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## Vol. 14 No. 4 (2020): Indian Journal of Forensic Medicine & Toxicology



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### Articles

#### Age Estimation from Second & Third Molar by Modified Gleiser and Hunt Method : A Retrospective Study

Abirami Arthanari<sup>1</sup>, Nagabhushana Doggalli<sup>2</sup>, Vidhya A<sup>3</sup>, Sushma Rudraswamy<sup>4</sup>

1-8



#### Knowledge Attitude and Practice of Usage of Anti-Anxiety Drugs in Paediatric Patients among General Dental Practitioners

Ahsana A<sup>1</sup>, M. Dhanraj<sup>2</sup>

9-13



#### Effect of Silver Diamine Fluoride Treatment on the Quality of Life of Children

Ahsana. A<sup>1</sup>, Deepa Gurunathan<sup>2</sup>

14-18

 PDF

### **A Pilot Study on Snake Bite Deaths in the District of Malda, West Bengal, India**

Aloke Mazumder<sup>1</sup> , Tanushree Mondal<sup>2</sup> , Partha Pratim Mukhopadhyay<sup>3</sup>  
19-25

 PDF

### **Profile of Dowry Death Cases Reported for Autopsy in Thoothukudi Medical College - A Retrospective Study**

Seethalakshmi.M <sup>1</sup> , Rajalakshmi. S.<sup>2</sup> , Rajesh.R<sup>3</sup> , Vinoth K.V.<sup>4</sup> ,  
26-28

 PDF

### **Closed Mitral Valvotomy (as a Surgical Treatment Option) – Does it has any Medico Legal Bearings in the Present Scenario**

Amit Chaudhary, Amit Singh<sup>2</sup>  
29-32

 PDF

### **Chest Injury Outcomes due to Road Traffic Accidents – an Institutional Experience**

Amit Chaudhary<sup>1</sup>, Amit Singh<sup>2</sup> , Roopak Aggarwal<sup>3</sup>  
33-38

 PDF

### **Assessment of Factors Influencing Academic Performance of Nursing Students**

Amoldeep<sup>1</sup> , Bhawna Sharma<sup>2</sup> , Sudhir Gupta<sup>3</sup> , Jyoti Sarin<sup>4</sup>  
39-45

 PDF

### **A Descriptive Correlation Study on Stress and Psychological Wellbeing among Nursing Students in Selected Nursing Institutes of Ambala, Haryana**

Anamika Gautam<sup>1</sup> , Srinivasan.P<sup>2</sup> , Gurvinder Kaur<sup>3</sup> , Jyoti Sarin<sup>4</sup>  
46-50

 PDF

### **Observations on Inter-Rater Agreement in Assessing Fusion Activities at Elbow, Wrist and Pelvis by Conventional and Digital X-Ray Films**

Aniruddha Das<sup>1</sup> , Srimoyee Mukherjee <sup>2</sup> , Arani Majumder<sup>3</sup> , Debasis Sarkar<sup>4</sup> , Partha Pratim Mukhopadhyay<sup>5</sup>  
51-57

 PDF

### **Contriving an Opinion of Cause of Death in Autopsies**

Anu Sasidharan<sup>1</sup> , Nadia M. Al-Kandary<sup>2</sup>

58-62

 PDF

### **A Study of Patterns of Thoracic Injuries in the Victims of Vehicular Incidents Brought to the GMCH Mortuary for Medicolegal Autopsies**

Anurupa Choudhury<sup>1</sup> , Himangshu Das<sup>2</sup> , Ravi Kumar Sharma<sup>3</sup>

63-70

 PDF

### **Assessment of Knowledge and Attitude Towards Medical Ethics among Medical Students**

Arti Ajay Kasulkar<sup>1</sup> , Ayushi Keoliya<sup>2</sup> , Madhur Gupta<sup>3</sup>

71-76

 PDF

### **Different Types of Diatoms Seen in the River Narmada in India**

Arushi Chawla<sup>1</sup> , Dhvani Patel<sup>2</sup>

77-83

 PDF

### **A Study of Age Estimation by Radiological Assessment of Ossification Centers at Hand Wrist and Elbow Joint at a Tribal Teaching Medical College of Adilabad**

B Shekhar Rao<sup>1</sup> , Mohd Inayatulla Khan<sup>2</sup>

84-89

 PDF

### **Admission, Transfer and Discharge Procedure in Hospital: Review Article**

Bhawna Sharma<sup>1</sup> , Pratibha Thakur<sup>2</sup> , Amoldeep<sup>3</sup> , Sudhir Gupta<sup>4</sup>

90-96

 PDF

### **Descriptive Study on Emotional Intelligence Amidst Nursing Undergraduates of Chosed Nursing Institute**

Binal Joshi<sup>1</sup> , Franny Joel Emmanuel<sup>2</sup> , Vipin Vageriya<sup>3</sup>

97-101

 PDF

### **Role of Cardiac Troponin I in Case of Death Time EstimationAn in Vitro Study**

Boddupally Ravi Kumar<sup>1</sup> , Prakash Chandra Jha<sup>2</sup> , Shakti Shishodia<sup>3</sup> , Dattatraya P. Kale<sup>4</sup>

102-107



### **Estimation of Stature from head length in medical students.**

Chandrakant M Kokatanur<sup>1</sup> , Ajay V Patil<sup>2</sup> , Vinay Bannur<sup>3</sup> , Karan Deep Mishra<sup>4</sup>

108-114



### **Comparative Evaluation of Stress Distribution Around Mandibular Overdentures Surrounded by Three and Four Implants: A Three-Dimensional Finite Element Analysis**

Ashrath Azwin<sup>1</sup> , Mayur Hegde<sup>2</sup> , Vidya K Shenoy<sup>3</sup> , Sathish Shenoy<sup>4</sup> , Bharath Prabhu<sup>5</sup> , Mithun Upadhy<sup>6</sup> , Shobha Rodrigues<sup>7</sup>

115-121



### **A Study on Oxygen Consumption in Freshwater Fish Labeo Rohita Exposed to Lethal and Sub Lethal Concentrations of Ethion 50%Ec**

Ch.Prasanna<sup>1</sup> , Ch.Anithasmruthi<sup>2</sup> , V.Venkatathnamma<sup>3</sup>

122-127



### **Effectiveness of Faradism Under Pressure Versus Complex Decongestive Therapy in Subjects with Peripheral Oedema**

Chelcy S Jaju<sup>1</sup> , Mandar Malawade<sup>2</sup>

128-134

### **The Effectiveness of Hot Fomentation with Epsom Salt Versus Common Salt on Knee Joint Pain among Elderly in the Selected Old Age Home of Pune City**

Derin Okram<sup>1</sup> , Dipak Sethi<sup>2</sup> , Ranjana Chavan<sup>3</sup> , Sharadha Ramesh<sup>4</sup>

135-138



### **Effectiveness of Postural Drainage and Forced Expiratory Technique Along with Segmental Relaxation Technique on Airway Clearance in Chronic Obstructive Pulmonary Disease Patients**

Apurva Girish Mehta<sup>1</sup> , Smita Chandrakant Patil<sup>2</sup> , Chandrakant Babaso Patil<sup>3</sup> , Khushboo Trishant Chotai<sup>4</sup>

139-146





## **The Perturbation Encompassing Dental Amalgam Toxicity: A Review**

Arjun Hegde<sup>1</sup> , Ganaraj Shetty<sup>2</sup> , Nishi Jayasheelan<sup>3</sup>

147-153



## **A Study to Evaluate The Influence of Thread Designs Affecting Stability of Immediately Loaded Implant at Maxillary Posterior Region**

Athma Shetty<sup>1</sup> , Manoj Shetty<sup>2</sup> , Chethan Hegde<sup>3</sup> , Nivya John<sup>4</sup>

153-159



## **Modes, Manner of deaths and types of injuries in Warangal Municipal Limits**

D. Vijaya Raghavendra<sup>1</sup>, K. Ravimuni<sup>2</sup> , K. Usha Rani<sup>3</sup>

160-166



## **Pattern of Suicidal Deaths in the First Month of Lockdown at A Tertiary Care Hospital: a Time Trend Analysis**

Deepmalya Sengupta<sup>1</sup> , Saikat Saha<sup>2</sup> , Pranabesh Bharatee<sup>2</sup> , Rajib Prasad<sup>3</sup>

167-172



## **Radiological Study of Ossification Centres Around Pelvis to Determine Their Age of Appearance and Fusion among Bengali Population – A Retrospective Study**

Deepsekhar Dalal<sup>1</sup> , Abhishek Das<sup>1</sup> , Sujash Biswas<sup>2</sup>

173-179



## **A Quasi Experimental Study to Assess the Effectiveness of Assertiveness Training Program on Self Esteem and Interpersonal Communication Satisfaction among Nursing Students in Selected Nursing Institutes of Ambala, Haryana**

