889


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 Rand O. Aljamil, Rasheed M. Yousuf, Nabhan A. Hamdoon

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Comparison of neutrophils lymphocyte ratio in type-2 diabetes mellitus patients and healthy individuals


 Sanjeev K Yadav, Ailani Vinita, Ahmad Nisar, Pal Alok, Yadav Amit K

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Induction of mutation by treatment with colchicine and spraying with progesterone for two cultivars of zinnia elegans by using DNA molecular

 Maab M. O. Mohammed, Hala Abd. Abdul-kader, Kamal B. Esho

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A rare case report

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 Bandita Priyadarsini, Kirtirekha Mohapatra, Madhushree Naik, Sasmita Behuria

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Buccal drug delivery system

A review

 Ramdas T. Dolas, Abhijeet D. Kulkarni, Vishal S. Gulecha, Amar G. Zalte, Swati G. Talele, Gayatri N. Bedarkar


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Low level quantification of two potential genotoxic impurities in rilpivirine hydrochloride drug substance by HPLC technique

 N. G. Dumbre, V. V. Chopade, P. D. Chaudhari

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Analysis of clinical profile of malaria patients attending a tertiary care centre in Central India

 Harendra Singh Jadon, Jitendra Mourya, Sachin Singh Yadav


 3020-3026

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Comparison of functional status, outcome of patient and complications in dynamic compression plating & interlocking nail in fixation of fracture shaft humerus

 Fahad Bin Hamid, Gurminder Singh Bedi, Shardaindu Sharma, Farah Ahmed

 3027-3036


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A study of clinical profile of patients with chronic obstructive pulmonary disease

 Jitendra Mourya, Harendra Singh Jadon, Sachin Singh Yadav

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Comparative evaluation of chemiluminescence immunoassay and immunochromatographic test with ELISA for detection of HCV infection

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The effect of slow stroke back massage nursing (SSBMN) cutaneous stimulus on pain intensity on sweet food crawlings (Arai Pinang) suffering low back pain (LBP)

 Aulia Asman, An Aulingga Asman, Auzia Asman, An Autika Asman


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 Mona Shahin, Ali Eldeeb, Hanaa Elbanna, Marwa Aboelhawa



Investigation of sod2 gene polymorphism in the patients associated with type-2 diabetes mellitus patients

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Evaluation of the immune response to silver nanoparticles against cutaneous leishmaniasis in albino mice Balbs/c

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Assessment of nutritional status and adequacy of fiber intake in a group of obese children and adolescents

Marwa Ahmed Sedkey, Hannah Mohamed Aboulghar, Hend Mehawed Abdel Latif Soliman, Radwa Marawan Abdel Halim, Shrouk Moataz Abdallah

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Diagnostic role of fiberoptic bronchoscopy in various respiratory diseases at esic medical college hospital Sanath Nagar

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Assessment of impact of fluoride on mechanical properties of NiTi and Cu NiTi orthodontic archwires

Shailesh B Dongre, Varsha Merani, Waseem Khan, Suyog Shendage, Arun Mhaske

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Fascin expression in breast cancer

Prathipaa R, Divya J, Ashutosh JHA, Muthukrishnan. R

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Effect of asthma on the English-speaking and reading comprehension ability of dusty allergy affected school children


A pediatric research

Nawaf Al Anazi, Muhammad Khalid Mehmood Sajid, Ghassan Adnan Hasan, Zafar Iqbal

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A randomized control trial

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An in-vivo study



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Case study from Jordan


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A case control study

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Retrospective observational study

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A randomized controlled trial

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Clinical study

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A cross-sectional study

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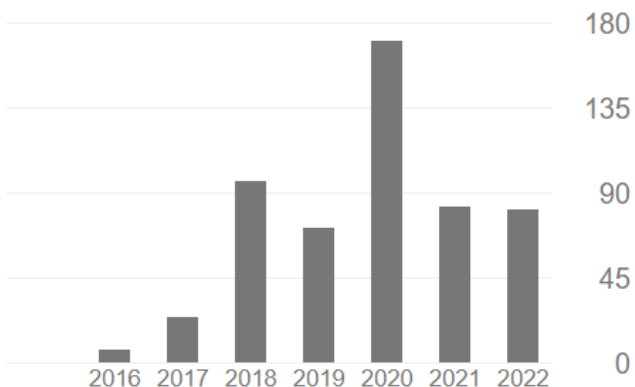
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Dexamethasone and olmesartan as potential antiremodelling agents of valvular interstitial cell into myofibroblast: *In vitro* study on α -smooth muscle actin expression