Diksha Sharma<sup>1</sup>, Manpreet Sharma<sup>2</sup> , Gurvinder Kaur<sup>2</sup>

180-185



## **Awareness of MBBS Students on Effects of Workplace Violence and Conflict Management in Hospital Scenario**

Divya Reddy Pannala<sup>1</sup> , S.M. Krishna Sagar<sup>2</sup> , Hemantha Rao Amara<sup>3</sup>

186-191



## **The Effectiveness of Information Education Communication (IEC) on Knowledge and Practices Regarding Patient Safety Measure among Nursing Officers in Selected Hospital of Pune City**

Divyank Kalal<sup>1</sup> , Dipak Sethi<sup>2</sup> , Ranjana Chavan<sup>3</sup> , Sharadha Ramesh<sup>4</sup>

192-195

 PDF

## **Study of Profile of Homicidal Deaths in South Chennai- A Retrospective Study**

Selvaraj.K<sup>1</sup> , Rajalakshmi. S.<sup>2</sup> , Vinoth K.V<sup>3</sup> , Sivakumar. S.<sup>4</sup> , Sudalaimuthu. R<sup>5</sup>

196-201

 PDF

## **YouTube TM Videos as a Source of Information on Clear Retainers**

Ramya Vijeta Jathanna<sup>1</sup> , Krishnaraj K.K<sup>2</sup> , P.N. Roopalekha Jathanna<sup>3</sup> , Vinod Rakesh Jathanna<sup>4</sup>

202-210

 PDF

## **Effect of Various Mesh Designs on Shear Bond Strength of New and Recycled Brackets –A Comparative, Invitro Study**

Kopal Agarwal<sup>1</sup> , Siddarth Shetty<sup>2</sup> , Asavari Desai<sup>3</sup>

211-218

 PDF

## **Medicolegal Aspects of COVID-19: Microbial Terrorism**

Ekampreet Kaur<sup>1</sup> , Jaskaran Singh<sup>2</sup> , Neeta Raj Sharma<sup>3</sup> , Gitanjali Dass<sup>4</sup>

219-227

 PDF

## **Effectiveness of Module on Knowledge and Practice Regarding Prevention of Medication Errors among Critical Care Nurses in Selected Hospital**

Jeba B<sup>1</sup> , Jasneet Kaur<sup>2</sup> , Deepak Sethi<sup>3</sup>

228-232

 PDF

## **Non-Compressive Mechanical Injuries to the Neck: An Autopsy based Study**

Jeeveswararao Bagadi<sup>1</sup> , Sadu Rammohana Rao<sup>2</sup>

233-238

 PDF

## **Evaluate the Effectiveness of Recreational Therapy on Level of Wellbeing and Hope among Psychiatric Inpatients**

Luxmipyari Soubam<sup>1</sup> , Ms. Parul<sup>2</sup> , Manpreet Sharma<sup>3</sup>, Jyoti Sarin<sup>4</sup>  
239-245



### **A Study of Autopsy Findings in Cases of Sudden Cardiac Death at Kakatiya Medical College and Hospital Warangal**

Mohd Raza Malik Khan<sup>1</sup> , B Shekhar Rao<sup>2</sup>  
246-251



### **Radiographic Evaluation of Third Molar Development in Relation to Chronological Age among South Indian Population**

Nanditha Sujir,<sup>1</sup> Nidhi P Chauhan,<sup>2</sup> Keerthilatha M Pai,<sup>3</sup> Junaid Ahmed,<sup>4</sup> Ceena Denny<sup>5</sup> , Nandita Shenoy<sup>5</sup>  
252-256



### **Soft Tissue Cephalometric Norms for Orthognathic Surgery in South Indian Population**

Murali P S<sup>1</sup>, K Saidath<sup>2</sup> , Sonika Achalli<sup>3</sup> , Azhar Mohammed<sup>1</sup> , Crystal Runa Soans<sup>1</sup> , U S Krishna Nayak<sup>4</sup>  
257-265



### **Assessment of Extraction Technique Using Physics Forceps and the Conventional Method : A Comparative Study**

Prasanna Shetty<sup>1</sup> , Sharma<sup>2</sup> , Nivya John<sup>3</sup>  
266-272



### **Correlation between Gestational Age and Head Circumference in Third Trimester**

Pande Varsha <sup>1</sup> , Patond Swapnil <sup>2</sup>, Patil Bharat<sup>3</sup>  
273-279



### **Assessment of Knowledge, Attitude and Practices of Allied Health Care Professional Students Towards Universal Precaution**

Patond Swapnil<sup>1</sup> Dakhode Sarika<sup>2</sup> , Rawekar Alka<sup>3</sup> , Pande Varsha<sup>4</sup>  
280-285



### **An analysis of the Inventory Level of Consumables in the Wards of a Tertiary Care Teaching Hospital in South India**

Bhargav Kumar, Rajesh Kamath<sup>2</sup>, Jibu Thomas<sup>3</sup>, Karan Giriyan<sup>4</sup>, Nagraj<sup>5</sup>, Abhishek Pallerla<sup>6</sup>  
286-291

 PDF

### **COVID -19 Pandemic: Immediate Psychological Impact During The 3TD Stage of Lockdown among General Population of Selected Areas of Vadodara City**

Ishita Shah<sup>1</sup>, Jalpa Prajapati<sup>1</sup>  
292-296

 PDF

### **Study of Pattern of Fusion of Coronal Suture Using Skull Radiography and its Association with Documented Age in Males**

Ishita Thakur<sup>1</sup>, Rashid Nehal Khan<sup>2</sup>, Thippesh Kumar<sup>3</sup>, Abhijit Rudra<sup>3</sup>  
297-304

 PDF

### **Pattern of Firearm Injuries: A Five Year Retrospective Study at a Tertiary Care Centre in Central India**

Juglan Sarthak<sup>1</sup>, Mustaria Pankaj Kumar Shushil<sup>2</sup>, Singh Sandeep<sup>3</sup>, Dhingra Vinod<sup>4</sup>  
305-311

 PDF

### **Accuracy of Mesiodistal Crown Measurements using CBCT Images: A Comparison with Plaster Models**

Kanak Priya<sup>1</sup>, Poonam Agrawal<sup>2</sup>, Dinesh Kumar Bagga<sup>3</sup>  
312-318

 PDF

### **The Personal Identification by Means of Cheiloscopy among People of North India**

Mukul Chopra  
319-325

 PDF

### **An Autopsy Study of Medicolegal Aspects of Burn Death among Married Females**

Neelesh Kumar Shakya<sup>1</sup>, Pratibha Shakya<sup>2</sup>, S.K. Panday<sup>3</sup>  
326-329

 PDF

### **Parental Perception on the Effect of Various Factors in a Dental Clinic Affecting Child's Behaviour**

Neethu Ann Preethy<sup>1</sup> , Deepa Gurunathan<sup>2</sup>

330-335

 PDF

### **Effect of Music on the Vital Signs and Behaviour of Children During the Recovery Period after General Anaesthesia Procedure - A Randomized Controlled Trial**

Neethu Ann Preethy<sup>1</sup> , Deepa Gurunathan<sup>2</sup>

336-341

 PDF

### **Effectiveness of Nursing Handoff Education Programme on Competencies of Handing Over among Nursing Students Posted in Selected Hospital of Ambala, Haryana**

Nidhi Sahu<sup>1</sup> , Jyoti Sarin<sup>2</sup> , Parvinder Kaur<sup>3</sup>

342-348

 PDF

### **Study of Spectrum of Various Pressure Abrasions Caused by Ligature Material in Violent Asphyxial Deaths**

Nirjhar Mathur<sup>1</sup> , Ravi kantsaini<sup>2</sup> , R. K. Mathur<sup>3</sup>

349-354

 PDF

### **Observational Study of Sociodemographic Profile on Female Autopsy Cases of J.L.N Medical College, Ajmer**

Nirjhar Mathur<sup>1</sup> , Sumer Singh<sup>2</sup> , R K Mathur<sup>3</sup>

355-361

 PDF

### **Study of Incidence and Pattern of Snake Bite Cases Brought in Tertiary Care Hospital, Rims, Ranchi, Jharkhand**

Nityanand Kumar<sup>1</sup> , Rohan Kumar<sup>2</sup> , Ajit Kumar Chaudhary<sup>3</sup> , Tulsi Mahto<sup>4</sup>

362-368

 PDF

### **Profile of Organophosphorus Poisoning Cases in a Tertiary Care Hospital in Central India**

Pande Varsha<sup>1</sup> , Patil Bharat<sup>2</sup> Patond Swapnil<sup>3</sup> , Gore Raju<sup>4</sup>

369-372

 PDF

### **Effect of Embalming Fluid on the Histological Appearance of Organs - A Cadaveric Study**

Swapna R1 , Annie Jojo2 , Tintu TS3 , Asha G4, Pillay VV5 , Minnie Pillay6  
373-378

 PDF

### **Statistical Analysis of Rheumatoid Arthritis in Association With Demography and Biochemical Investigation**

G Y Mythili 1 , B. Venkateswarlu2, T. Subramanyam3 , C. Narayana4  
379-385

 PDF

### **Surrogacy in India: Ethical and Legal Aspect**

Gerard Pradeep Devnath1 , Senthil Kumaran2  
386-392

 PDF

### **Literature Review in Forensic Practitioner and COVID-19**

Ishita Manral1 , Thippesh Kumar2 , KV Radhakrishna3 , Ravi Rautji4  
393-397

 PDF

### **Hypertrophic Cardiomyopathy as a cause of Sudden Cardiac Death—An Autopsy Study**

Kanwardeep Kaur Tiwana, Manmeet Kaur2 , Mohanvir Kaur3, Shaffaly Gagneja4 , Haspreet Kaur5  
398-401

 PDF

### **Attitude and Knowledge toward Organ Donation among Arts and Science Students**

Khan F1 , Latif M2 , Bashir S3  
402-407

 PDF

### **The Study of Finger print Patterns among Male and Female**

Mukul Chopra  
408-413

 PDF

### **Midazolam as A Sedative Agent in Paediatric Dentistry- A Literature Review**

Neethu Ann Preethy1 , Sujatha Somasundaram2  
414-419

 PDF

### **Study on the Appearance and Fusion of Iliac Crust Epiphyseal Center among Natives of Ajmer Region**

Nirjhar Mathur<sup>1</sup> , Vipin Gupta<sup>2</sup> , R. K. Mathur<sup>3</sup>

420-427

 PDF

### **Google Site as a Tool for Teaching Undergraduate Students in Forensic Medicine**

Pragnesh Parmar<sup>1</sup> , Swapnil Patond<sup>2</sup> , Gunvanti Rathod<sup>3</sup> , Sudhir Ninave<sup>4</sup>

427-431

 PDF

### **Clinical Profile of Snake Bite in Children at a Tertiary Care Center**

Rahul Yashavant Surve<sup>1</sup> , Anupama V. Mauskar<sup>2</sup> , Vishal Sawant<sup>3</sup> , Rajesh Nare<sup>4</sup>

432-439

 PDF

### **Soft Palate Dimensions and Configuration Changes in Oral Submucous Fibrosis (OSMF)- A Radiographic Study**

Ravindra. S. V1, Aiman Mahfooz<sup>2</sup> , M.K. Sunil<sup>3</sup> , Anudeep Raina<sup>4</sup> , Surangama Lehri<sup>5</sup> , Lucy Pradhan<sup>6</sup> , Monika Singh<sup>7</sup>

440-444

 PDF

### **An Observational Analysis of Suicidal Deaths during COVID 19 Pandemic Lockdown at Lucknow, India**

Richa Choudhury

445-449

 PDF

### **Forensic Odontology in India: Present Scenario, Hurdles & The Way Forward**

Rohan Ashok Gawali

450-456

 PDF

### **Impact of Nutrient-Gene-Interaction on Periodontal DiseasesAn Over View**

Razia Haidrus<sup>1</sup> , Sangeeta U Nayak<sup>2</sup> , Deepa G Kamath<sup>3</sup>

457-463

 PDF

### **Comparison of Bone Mineral Density in Patients with Celiac Disease and Healthy Individuals**

Roymon Joseph<sup>1</sup>, Ranjna Janagal<sup>2</sup>, Mohan Singh<sup>3</sup>, Dimple Patel<sup>4</sup>, Rohit Zariwala<sup>5</sup>, Garima Khatri<sup>6</sup>,  
Khushboo Joshi<sup>7</sup>

464-467

 PDF

### **Study of Profile of Accused of Sexual Offences in Thoothudi Medical College- A Retrospective Study**

Seethalakshmi.M<sup>1</sup>, Rajalakshmi. S.<sup>2</sup>, Rajesh.R.<sup>3</sup>, Vinoth K.V.<sup>4</sup>

468-472

 PDF

### **Profile of Medicolegal Death in Females in Thoothudi Medical College- A Retrospective Study**

Selvaraj. K<sup>1</sup>, Rajalakshmi. S<sup>2</sup>, Vinoth K.V.<sup>3</sup>, Sudalaimuthu. R<sup>4</sup>

473-476

 PDF

### **Estimation of Stature using Ulnar Length in a Population of Chennai**

S. Siva Kumar<sup>1</sup>, P. Vinod Kumar<sup>2</sup>, R. Selva Kumar<sup>3</sup>

477-482

 PDF

### **A Study of Pattern of Thoracic Injuries in Cases of Railway Traffic Accidents Brought to the Post Mortem Centre**

Surwade V B<sup>1</sup>, Meena S.K<sup>2</sup>, Tirpude B.H.<sup>3</sup>, Murkey P N<sup>4</sup>

483-487

 PDF

### **Awareness among Medical Students about Forensic Odontology**

S.M.Krishna Sagar<sup>1</sup>, Divya Reddy Pannala<sup>2</sup>, Rithya Kuppam<sup>3</sup>

488-493

 PDF

### **Google Classroom - An Effective Tool for Online Teaching and Learning in this COVID era**

Santosh Sheelavant

494-500

 PDF



## **A Study of Autopsy Cases - Viscera Sample Reports A Retrospective Study**

Selvaraj. K1 , Vinoth K.V2 , Sudalaimuthu. R3

501-504



## **Digital Visualizer as an Aid-assisted Teaching in Anatomy**

Senthil kumar.B1, Deepti Shastri2 , Samraj. T3

505-510



## **Assessment of Assertiveness and Self Esteem among Nursing Students in Selected Nursing Colleges: A Systematic Review**

Shilpa1 , Shalini2

511-514



## **Cryotherapy as an Adjunct to Cleaning and Shaping in Endodontics: A Review**

Shivangi Vats1 , Vinod Jathanna2

515-518



## **Prevalence of Pelvic Girdle Pain in Post Partum Women**

Shraddha Dhongade1 , G. Varadharajulu2

519-523



## **Practices of Documentating the Pre-Operative Informed Consent in Obstetrics and Gynaecology in a Tertiary Care Hospital – An Interventional Study**

Shreen R.1 , Kagne R.N.2 , Ganesh Rajendiran3 , Jayasree M4

524-528



## **Otitis in Aerospace-Aerotitis: A Review**

Shweta Sharma1 , Heeral Joshi1 , Pooja Anand2

529-530



## **A Morphometric Study to Find Correlations among Various Dimensions of Proximal End of Humerus of Telugu Population**

Sinchal Datta

531-537



### **A Comparative Study of Facial Indices of Maharashtrian Adults with Other Ethnic Group and Test For Reliability of Sexual Dimorphism**

Sinchal Datta<sup>1</sup> , Sawant V.G<sup>2</sup>

538-544



### **Awareness of Forensic Dentistry among Dental Practitioners**

Smita Dutta

545-550



### **Effectiveness of Ergonomic Training Program on Knowledge, Self-efficacy and Practice on Prevention of Work Related low Back Pain among Staff Nurses**

Sosunika Hijam<sup>1</sup> , Uma Deaver<sup>2</sup> , Kanika<sup>3</sup> , Jyoti Sarin<sup>4</sup>

551-556



### **Adolescent Behavior: Understanding in Relation to Mental Health Issues with Implication in Nursing- Review Article**

Srinivasan.P<sup>1</sup> , R. Sreevani<sup>2</sup>

557-563



### **Study of Limb Injuries in Road Traffic Accident Cases-An Analytical Study in IMS And SUM Hospital, Bhubaneswar, Odisha**

Srimannarayan Mishra<sup>1</sup> , Tapaswini Mishra<sup>2</sup> , Pusparaj Samantsinghar<sup>3</sup> , Nirupama Samantaray<sup>4</sup>

564-569



### **Reconstruction of Length of Femur from Its Fragments- A Preliminary Study in Eastern Indian Population**

Srimoyee Mukherjee<sup>1</sup> , Aniruddha Das<sup>2</sup> , Debasis Sarkar<sup>3</sup> , Arani Majumder<sup>3</sup> , Partha Pratim

Mukhopadhyay<sup>4</sup>

570-575



## **Epidemiological Study of Drowning Deaths in Madurai Region**

T.Selvaraj1 , .V.Rama2

576-579



## **Determination of Sex and Estimation of Age by Mandibular Basal Bone Height**

Vidhya Arumugam1, Nagabhushana Doggalli2, Abirami Arthanari3

580-586



## **A study on the Aspiration Procedures in Syringes among Health Care Professionals**

Vijay Anand1 , Rathika Rai2 , Nirmal Famila Bettie3 , Shantha Priya4

587-592



## **A Study to Assess the Opinion of Students about Uses of Digital Education Technology during COVID-19 Lockdown in India**

Yogesh Kumar1 , Jasoda Sanasam2 , Dhanesh Garg3 , Jyoti Sarin4 , Parvinder Kaur5 , Chetna Kumari6