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Abstract--Rheumatic heart disease is a late complication of valvular inflammation caused by rheumatic fever. Studies have shown that the differentiation of valvular interstitial cells (VIC) into fibroblasts plays an important role in valvular remodeling and fibrosis. Various strategies to minimize valvular fibrosis has increased recently. This study aims to analyze the effect of dexamethasone, olmesartan, and its

combination in inhibiting TGF- β 1-induced VIC differentiation into myofibroblast. In vitro laboratory experimental-posttest only control group design was conducted. Isolated VIC of *Oryctolagus cuniculus* was pretreated using 2,5 ng/mL of TGF- β 1 and divided into groups of dexamethasone (0.1 μ M/L), olmesartan (100 nmol/L), and its combination. Inhibition of myofibroblast differentiation was quantified by the expression of α -SMA levels detected by immunofluorescence. Dexamethasone, olmesartan, and its combination administration were significantly reduced TGF- β 1-induced VIC differentiation into myofibroblast expressed by α -SMA levels (dexamethasone 6823 ± 1735.3 , olmesartan 6683.7 ± 2795.05). Combination of dexamethasone and olmesartan exhibit the most potent inhibition compared to control (5051.87 ± 1612.210 vs 22286.73 ± 2780.2 ; $p < 0.000$). In conclusion, dexamethasone, olmesartan, and the combination can significantly reduce the differentiation of VIC into myofibroblasts. The greatest potential is the combined effect of dexamethasone and olmesartan, while dexamethasone and olmesartan have the same potential.

Keywords---Valvular interstitial cell, myofibroblast, α -Smooth muscle actin, dexamethasone, Olmesartan

Introduction

Rheumatic heart disease (RHD) is a sequela of acute rheumatic fever with serious long term complication and morbidity which still a significant public health problem with 33.4 million cases and mortality rate up to 319,400 in 2015.(Guilherme & Kalil, 2020; Ordunez et al., 2019; Watkins et al., 2017) One of the serious sequela of RHD is the progression of mitral valve damage in 50-60% of cases, multivalvular and mixed lesions are found in 20%, with the most common pathology being stenosis (34% mitral; 9% aortic). The long-term effectiveness of mitral valve surgery, both mitral valve replacement and mitral valve repair, has not shown satisfactory results with 10-year survival rate of 62% in the mitral valve replacement group and 46% in the mitral valve repair group.(Thourani et al., 2003) Meanwhile, in individuals undergoing percutaneous mitral commissurotomy (PMC), prior prospective cohort study showed a survival rate of 68% at 20 years and only 16% of patients did not require reinvention.(Bouleti et al., 2013)

The use of PMC and valve replacement or repair surgery as the currently available standard therapy, shows the low long-term effectiveness of valve intervention measures. Interventions using surgery and minimally invasive procedures are practically only focused on the valve leaflets and annulus, even though the pathological process also occurs at the cellular level, especially fibrosis in the sub-valvular apparatus, ventricular, and atrial myocardial remodeling due to pressure/volume overload exposure and chronic inflammation.(Yankah et al., 2011) Intervention in the early inflammatory phase using antifibrotic agents is a new hope to break the bridge of acute rheumatic fever into valvular pathology in RHD. Chronic inflammatory exposure involving TGF- β 1 in valve interstitial cells

(VIC) due to cross-reactivity of group A streptococcal M protein, induces activation of the *Smad* signaling pathway, mitogen-activated protein kinase (MAPK), and extracellular signal-regulated kinase 1/2 (ERK1/2) will cause myofibroblastic differentiation, induction of extracellular matrix synthesis resulting in fibrosis and distortion of valve architecture.(Vaideeswar & Butany, 2016)