593-600



## **Accessible India Campaign “A Silver Lining for the Persons with Disability”- An Analysis**

Yogesh Mishra1 , Nilanjan Chakraborty2 , Debalina Sengupta3 , Ankita Mishra4

601-606



## **Identification of Sex from Maxillary Sinus in Western Maharashtra Population**

Umang P Patel 1 , Makardar U K2 , Karkhyle Md. Layeeqeu3 , Kailash Balkund4

607-611



## **COVID-19: Analysing the Legal Nuances of the Lockdown Order**

Swati Mohapatra1 , Yogesh Mishra2 , Lipsa Dash3

612-616



## **Pattern of Finger Prints in Raichur District Population A Prospective Study Conducted at Raichur Institute of Medical Sciences, Raichur**

Gopinath M1 , Suraj2 , Priyanka A Ghuli3 , Sharanabasavappa Karaddi4

617-622

 PDF

### **Management of the Diagnostic and Therapeutic Obstacle to Successful Endodontics in C Shaped Canals: A Case Series**

Raksha Bhat1 , Preethesh Shetty1 , Abhishek MA2 , Prajwal Shetty3

623-631

 PDF

### **Profile of Victims of Rape in the Transkei Sub-Region of South Africa (2006-2014)**

B MEEL

632-636

 PDF

### **Child Homicide in the Mthatha region of South Africa of South Africa- Case Reports**

B Meel

637-642

 PDF

### **Poverty and non-natural deaths among former mineworkers and their families in Transkei region of South Africa**

B Meel

643-646

 PDF

### **A case report on Shortfall in Pension in a Dual Employment in Health and Rural University, South Africa**

B Meel

647-651

 PDF

### **Complexity of Dual Employment in Department of Health and university, South Africa**

B Meel

652-657

 PDF

### **Biometric Cardiac Profile as a Possible Identifier for sex among Sample of Egyptian Population**

Walaa Abd el Hady Abd el Halim Abo-Zeid1 , Abeer Ahmed Ahmed Zayed2 , Heba Gamal Kotb2 , Reham Nafad Elbendary3 , Emmanuel Fares Aziz4

658-664

 PDF

### **Expression of MRHR-CRHR1 (Type I CRH Receptor) and the Effects of Prenatal Yoga on Mother Primagravida Trimester III**

Ruqaiyah<sup>1,2</sup>, Nusratuddin Abdullah<sup>3</sup>, Mochammad Hatta<sup>4</sup>, Nasrudin A Mappeware<sup>5</sup>, Ayatullah Harun<sup>2</sup>, Fatmawati Amir<sup>2</sup>, Alfina Baharuddin<sup>6</sup>

665-670

 PDF

### **The Measurement of Cortisol Levels in the Blood Pregnant Trimester III with ELISA (Enzyme Linked Immunosorbent Assay ) Method**

Ruqaiyah<sup>1,2</sup>, Nusratuddin Abdullah<sup>3</sup>, Mochammad Hatta<sup>4</sup>, Nasrudin A Mappeware<sup>5</sup>, Ayatullah Harun<sup>2</sup>, Fatmawati Amir<sup>2</sup>, Alfina Baharuddin<sup>6</sup>

671-676

 PDF

### **Contamination of Polynuclear Aromatic Hydrocarbons- (PNAH) in Sediments: Identification and Distribution in the River of Shatt Al-Arab-(ROSA)**

Wisam Abdul-Ameer Farid<sup>1</sup>, Wasen Abdul-Ameer Ali<sup>1</sup>, Aseel Nadum Al-Salman<sup>2</sup>

677-683

 PDF

### **Serum Androgens in Men with Rheumatoid Arthritis**

Abdulsatar J. Mathkhor<sup>1</sup> . Abdulnasser H. Abdullah<sup>2</sup> . Amer S. Khudhairi<sup>3</sup>

684-688

 PDF

### **Disposition of Policy Implementers: Study on The Hospitals Payment System Change in The Indonesian National Health Insurance Era**

Abu Khoiri<sup>1,2</sup>, Widi Hidayat<sup>3</sup>, Djazuly Chalidyanto<sup>4</sup>, Fendy Suhariadi<sup>5</sup>

689-696

 PDF

### **How to Teach Intramuscular Injection Through Virtual Learning Environment in Covid-19 Pandemic Time for Nursing Students**

Adam Mahmoud Salameh Khraisat<sup>1</sup>, Hermizi Hapidin<sup>2</sup>, Nik Siti Hanifah Nik Ahmad<sup>3</sup>, Mohammad Dzulsyafiq Mohammad Yusoff<sup>4</sup>, Mohd Said Nurumal<sup>5</sup>, Kasmah Wati Pardi<sup>6</sup>

697-704

 PDF

## **A Virtual Learning Environment for Teaching Intramuscular Injection: A Focus Group Discussion Analysis**

Adam Mahmoud Salameh Khraisat<sup>1</sup> , Hermizi Hapidin<sup>2</sup> , Nik Siti Hanifah Nik Ahmad<sup>3</sup> , Mohammad Dzulsyafiq Mohammad Yusoff<sup>4</sup> , Mohd Said Nurumal<sup>5</sup> , Kasmah Wati Pardi<sup>6</sup>  
705-709

 PDF

## **Risk Factors of Type 2 Diabetes Mellitus in Hospital of Bengkulu City, Indonesia: Case Control Study**

Agung Sutriyawan<sup>1</sup> , Tenike G Miranda<sup>2</sup> , Hairil Akbar<sup>3</sup> , Riswan<sup>4</sup>  
710-716

 PDF

## **Validity and Reliability of the Questionnaire Assessing the Healthcare Delivery Status in Iran's Prisons**

Ahmad Hajebrahimi<sup>1</sup> , Khalil Alimohammadzadeh<sup>2,3</sup> , Seyed Mojtaba Hosseini<sup>4</sup> , Ali Maher<sup>5</sup> , Mohammadkarim Bahadori<sup>6</sup>  
717-723

 PDF

## **Outcome of Anterior Cruciate Ligament Reconstruction using Femoral Intrafix for a Single Tunnel Double Bundle Anterior Cruciate Ligament Reconstruction**

Ahmed Mahmoud Gad<sup>1</sup> , Mohamed Kamal Ismail<sup>2</sup> , Ramy Salah Morsy<sup>3</sup>  
724-730

 PDF

## **PON1 Q192R Gene Polymorphism and Pesticide Exposure Status of Rice Farmers, Suphan Buri, Thailand**

Airada Sridon<sup>1</sup> , Kampil Ruchiwit<sup>2</sup> , Kowit Suwannahong<sup>3</sup> , Wanida Pongstaporn<sup>4</sup> , Yuttana Sudjaroen<sup>5</sup>  
731-738

 PDF

## **Effect of Oral Contraceptive Pills on Levels of Calcium and Vitamin D in women in Al-Najaf Province**

Alaa Abd Kadim<sup>1</sup> , Suha Hussein Mohammed<sup>1</sup>  
739-743

 PDF

## **Clinical study on the therapeutic effects of Quercus infectoria galls as oral powder in gingivitis and plaque patients**

Ali Hasanain Alhamadani<sup>1</sup> , Hiba Ahmed Saeed<sup>2</sup> , Hussein Asli Khayoon<sup>3</sup>

744-748

 PDF

### **Effectiveness of Nursing Management Educational Program upon Head Nurses Knowledge Regarding Patient Management**

Ali Hussein Ali Al-Jazaeri.1 , Muna. A. Khaleel

749-751

 PDF

### **The Willingness of Dental Medicine Students in Handling Positive HIV Patients**

Alya Sahilla Fahri1 , Nurhana Binti Suhaimi1, Balwinjit Kaur Sidhu1 , Yaaliny A/P Krishnamurthi1 , Oretha Patrick Balinu1 , Mohd Rashid Bin Mohd Abu Bakar1 , Regina Purnama Dewi Iskandar2

752-756

 PDF

### **Drugs used for Weight Reduction among Iraqi Population Sample, a Comparative Study**

Amani Anees Aboddy1 , Rua Abbas Alhamdy2 , Zakariya Al-Mashhadani 3, Nabaa Nabeel4, Hussien Ahmed5

757-763

 PDF

### **Nanocurcumin Protective Effect on Lipid Peroxide of Lead Acetate Induced White Rats (*Rattus norvegicus*) Ovarian Granulosa Cells**

Anis Satus Syarifah1,2, Sri Agus Sudjarwo3 , Hendy Hendarto4 , Reny I'tishom5

764-769

 PDF

### **Effectiveness of Educational Program on Nurses-Midwives' Preventive Measures about Puerperal Sepsis at Baghdad Maternity Hospitals**

Anwar Saad Qasim1 , Shukriya Shadhan Chyad2

770-776

 PDF

### **Mean Circumferential Fibers Shortening Velocity Assessment in Patients with type II Diabetes Mellitus in relation to their Diastolic Function Status**