Valve stenosis is a terminal process of the acute phase of valvular disease inflammatory response. Various methods of treating valve stenosis surgically and minimally invasive methods have been developed, but limitations in terms of long-term benefit, survival, patient selection, and complications limit the application of this method. This issue has recently attracted interest to find novel antiremodelling agents. The antifibrotic effect of angiotensin receptor blockers (ARBs) are demonstrated through antagonism of the angiotensin II type I receptor (AT1R) on local Ang II exposure in inflamed tissues, resulting in inhibition of myofibroblast growth and induction of extracellular matrix synthesis.(Yang et al., 2005) In addition, early studies revealed that glucocorticoids also may exert some anti-atherosclerosis effects with reduced atherosclerosis severity in animal model.(Asai et al., 1993; Makheja et al., 1989) The onset of atherosclerosis is triggered by proinflammatory mediators, which induce adhesion molecules in endothelial cells by activating MAPK, especially p38 MAPK.

Furthermore, inhibition of fibrogenesis via transforming growth factor (TGF- β 1) pathway using various agents has been investigated in pulmonary alveolar cells (Guzy, 2020), renal glomerular cells (Isaka, 2018), hepatocytes(Masuda et al., 2020), myocytes (Parichatikanond et al., 2020), and fibroblasts ventricles(Peng et al., 2010; Zhou et al., 2015). However, to the best of our knowledge, there have been no studies examining the antifibrotic properties of ARBs and steroids on VIC. Based on these considerations, this study aimed to assess the effectiveness of dexamethasone and olmesartan in inhibiting fibrogenesis of VIC in the early inflammatory phase, so that the process of valve fibrosis can be prevented in the acute phase of the disease and patients can avoid long-term sequelae.

Method

1.1 Study design and animal model

This in vitro post-test control group only study was conducted in Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia. Some of the interventions given were (1) Dexamethasone; (2) Olmesartan; (3) Dexamethasone-Olmesartan combination. The experimental subject used in this study was valvular interstitial cell (VIC) induced by TGF- β 1. The experimental unit was obtained from VIC cultures taken from the valves of New Zealand rabbits (*Oryctolagus cuniculus*), aged 12-13 weeks with weight of 2.5-3 kg. The model then divided into three groups, namely the dexamethasone group, olmesartan group, and a combination group. The sample size calculated using the Higgins and Klinbaum formula was 6 replications.

1.2 Isolation, culture, and characterization of VIC

Isolation of rabbit VIC was performed as previously described by Linn et.al with some modification on animal model selection.(Lin et al., 2017) Rabbits which met the standardized inclusion criteria were terminated by lethal injection using midazolam 5 mg/kg and phenytoin 125 mg/kg intraperitoneally. Surgically extracted rabbit leaflets were then separated from myocytes and valvular endothelial cells by digestion method using collagenase II enzyme followed by centrifugation and seeding of cell suspensions. Adhered cells after centrifugation at 100g were transferred to culture media.

Disintegrated VIC cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL, Grand Island, NY, USA) with supplementation of 10% foetal bovine serum (FBS), and 1% penicillin/streptomycin solution at 37°C with 95% humidity and 5% CO₂. After reaching 80% confluency, cells from the second passage were then transferred to non-coated six wells with DMEM and 10% FBS at a density of 2600 cells/dish. After 24 hours, the media was replaced with new DMEM containing 10% FBS and TGF-β1 (2.5 ng/ml) and then incubated for one to seven days.

Characterization of VIC was done through measuring vimentin expression. After washing with phosphate buffer saline (PBS), cells were fixed with 3% formaldehyde for 15 minutes. In order to inhibit non-specific binding, PBS containing 1% serum was added for 15 minutes and washed again using PBS. Subsequently, the fluorescein isothiocyanate (FITC)-labeled anti-vimentin from Thermo Fisher Scientific (Schwerte, Germany) reagent was added, and washed again with PBS. Vimentin expression was documented by fluorescent microscopy.