Asaad Hasan Noaman1 , Amina A. B. Al-Dejeli2 and Falah Mahdi Dananah

777-781

 PDF

### **Some Histogenetic Aspects of Methotrexate Associated with Juvenile Idiopathic Arthritis (JIA) Treatment Doses**

Asal A. Tawfeeq1 , Sundus M. Taifoor2

782-787



### **X-Chromosome Markers Used in Deficiency of Paternity Case**

Azzah Bahaa Abdulazeez 1,2, Ismail Hussein Aziz 1 , Mohammed Mashni Farhan 2

789-793



### **Design and Manufacture of Nanomedicine for Aflax Pump Inhibitors in Clinical Isolates of Methicillin-Resistant Staphylococcus Aureus (MRSA) Bacteria**

Banafshe Sayadi Rad1 , Anoosh Eghdami21

794-801



### **Effectiveness of Security Incident Event Management (SIEM) System for Cyber Security Situation Awareness**

Bandr Siraj Fakiha

802-808



### **Barcodes in Naming Basic Denture for Forensic Identification Needs**

Barcode, Denture Base, Forensic identification

809-813



### **Knowledge, Attitudes, and Practices of Staff and Students at Sulaimani Polytechnic University towards COVID-19/Iraq**

Bestun Ibrahim Hama Rahim

814-821



### **Histopathological Changes in Skin-A Tool to Establish Time Since Death in First 24 Hours**

Boban Babu1 , Vina Vaswani2 , Kishor Kumar3 , Rani Nallathamby4

822-828



### **Anterior Lamellar Keratoplasty Using Artificial Biotransplant in Veterinary Ophthalmology**

Boris V. Usha1, Vladimir I. Lutsay1, Inga M. Nityaga2, Uliyana E, Lukashina3, Evgeny V. Davidov4



829-834

 PDF

### **Modern Mycobacterium Tuberculosis Strain in Bronchoalveolar Lavage from Tuberculosis Patients Associated with Lung Tissue Damage Severity**

Budi Yanti<sup>1</sup> , Muhammad Amin<sup>2</sup> , Ni Made Mertaniasih<sup>2</sup>

835-843

 PDF

### **Effect of Cinnamon (*Cinnamomum burmannii*) Bark Oil on Pancreatic Histopathology of white Rats (*Rattus norvegicus*) Induced with Streptozotocin**

Budiastuti<sup>1</sup> , Reni Utami<sup>2</sup> , Mustofa Helmi Effendi<sup>3</sup> , and Hani Plumeriastuti<sup>4</sup> \*

844-850

 PDF

### **Hypolipidemic Effect of Fermented Soymilk Related with Lactic Acid Bacteria in Feaces**

Darimiyya Hidayati<sup>1,3</sup> , Soetjipto<sup>2</sup> , Annis Catur Adi<sup>1</sup>

851-856

 PDF

### **The Culture of Life's Consumptive Behavior Against Potential Hypertension at Pangkep Community in South Sulawesi Indonesia**

Darwis<sup>1</sup> , Muzakkir<sup>1</sup> , Masriadi<sup>2</sup>

857-861

 PDF

### **Neonatal Therapeutic Intervention Scoring System (NTISS) in Rural Country: Mortality and Length of Stay (LOS) Predictive Score in Preterm Infant**

Desy Nurrosalia Wisnuwardani<sup>1</sup> , Mahendra Tri Arif Sampurna<sup>2</sup> , Martono Tri Utomo<sup>2</sup> , Risa Etika<sup>2</sup>

862-867

 PDF

### **Correlation of Toluene Safe Duration (Hours/Day) and Glutathione Concentration, Malondialdehyde and Neurotoxic Symptoms in Osowilangun Shoe Home Industry Workers**

Diah Pramesthi Ningrum<sup>1</sup> , Abdul Rohim Tualeka<sup>2</sup> , Juliana Jalaludin<sup>3</sup> , Syamsiar S Russeng<sup>4</sup> , Pudji Rahmawati<sup>5</sup> , Ahsan Ahsan<sup>6</sup> , Indri H Susilowati<sup>7</sup>

868-874

 PDF

**Deleted**

### **Skin Penetration of Topical Epigallocatechin-3-Gallate (EGCG) as an Alternative Agent for Photoaging Prevention**

Damayanti<sup>1,2</sup>, Cita Rosita Sigit Prakoeswa<sup>2</sup>, Djoko Agus Purwanto<sup>3</sup>, Anang Endaryanto<sup>4</sup>, Widjiati Widjiati<sup>5</sup>  
882-886



### **Methods for Determination of Reactive Metabolites of Thiopurines**

Dardan Dreshaj<sup>1</sup>, Flaka Pasha<sup>2</sup>  
887-893



### **Identification of Inhibiting Factors as a basis for Formulating Strategies to Tackle HIV/AIDS in Tulungagung Regency, Indonesia**

Desy Puspitasari<sup>1</sup>, Farida Handayani<sup>1</sup>, Mamik Hidayah<sup>1</sup>, Ratna Dwi Wulandari<sup>2</sup>, Agung Dwi Laksono<sup>3</sup>  
894-900



### **Relation between Serum Lipids and Thyroid Hormones in Hypothyroidism Patients**

Dheyaa Shinyar Hamad  
901-907



### **Evaluation of Storage Length to Blood Component Platelet Concentrate Quality in the Blood Bank, Dr. Soetomo General Hospital, Surabaya, Indonesia**

Dhinasty Armenia<sup>1</sup>, Betty Agustina Tambunan<sup>2,3</sup>  
908-913



### **Pediatric Biliary Atresia: Prenatal and Postnatal Risk Factors**

Dina Aristiya Sumarno<sup>1</sup>, Sjamsul Arief<sup>2</sup>, Bagus Setyoboedi<sup>3</sup>  
914-919



### **Distribution of Snail Mucous Extract (*Achatina Fulica*) on the Number of Wound's Basal Epithelial Cells in Rats of Wistar Strain**

Dina Karimah Putri<sup>1</sup>, Titiek Berniyanti<sup>2</sup>, Edhi Jularso<sup>3</sup>  
920-927

 PDF

### **Do Turbans Provide Protection Against Cranial Trauma in Two-Wheeler Accidents? A Review of the Indian Medical Literature**

Dirk HR Spennemann

927-932

 PDF

### **The Influence of Baby Spa Toward Growth and Development Rough and Smooth Motoric Babies, Age 3 – 12 Months in the Subdistrict Lapongkoda District of Tempe Wajo Regency**

Een Kurnaesih<sup>1</sup> , Ariesty AR<sup>2</sup>, Masriadi<sup>3</sup>

933-938

 PDF

### **Working and Resting Period based on Inclination, Loads, and Lift Methods on Manual Material Handling in Mining Environment**

Eko Nurmianto<sup>1</sup> , Udisubakti Ciptomulyono<sup>2</sup> , Suparno<sup>3</sup> , Sudiyono Kromodihardjo<sup>4</sup> , Hari Setijono<sup>5</sup> , Nur Ahmad Arief<sup>6</sup> , Abdul Rohim Tualeka<sup>7</sup>

939-944

 PDF

### **Oral Care Colostrum Effect on Preterm Infants Fecal Immunoglobulin A Secretory Level**

Eko Wahyudi<sup>1</sup> , Martono Tri Utomo<sup>2</sup> , Risa Etika<sup>2</sup>

945-949

 PDF

### **Correlation of Toluene Safe Concentration, Malondialdehyde and Glutathione Levels in Osowilangun Shoe Home Industry Workers**

Emilia Yanita Rachma<sup>1</sup> , Abdul Rohim Tualeka<sup>2</sup> , Juliana Jalaludin<sup>3</sup> , Syamsiar S Russeng<sup>4</sup> , Pudji Rahmawati<sup>5</sup> , Ahsan Ahsan<sup>6</sup> , Indri H Susilowati<sup>7</sup>

950-958

 PDF

### **Causes of Death Mother and Efforts to Reduce Mother Death Numbers in Luwu District, South Sulawesi Province**

Erni<sup>1</sup> , Muhammad Syafar<sup>2</sup> , Irwandy<sup>3</sup>

959-964

 PDF

## **The Effect of Proactive Personality, Professionalism, and Work Engagement on Performance among Village Midwives in Neonatal Complication Management Using the Integrated Management of Childhood Illness**

Farouk Ilmid Davik<sup>1</sup> , Fitri Widyacahya<sup>1</sup> , Muhammad Ardian Cahya Laksana<sup>2</sup> , Nyoman Anita Damayanti<sup>1</sup>, Dewi Retno Suminar<sup>3</sup> , Ratna Dwi Wulandari<sup>1</sup>