1.3 Intervention and myofibroblast differentiation assessment protocol

After the cells reached 80-90% confluency, the medium was replaced with serum free DMEM. Afterwards, pre-treatment condition was carried out with Olmesartan 100 nM, Dexamethasone 1000 nM, along with antifibrotic combination for 3 hours followed by incubation with TGFβ1 2.5 ng/ml for 24 hours. Myofibroblast characterization was carried out by fixation with 2% paraformaldehyde/PBS and 1% Triton X-100/PBS for 30 minutes. Then the cells were blocked with 2% BSA/PBS and incubated with mouse monoclonal antibody against FITC-labeled α-SMA, then washed again with PBS. α-SMA expression was documented by fluorescent microscopy.

1.4 Statistical analysis

After each experiment was carried out six times, the data were then collected and tabulated using Microsoft Office Excel 2016 and were further processed using IBM SPSS ver.26 for Windows (SPSS Inc, Chicago, IL). After the data cleaning, descriptive analysis was performed to calculate the mean, standard deviation, and box-plot diagrams for each group. In addition, unpaired numerical comparative statistical analysis in both groups was carried out using unpaired T-test for normal distributed data, otherwise the Mann-Whitney test was used. For comparison among three or more groups, one way analysis of variance (ANOVA)

with post hoc Bonferroni was conducted for the same variance, otherwise ANOVA with post hoc Games Howell was performed, but if the data not normally distributions then Kruskal-Wallis with post hoc Mann-Whitney will used. p-value <0.05 was considered as statistically significant.

Discussion

The results of the cultured and isolated rabbit *Oryctolagus cuniculus* valvular' cells in this study showed islands of cuboidal-spindle shape elongated cells with regular cell membranes that visually under light microscope, as demonstrated in figure 1. The characterization of VIC was carried out with positive markers of vimentin through immunocytochemical examination. Immunocytochemical analysis showed that there was a positive expression (luminescence above 95% of cell luminescence per field of view) of vimentin. Immunocytochemical staining results showed positive markers for vimentin which is a marker of valve interstitial cells (figure 2). The vimentin marker expression in the process of VIC isolation after incubation on the 9th day was detected with median of 6,585.4 (384 – 16,909).

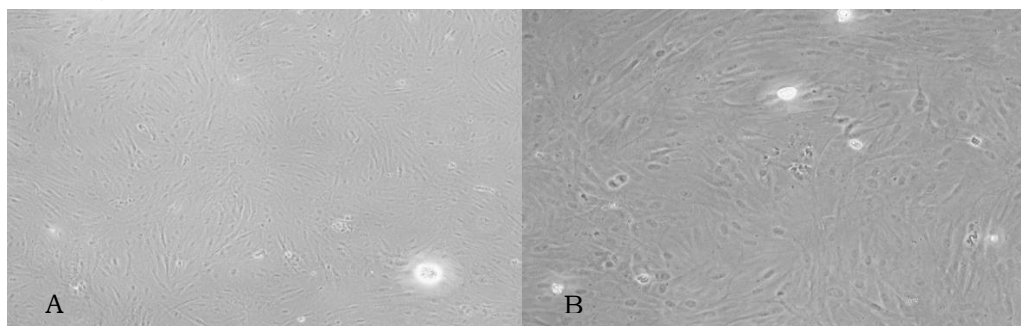


Figure 1 Valve Interstitial Cell Morphology A) Magnification 4 x 10; B) Magnification 10x10. The spindle-elongated cells are seen as valve interstitial cells.

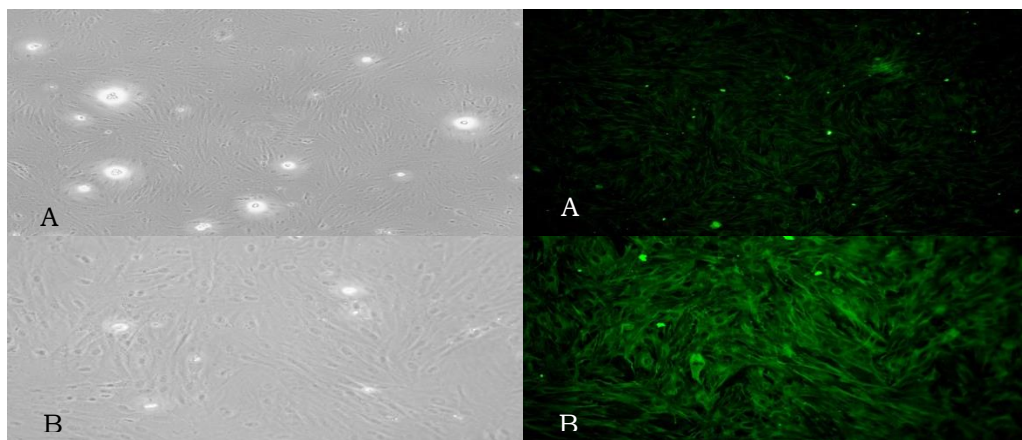


Figure 2 Vimentin Expression in Valve Interstitial Cells A) Magnification 4 x 10; B) Magnification 10 x 10. Valve interstitial cells appeared evenly distributed across the visual field tightly with strong luminescence

Subsequently, after exposure to TGF- β 1, VIC differentiated into myofibroblasts with characterized as irregular surfaces and cytoplasm filled with stress fibers. The Wilcoxon test results showed in the group exposed to TGF- β 1 were significantly different ($p < 0.001$) compared to the control group. This suggests TGF- β 1 as a potent cytokine that induce VIC differentiation into myofibroblasts. Furthermore, immunocytochemical staining after exposure to TGF- β 1 showed a significant increase in α -SMA expression (fluorescence above 95% luminescence of cells per field of view) when compared to controls. This indicates the process of differentiation of VIC into myofibroblasts (figure 3). The median quantity value of α -SMA expression in VIC exposed to TGF- β 1 as an inductor of myofibroblastic differentiation obtained a median of 23,474 (18,452 – 25,671), whereas in the control group the median expression of α -SMA was only 1,075 (118 – 7,696).

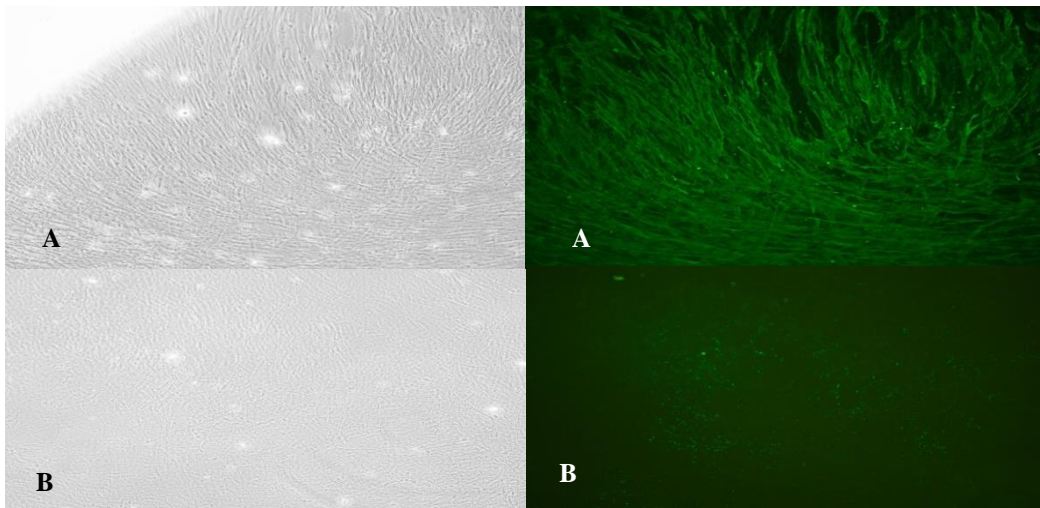


Figure 3 A) Myofibroblast Cell Morphology and α -SMA Expression in Immunocytochemistry with TGF- β 1 Induction B) Myofibroblast Cell Morphology and α -SMA Expression in Control Group Immunocytochemistry

Quantity of α -SMA expression in VIC exposed to TGF- β 1 and treated with dexamethasone, olmesartan, and combination showed that the lowest α -SMA expression was observed in the combination group of dexamethasone and olmesartan with a mean of $5,051.8 \pm 1,612.2$. The α -SMA expression in the dexamethasone group was $6,823 \pm 1735.3$ and the α -SMA expression in the olmesartan group was $6,942.7 \pm 2,455.9$. Statistical analysis using Kruskal-Wallis showed significant difference in these groups (p -value < 0.001).