965-971



## **Causative Factors of Increasing Prescription Drug Abuse based on Transcultural Nursing Theory**

Ah Yusuf<sup>1</sup> , Muhammad Tarmizi<sup>2</sup> , Makhfudli<sup>3</sup>

972-979



## **The Synergistic Effect of Ascorbic Acid and Selenium on Serum Testosterone in Restraint Stressed Rats**

Alaa K. M. Al-amery<sup>1</sup> , Ali A. Al-Fahham<sup>2</sup>

980-985



## **Comparative Test of Midazolam Hydrochloride Stability in Different Storage and Temperature Container**

Anang Endaryanto<sup>1</sup> , Andang Miatmoko<sup>2</sup> , Mela Dwi Wulandari<sup>3</sup> , Novi Aryanti<sup>3</sup>

986-991



## **Relationship of Age, Work Period, and Education Level with Occupational Accidents in the South Borneo Coal Industry**

Arahnca Sevanya Tanaya<sup>1</sup> , Tri Martiana<sup>2</sup> , Zikri Fathur Rahman<sup>3</sup>

992-997



## **Effect of SafMannan on Local Iraqi Laying Hens Performance**

Yasser J. Jameel<sup>1</sup> , Mohannad M. Alrekabi<sup>2</sup> , and Nihad A. Ali<sup>3</sup>

998-1003



## **In vitro measurement volume of the neonate thyroid gland**

Atyaf Mohammed Ali<sup>1</sup> , Anas Hamed Musleh<sup>1</sup>

1004-1010

 PDF

## Deleted

1011-1014

## Effect of Alpha Lipoic Acid on Polycystic Ovary Syndrome with Insulin Resistance

1015-1020

 PDF

## Assessment of Women's Knowledge Concerning Hydatidiform Mole at Maternity Hospitals in Baghdad City

Fatima Fadel Benyian

1021-1027

 PDF

## Adherence and Satisfaction of Deferasirox versus Deferoxamine in Transfusion-Dependent Beta-Thalassemia

Mustafa Al-Khyate<sup>1</sup>, Zeina A. Althanoon<sup>2</sup>, Nasih A. Alkazaz<sup>3</sup>

1028-1033

 PDF

## Clinical Treatment of UTI in Rats Induced by Pathogenic E. Coli

Wissam Qadry Mutaab Alqaissy<sup>1</sup>, Thaar Mohammed Najim<sup>2</sup>, Bahjat Fakhri Mahmood Al-shammari<sup>1</sup>, Mustafa Salah Hasan<sup>2</sup>, Marwa Raad Jead<sup>3</sup>

1034-1038

 PDF

## Artemesia annua L. Silver Nanoparticles Exhibit Anticancer Activity by ROS mediated apoptosis in MDA-MB-231 Cells

Renugadevi Karthikeyan<sup>1</sup>, Julius Amaldas<sup>2</sup>

1039-1045

 PDF

## Implant Screw Breakage: A Review Article

Vijay Ebenezer

1046-1048

 PDF

## Fate of Immediate Implants Vs Single Tooth Implants: A Review Article

Balakrishnan1 , Vijay Ebenezer2

1049-1050

 PDF

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Balakrishnan1 , Vijay Ebenezer2

1051-1053

 PDF

### **Tilted Implants: An Alternative to Conventional Fabricated Dentures**

Vijay Ebenezer1

1054-1057

 PDF

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Balakrishnan1 , Vijay Ebenezer2

1058-1060

 PDF

### **A Beginner’s Guide to Surgical Treatment Objective – An Orthodontist’s Point of View**

Diana Ashok1 , Thulasi Ram E2 , Kannan M S.3

1061-1065

 PDF

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Jeenu Raj1 , M.S Kannan2

1066-1072

 PDF

### **Key Stone Entity in Paediatric Dentistry: Tell Play Do**

Sabari Lavanya, PonnuduraiArangannal, Jeevarathan J, Amudha S, Aarthij, Vijayakumar M

1073-1075

 PDF

### **Naturally Available Transport Medium For Avulsed Teeth- A Review**

Ramya Chellammal1 , Jeevarathan J2 , PonnuduraiArangannal3 , Vijayakumar M4 , Amudha S5 , Aarthi J6

1076-1080

 PDF

### **Gymnema Sylvestre - A Review**

Arumugam Rajalakshmi 1, 2, Govindarajan Sumathy3  
1081-1084



### **Methods for Distalizing Molar – A Review**

B Raghavendra1 , E.thulasi Ram2 , Harini R1  
1085-1089



### **Frozen Section-A Short Review**

Ryhanath Gulshan1 , N. Aravindha Babu2  
1090-1092



### **Potential Triggers of Lichen Planus: A Review**

E.P. Sridevi Anjuga1 , N. Aravindha Babu2  
1093-1098



### **Guidelines for Diagnosis and Treatment of Recurrent Aphthous Stomatitis for Dental Practitioners**

E.P. Sridevi Anjuga1 , N. Aravindha Babu2  
1099-1104



### **Holistic or Biologic Dentistry : Future Alternative or Not**

Sudakshina Mukherjee1 , N. Aravindha Babu2 , E. Rajesh3, K.M.K.Masthan4  
1105-1107



### **Viral Lesions of Oral Cavity**

Sudakshina Mukherjee1 , N. Aravindha Babu2 , E. Rajesh3, K.M.K.Masthan4  
1108-1113



### **Endodontic-Periodontic Relationship - A Short Review**

Ravindranath Megha1 , Alagarsamy Venkatesh2 , Nagarajan Geethapriya3 ,Balasubramaniam Anuradha3  
1114-1118

 PDF

### **A Review on Cracked Tooth Syndrome**

Loganathan Saatwika<sup>1</sup> , Venkatachalam Prakash<sup>2</sup> , Dhakshinamoorthy Malarvizhi<sup>2</sup> , Arunajetasan Subbiya<sup>3</sup>  
1119-1122

 PDF

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Balakrishnan Ramalingam  
1123-1125

 PDF

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Jayamathi Govindaraj<sup>1</sup> , Keerthidaa Govindaraj<sup>2</sup> , S.Raghavendra Jayesh<sup>3</sup> , Kesavaram Padmavathy<sup>4</sup> ,  
Mathangi Ramalingam<sup>5</sup> , Vidyarekha U<sup>6</sup>,  
1126-1130

 PDF

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Aravindha Babu.N<sup>1</sup> , Nishanth.G<sup>2</sup> , B. Krishna Prasanth<sup>3</sup> , K.M.K.Masthan<sup>4</sup>  
1131-1134

 PDF

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B. Kanchana, N. Sundharrajan<sup>2</sup> , B. Krishnaprasanth<sup>3</sup> , N. Aravindha Babu<sup>4</sup>  
1135-1139

 PDF

### **Auditory Hallucination in Hearing Loss Patient: A Case Report**

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1140-1142

 PDF

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Pavan Kumar N<sup>1</sup> ,Venkatraman Indiran<sup>2</sup> , Ramachandra Prasad<sup>3</sup> , Prabakaran Maduraimuthu<sup>4</sup> ,Krishna Prasanth<sup>5</sup>  
1143-1149

 PDF



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Sherly.K1 , Ambar Gupta1 , Aishwarya.P1 , Krishna Prasanth2 , Aravindha Babu.N3

1150-1154

 PDF

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Jamuna Dayalan1 , Suman Maji1 , Jerusha Biju1 , Subhashini Dhandayutham2 , Krishna Prasanth3, Bhuminathan.S4

1155-1159

 PDF

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Aravindha Babu.N, Sudakshina Mukherjee2 , N. Anitha3 , K.M.K.Masthan4

1160-1162

 PDF

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Vijay Ebenezer1 , Balakrishnan Ramalingam2 , M.Elumalai3

1163-1165

 PDF

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1166-1172

 PDF

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Suresh Mitthra1 , Manasa Narasimhan2 , Ragupathy Shakila2 , Balasubramaniam Anuradha3

1173-1178

 PDF

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Suresh Mitthra1 , Regunathan Hema2 , Balasubramaniam Anuradha3 , Tirpuravaram Vinay Kumar Reddy3 ,

1179-1183

 PDF

## **Morphologic Aberrations of Mandibular Premolar with two and Three Canals: Case Report**

Sahithi Reddy1 , Venkatachalam Prakash2 , Ramachandran Tamilselvi3, Arumugam Karthick4 ,

1184-1188

 PDF

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Balasubramaniam Anuradha<sup>1</sup> , Narasimhan Manasa<sup>2</sup> , Suresh Mitthra<sup>3</sup>, Balasubramaniam Anuradha<sup>1</sup> ,  
Narasimhan Manasa<sup>2</sup> , Suresh Mitthra<sup>3</sup>

1189-1192

 PDF

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1193-1197

 PDF

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1198-1202

### **100 Years of Calcium Hydroxide in Dentistry: A Review of Literature**

Sahithi Reddy<sup>1</sup> , Venkatachalam Prakash<sup>2</sup> , Arunajatesan Subbiya<sup>3</sup> , Suresh Mitthra<sup>4</sup>