Visual expression of α -SMA were shown in figure 4. These data were further analyzed by the Mann-Whitney post hoc test, and it was found that there were significant differences between the dexamethasone and control groups ($p < 0.001$), dexamethasone in combination ($p < 0.001$), olmesartan with control ($p < 0.001$), olmesartan with combination ($p < 0.004$) and combination with control ($p < 0.001$), but there was no difference between the dexamethasone and olmesartan groups ($p < 0.705$) (figure 5). According to these results, it demonstrated that the combination of dexamethasone and olmesartan can significantly reduce the

differentiation of rabbit VIC into myofibroblasts based on the expression of α -SMA, while dexamethasone and olmesartan alone have same potential

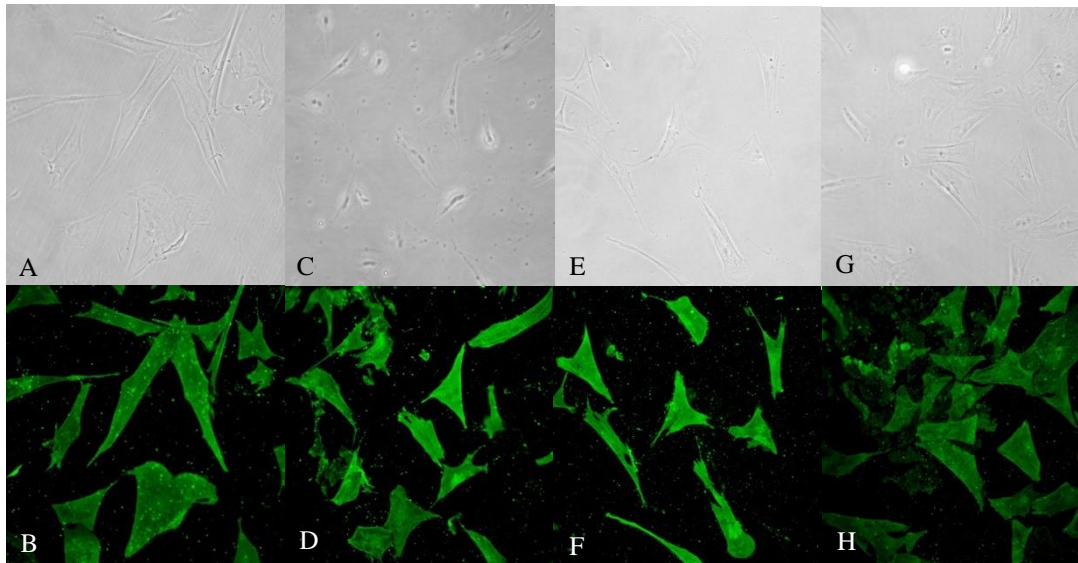


Figure 5 (A) Dexamethasone Group in Phase Contrast. (B) α -SMA Immunofluorescence Expression Group in the Dexamethasone Group. (C) Olmesartan Group in Phase Contrast. (D) Expression of α -SMA Immunofluorescence in the Olmesartan Group. (E) Combination Group in Phase Contrast. (F) Expression of α -SMA Immunofluorescence in the Combination Group. (G) Control Group on Contrast Phase, (H) Expression of α -SMA Immunofluorescence on contrast phase microscope.