1203-1219

 PDF

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1210-1214

 PDF

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1215-1220

 PDF

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1221-1225

 PDF

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Malvika Raghuvanshi<sup>1</sup> , E.Rajesh<sup>2</sup> , Shruti Sinha<sup>3</sup> , N.Aravindha Babu<sup>4</sup>

1226-1229



## **Alternative Medicines in Covid 19 – A Review**

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1230-1242



## **Artifacts in Histopathology – A Review**

N. Aravindha Babu<sup>1</sup> , E.P. Sridevi Anjuga<sup>2</sup> , K.M.K.Masthan<sup>3</sup> , E.Rajesh<sup>4</sup> ,

1243-1250



## **Artifacts in Histopathology – A Microscopic View**

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1251-1255



## **Management of Biomedical Waste in Hospital and Health Care Organizations- A Review**

Shruti Sinha<sup>1</sup> , N.Aravindha Babu<sup>2</sup> , Shyam Sundar Behura<sup>3</sup> , E.Rajesh<sup>4</sup>

1256-1260



## **Blood Transfusion and Its Effects**

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1261-1266



## **Corona Virus- An Overview**

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1267-1282



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1283-1290

 PDF

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1283-1290

 PDF

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1300-1302

 PDF

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1303-1306

 PDF

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1311-1314

 PDF

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1315-1320

 PDF

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R.Hariharan<sup>1</sup> , N.Anitha<sup>2</sup> , E.Rajesh<sup>2</sup> , AravindhaBabu<sup>3</sup>  
1321-1324

 PDF

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1325-1329



### **An Infected Sac in the Mandible – A Case Report.**

Harshada Ragunathan<sup>1</sup> , Sankar Narayanan R<sup>2</sup> , Nalini Aswath<sup>3</sup>  
1330-1334



### **Parathyroid Hormone Related Protein - A Skeletal Biomarker – A Review**

A. Arif Yezdani,  
1335-1339



### **Principles of Tooth Preparation - Review Article**

Ganesh Ramesh<sup>1</sup> , Sanjna Nayar<sup>2</sup> , S.Chandrakala<sup>3</sup>  
1340-1346



### **An Review on Occlusal Plane Analysis in Dentulous Patients**

S. Chandrakala<sup>1</sup>, Ganesh Ramesh<sup>2</sup> , Sanjna Nayar<sup>3</sup>  
1347-1351



### **Dental Articulator – Hanau Wide Vue**

Monika.D1 , Sanjna Nayar 2  
1352-1359



### **A Review on Occlusal Plane Analysis in Edentulous Patients**

Lakshmi.S1 , Sanjna Nayar<sup>2</sup>  
1360-1369



### **Nano Modification of Titanium Dental Implants- A MetaAnalysis**

Monika D1 , SanjnaNayar 2  
1370-1379



## Diagnosis and Treatment Modalities of Open Apex – A Review

Ravindranath Megha<sup>1</sup> , Newbegin Selvakumar Gold Pearlin Mary<sup>2</sup> , Arumugam Karthick<sup>3</sup> , Paramasivam Vivekanandhan<sup>3</sup>

1380-1384



## A Recurrent Case of Oral Squamous Cell Carcinoma

Hemalatha V.T<sup>1</sup> , Sindhu P<sup>2</sup>

1385-1388



## Oral and Perioral Piercing and Its Implications on Hard and Soft Tissues

Aravindh Swamy

1389-1392

## Short Communication -Oro dental Perspective of Substance Abusers

S.Angelinteena,

1393-1395



## Laboratory Diagnosis of Covid 19 – A Review

1396-1404



## Lingual Thyroid- A Review

G. Nishanth, N.Anitha<sup>2</sup>, N. Aravindha Babu<sup>2</sup> , L.Malathi<sup>3</sup>

1405-1407



## Nutrigenomics and its Role in Periodontal Health – A Review

Shyam Sundar Behura<sup>1</sup> , E.Rajesh<sup>2</sup> , Abinash Patra<sup>3</sup> , N.Aravindha Babu<sup>4</sup>

1408-1411



## Syndromes of Head and Neck – A Quick Review

Sangeetha Priya.P<sup>1</sup> , Aravindha Babu<sup>2</sup> , K.M.K.Masthan<sup>3</sup> , N.Anitha<sup>4</sup>

1412-1418



## **Effects of Yoga in Dentistry- A Review**

G. Nishanth1 , L.Malathi2 , N. Aravindha Babu3 , E.Rajesh4

1419-1422



## **Use of Agar as a Dental Impression Material**

Niha Naveed1 , Kishorekumar S2

1423-1426



## **Bone Morphogenetic Protein-1 and Its Regulation - A Review**

Niha Naveed1 , Kishorekumar S2

1427-1430



## **Curing Techniques of Composite Resin: Continuous or Intermittent?**

Niha Naveed1 , Kishorekumar S2

1431-1435



## **Efficacy of Clear Aligners in Correcting Anterior Open Bite- A Systematic Review**

Niha Naveed1 , Kishorekumar. S2

1436-1443



## **In Situ Hybridisation-A Review**

E.Rajesh1 , R.Hariharan2 , N.AravindhaBabu3 , L.Malathi4 ,

1444-1449



## **Oral Manifestations of Hyperparathyroidism and Its Management-A Review**

R.Hariharan1 , N.Anitha2 , L.Malathi2 , N. Aravindha Babu3

1450-1454



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Niha Naveed1 , Kishorekumar S2

1455-1458

 PDF

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1459-1463

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S.KishoreKumar1 , T.Shoba2 , Prashanth Sreenivasan3  
1464-1468

 PDF

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Tamizhesai Balavadivel1 , Raghavendra Jayesh2 , Sanjna Nayar3 , Venkateshwaran Rajendran4  
1469-1475

 PDF

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S. Chandrakala1 , Ganesh Ramesh2, Sanjna Nayar3  
1476-1482

 PDF

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Praveen P1 , Ragavendra Jayesh2 , Sanjna Nayar3  
1483-1487

 PDF

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Dhanasekaran Sihivahanan1,Vidyashree Nandini2 , E.Rajesh3 , N.Anitha3  
1488-1491

 PDF

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1492-1497

 PDF



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Niha Naveed<sup>1</sup> , Kishorekumar S<sup>2</sup>

1498-1501



## **Esthetic Replacement of Missing Anterior Tooth with All Ceramic Restoration (IPS E.max Cad) – A Case Report**

Praveen P<sup>1</sup> , Ragavendra Jayesh<sup>2</sup> , Sanjna Nayar<sup>3</sup> .

1502-1505



## **Role of Platform Switching in Success of Dental Implants- A Meta-Analysis**

Lakshmi.S<sup>1</sup> Post graduate student, Sanjna Nayar<sup>2</sup> MDS

1506-1515



## **Microbial Etiology of Acute Diarrhea in Children Under Ten Years of Age in Diyala, Iraq / Hospital-Based Study**

Adawia Fadhel Abbas Alzubaidi<sup>1</sup> , Mustafa Ali Abbas<sup>2</sup>

1516-1522



## **Influence of Sesame Oil on Physiologic and Histopathological Changes in Alloxanized Male Guinea Pigs**

Ahlam A. AL-Rikaby<sup>1</sup>, Mitham A. AL-Rikaby<sup>2</sup> , Saleh K.Majeed<sup>3</sup>, Researcher/Hussein S. AL-Jaberi<sup>1</sup>

1523-1529



## **Prevalence and Determinants of unfavorable Maternal and Neonatal Outcome in Term Premature Rupture of Membrane**

Israa Kamal Qasim<sup>1</sup> , Ilham Ali Salman<sup>2</sup>

1530-1537



## **Detection of the Genetic Diversity and the DNA of Some Species of the Cruciferous Family (Brassicaceae) using the RAPD-PCR Markers**

Qusay Ahmed Olajj<sup>1</sup> , Abdul Majeed Abdul Aziz Humadi<sup>2</sup>

1538-1544



## **Study of Bacterial Infections Associated with Infection with Cytomegalovirus in Anbar Governorate**

Saddam Hussein Ali<sup>1</sup>Jamal<sup>1</sup> Abdul Rahman<sup>2</sup>  
1545-1550



## **The Effect of Pollen Type on Some Fruit Traits and Production Quantity of Date Palm Trees Phoenix Dactylifera L. For Agricultural Cultivar Al-Ibrahimi**

Uday Hatem Khaeer Abed<sup>1</sup> , Abed-Almajeed Abed-Alazeez<sup>2</sup>  
1551-1555



## **Isolation and Molecular Detection of Salmonella Infantis from Milk and Children with Gastroenteritis in Babylon Province, Iraq**

Niran Kadhim F. Al-Rubaey<sup>1</sup> , Alyaa Mohammed H. Al-Tabtabai<sup>2</sup> , Khaleid Yassen Al-Zamily<sup>3</sup> ,  
1556-1562