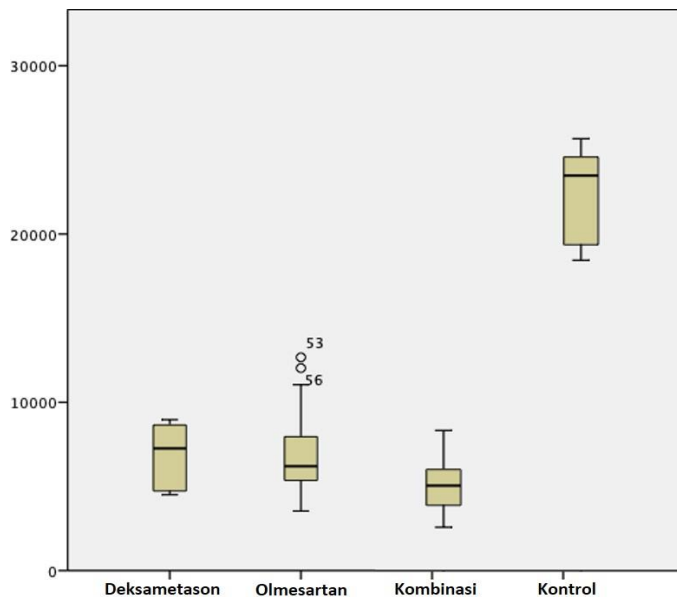


Figure 4 Comparison of each intervention treatment group on α -SMA expression. It can be seen that there is a significant difference between the groups against the control.

To the best of our knowledge, this is the first research that used rabbit VIC to make in vitro fibrosis models with single induction using TGF- β 1. This study proved the role of TGF- β 1 as an inducer of VIC differentiation as evidenced by changes in cell morphology and the presence of significant quantity of α -SMA luminescence. TGF- β 1 can activate the small-mothers-against-decapentaplegic (Smad) transduction pathway and initiate transcription of myofibroblastic pro-differentiation genes.(Cucoranu et al., 2005) TGF- β 1 has been shown to act as a key profibrogenic cytokine in vitro and in vivo studies(Bujak, 2001; Calderone et al., 2001; Frangogiannis et al., 2000), as well as an increase in TGF- β 1 levels in fibrotic heart organs.(Kim et al., 1995; Kupfahl et al., 2000) Prior study also revealed the effect of TGF-1 exposure on ventricular fibroblast cells to induce the fibrosis process through induction of collagen production by fibroblasts, myofibroblasts, and induction of fibroblast differentiation into myofibroblasts.(Lijnen et al., 2000; Petrov et al., 2002) Our study was successfully able to produce VIC isolates similar to the method used by Lin et al (2017), but with a lower potential for contamination of ventricular or atrial myocytes and fibroblasts through rational selection of experimental animals.(Lin et al., 2017)

TGF- β 1 as one of the main profibrotic cytokines is widely involved in the pathogenesis of RHD. This cytokine has the ability to stimulate myofibroblastic differentiation which will functionally increase the upregulation of α -SMA, to produce inflammatory mediators, growth factors, and increase the production of extracellular matrix such as the secretion of collagen I, III, and fibronectin which amplify the fibrosis process. Induction of TGF- β 1 will initiate signaling cascades through cell membrane and intracellular protein receptors Smad 2/3, the Mitogen-Activated Protein Kinase (MAPK) pathway, and the c-Jun N-terminal kinase (JNK) pathway.(Du et al., 2020; Heldin et al., 1997) The characteristics of myofibroblast cells that we found were similar to previous result reported with high content of exocytic vesicles, irregular cell membranes, and stress fibers in the cell cytoplasm.(Baum & Duffy, 2011; Petrov et al., 2002)

Myofibroblastic differentiation is a physiological response of VIC after exposure to pathological injury. This induces TGF- β 1 expression and initiates smooth muscle actin transcription. Myofibroblasts will increase the ability of collagen production and molecular adhesion (paxilin, tensin, ED-A fibronectin).(Frangogiannis et al., 2000; Gabbiani, 2003) Myofibroblasts also have high levels of microfilaments with abundant extracellular fibronectin, which can cause traction on histological tissue structures.(Gabbiani, 2003) The transformation of VIC into myofibroblasts will then cause excessive collagen deposition, thereby increasing the distance between functional VIC and disrupting cell-cell adhesion junctions (Connexin 43 and Connexin 45), as well as the effect of tissue contraction, which will bind to impaired valve function at the macroscopic level.(Baum & Duffy, 2011)

This study demonstrated the potential antiremodelling properties of dexamethasone and olmesartan as well as their combination in successfully inhibit VIC differentiation into myofibroblastic. Previous study has shown that dexamethasone treatment resulted in high extracellular matrix deposition.(Lo et al., 2003) It was also described that several components of the myofibroblast process could be inhibited by dexamethasone as well as inhibit α -SMA

amplification in endothelial injury.(Gu et al., 2010) Actin has been implicated as a major element in cellular movement and is associated with increased cellular migration.(Christian et al., 2001) Consequently, it is reasonable to assume that decreased expression of one of its isoforms would lead to decreased cell motility. In the study by Tsai et.al, cell migration decreased in parallel with decreased α -SMA expression after administration with dexamethasone.(Tsai et al., 2003) These findings demonstrate a direct relationship between cell migration and α -SMA expression and suggest that this protein plays a crucial role in driving cell migration. Moreover, the antifibrotic properties of dexamethasone can also be achieved through the induction of the MKP-1 pathway through increased levels of 15-deoxy-delta -12,14-PGJ2 (15DPGJ2), an endogenous MKP-1 ligand, via the p38-MAPK pathway. Activation of the MKP-1 pathway then act as an inhibitor of NF- κ B pathway activation.(Almawi et al., 1996)

Angiotensin receptor blocker (ARB) drugs, one of which is olmesartan, has an antiremodelling effect through inhibition of the RAAS system which suppresses the production of AngII. AngII has the role in enhancing the process of fibrogenesis in various organs has been demonstrated in various studies. Likewise, inhibition of the RAAS hormone using ARB has been shown to provide benefits in the process of organ fibrosis.(Kim et al., 1995) This Inhibition effect can be an intervention target to suppress the process of fibrosis and inflammation in VIC. Our results also in accordance with a previous study revealed that administration of 10 nM olmesartan was shown to reduce the levels of TGF- β 1 and α -SMA in cardiac fibroblasts.(Zhou et al., 2015) In addition, olmesartan administration 5 mg/kg/day in rats showed changes in profibrotic parameters, including: decreased activation of the TGF-1/Smad signaling pathway, increased levels of ACE2 and TNF- Converting Enzyme (TACE), and inhibition of apoptotic effects characterized by decreased levels of Caspase 3, BAX, Bcl-2.(Suh et al., 2019) The study using ARB as an inhibitory agent of fibroblast cell differentiation and its equivalent, is similar to the findings in this study, which showed that administration of olmesartan at a dose of 10 nanomol/L could significantly inhibit the differentiation of VIC.

In this study, the strongest potential in inhibiting myofibroblastic differentiation of VIC was found in the dexamethasone-olmesartan combination group. This is because the combination mechanism of action through different pathways that mutually potentiate each other. Olmesartan inhibits the interaction of ATII molecules at the ATII type 1 receptor, thereby reducing the synthesis of Ras molecules and activation of the JAK/STAT pathway, while dexamethasone acts as a negative inhibitor and regulator of Smad 2/3, TNF α , IL-1 and downstream NF- κ B signaling pathways. pro-myofibroblast differentiation. The combination of these different working points is mutually potentiating so that it provides the most potent inhibition effect of myofibroblast differentiation compared to the use of a single agent.

To date, there is no combination tested in the context of in vitro VIC model with TGF- β 1 induction. The finding of this potential inhibitory effect of VIC differentiation into myofibroblasts, especially the dexamethasone-olmesartan combination that we chose with in-depth consideration of its simultaneous and potentiating effects through various cell differentiation pathways, can be a

reference for further research in exploring more deeply the antifibrotic potential of dexamethasone, olmesartan, and dexamethasone. The combination in order to be an alternative to pharmacological therapy to inhibit the fibrosis process in RHD.

Conclusion

Dexamethasone and olmesartan has inhibitory effects on the differentiation of rabbit VIC induced by TGF- β 1 into myofibroblasts based on the expression of α -SMA. The combination of both drugs has a better potentiating effect in the ability to inhibit VIC differentiation into myofibroblasts. The combination of these two agents has great potential as an antiremodelling agent, especially in valvular disease due to RHD.

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