## **Evaluation Study of Different Techniques in Diagnosis of Acute Leukemia in Adults and Children Patients**

Rash Hamid Ayub<sup>1</sup> , Ayad Hameed Ibraheem<sup>2</sup>, Najwan Salih Nima<sup>3</sup>  
1563-1570



## **Analysis of Blood Spot in the Crime Scene in Relation to the Environmental Parameters**

Nesreen Muneam Yaseen<sup>1</sup> , Ayad M. J. Al-Mamoori<sup>2</sup>  
1571-1576



## **Determination of IL17 Plasma Levels and IL-17A gene Polymorphisms in Patients with Allergic Rhinitis**

Mohammed D. D. AL-Beagy<sup>1</sup> , Thanaa M. Jouda<sup>2</sup> , Safaa H. Alturaihy<sup>3</sup>  
1577-1580



## **Activity of Extract Seed Moringa Oleifera on Gene Expression of (EFB1) Gene in Candida dubliniensis Isolated from oral Candidiasis to Cancer Patients**

Zahraa Khudhair Abbas Al-Khafaji<sup>1</sup> Farqad Abdulelah Ali AL-Janahi<sup>2</sup> , Al-karrar Kais Abdul Jaleel Duaibel<sup>3</sup>  
1581-1587



## **Reducing the Environmental Impact of Cork Waste by Improving Natural Asphalt as Water Proofing Material**

Bashar Abdulazeez Mahmood<sup>1</sup>, Majid Ahmed Mohaisen<sup>2</sup>, Adil Hasan Mahmood<sup>3</sup>, Saadoon O. Eyada<sup>4</sup>  
1588-1595



## **Assessment the Effect of Lactobacillus Acidophilus on Escherichia Coli Serotype O157:H7 with Detection of Some Virulence Factors**

Mokhtar Jawad AL-Imam<sup>1</sup>, May Talib Flayyih<sup>2</sup>  
1596-1602



## **Effect of Conocarpus Erectus Extraction on Some Physiological and Biochemical Parameters on Male Rats**

Adel M. Al Zobidy  
1603-1607



## **Synergistic Effect of Amikacin and Ciprofloxacin on p<sub>elA</sub> and algD Genes in Pseudomonas aeruginosa**

Wathiq Abbas Hattie Al-draghi<sup>1</sup>, Rami Abd Al-Kareem Saeed<sup>2</sup>  
1608-1614



## **CA 27-29: A Valuable Marker for Breast Cancer Management in Correlation with CA 15-3**

Hiba Sarmad Kamal AL-Azzawi<sup>1</sup>, Manal Kamal Rasheed<sup>2</sup>, Manwar AL-Naqqash<sup>3</sup>  
1615-1621



## **Incidence of Mumps in Anbar province, West of Iraq during the Period 2009-2018**

Mothana Ali Khalil<sup>1</sup>, Hekmat Ahmed Owaid<sup>2</sup>, Shehab Ahmed Lafi<sup>3</sup>  
1622-1629



## **Effect of Insecticide "Maxxthor" on Some Hematological and Oxidative Stress Parameters in Male Albino Rats**

Entesar A. Essa<sup>1</sup>, Layla J. Mohammed<sup>2</sup>, Jabbar H. Yenzeel<sup>3</sup>, Sahar A. H. AL-Sharqi<sup>4</sup>  
1630-1636



## Using Probiotics, Antibiotics and their Combinations to Eradicate Biofilms Formed by Four Pathogenic *Klebsiella Pneumoniae* Strains

Omer N. Abbas<sup>1</sup> , Abdulwahid B. Al-Shaibani<sup>2,3</sup>, Ahmed A. Mhawesh<sup>2</sup>  
1637-1644

 PDF

## Study and Assess the Occupation Hazards to Health Workers in the City of Kut/ Wassit

Fatima Haran Daham<sup>1</sup> , Alaa Kadhim Jasim <sup>2</sup> , Khalied Yassen Zakair<sup>3</sup>  
1645-1651

 PDF

## Associated Factors of Adulthood Overweight/Obesity among Pharmacy Department Students in Al-Rafidain university College / Iraq

Samer Shukur Mohammed<sup>1</sup> , Wael Waleed Mustafa<sup>2</sup>  
1652-1658

 PDF

## Serological, Culture and Urea Breath Test for Detection of *H. Pylori* in Gastric Ulcers Patients

Nktel Faaz AL-Saad<sup>1</sup> , Nadhim M.H<sup>2</sup> , Nawar H.Y<sup>2</sup>  
1659-1664

 PDF

## Low Pollution Emission to Purify Fuel Cell Technology Comprehensively: A Review

Muhaned Mohammed Eteya<sup>1</sup> , Hayder Saleh Mahdi<sup>2</sup>  
1665-1669

 PDF

## Major Histocompatibility Complex gene Polymorphism in Systemic Lupus Erythematosus Patients

Zahraa Haleem Alqaim<sup>1</sup> , Methak J. Al-Jboori<sup>2</sup> , Mona N. Al-Terehi<sup>3</sup>  
1670-1673

 PDF

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1674-1678

## Association of Some NKG2 gene Family and it' s Polymorphism with Pulmonary Tuberculosis

Nktel Faaz AL-Saad <sup>1</sup> Mohammed A.K Alsaadi <sup>2</sup> Ali Hmood Al-saadi <sup>3</sup>  
1679-1685

 PDF

### **Evaluation of Breast Cancer Molecular Profile in Old Age Patients in Comparison to Young Age Patients**

Zahraa Ali Saleh AL-Tae1 , Asaad A. Al-Janabi2 , Ahmed Muhsin Yousif AL-Mayali3  
1686-1692

 PDF

### **Deleted**

1693-1699

### **Seroprevalence of Syphilis among Iraq's Blood Donors and Their Relation with Blood Group and Rh Factor**

Suad Azeez Hassan1 , Eman S. Nassir2 , Aseel I. Ibrahim1 , Luma Tareq3  
1700-1706

 PDF

### **Comparative Histomorphological Study of Kidneys in Pigeon (*Columba livia*) and Starling Birds (*Sturnus vulgaris*)**

Suhaib A. H. AL-Taai1 , Riyadh H. Nasif1  
1707-1713

 PDF

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Yusriyadi1 , Aditya Yuli Sulistyawan2  
1714-1718

 PDF

### **Variation of 16SrRNA gene for Some Nosocomial Bacteria Isolated from Ramadi Teaching Hospital for Women and Children**

Saade Abdalkareem Jasim1 , Sumaya Ayad abdulrazzaq1 , Saba ,a Muhsen Farhan1  
1719-1724

 PDF

### **Assessment of Knowledge and Performance Regarding Triage Among Nurses Working in Emergency Unit-Al Dawadmi General Hospital: A Cross Sectional Study**

Walaa Ahmad Eisa1 , Awatif Alhmeidi Alenizi2 , Entesar-makhlouf3  
1725-1731

 PDF

## **Effect of Orally-administered Silver Nanoparticles (Ag-NPs) on Some Biochemical Parameters in Kidney of Rats**

H. Kh. Ismail 1 , A. A. AlKshab2 , A. A. AL-Baker2

1732-1738



## **The Effect of a Mentoring Program on Competition Orientation for Football School Athletes**

Sarmad Abdul Ridha Abdul Hussain Al Hussaini 1, Mohamed Abdel-Reda2 , Khaled Mohamed Reda3, Haider Naji Habash Al-Shawi 4

1739-1744



## **The Synergistic Effect of Combining Some Antibiotics with Powder of Silver NPs, Copper NPs and Titanium NPs**

Raghad N. Shihab1 , Ali M. Al-Dahbi2 , Saif Ali Mohammed Hussein3

1745-1751



## **Phylogenetic Tree Analysis of First Psychrobacter Sp. Strain From Blood of Iraqi Patient; A Case Report**

Nuha S. Jassim1 , Sameer Abdul Ameer Alash2 , Najwa Shihab Ahmed2

1752-1756



## **Analytical Evaluation Study of Some Characterization of Potable water for an Old Well in Baghdad City**

Analytical Evaluation Study of Some Characterization of Potable water for an Old Well in Baghdad City

1757-1761



## **Prevalence of Hepatitis B, C and HIV Viruses among Hemophiliac Patients in Al-Anbar Governorate**

Hussein Ali AL-Enzy1 Fakhri Jamil Al-Dalla Ali2 , Adnan M Jasim3

1762-1767



## **Quantitative Genetics Analysis of Autism According to Sex Hormones**

A-S. U. Hassan1 , Najat Mohammed Flyyih2, Suhaib Khalid Ibrahim3

1768-1772



## **Association Between Carbamazepine Toxicity, Liver Bile Duct Injury, Granuloma and Inflammatory Cells Infiltration in Female Mice**

A.T. Nawar R. Jaber<sup>1</sup> , Nahla A. Al-Bakri<sup>2</sup> , A.T. Ola Al-Bhadly<sup>3</sup>

1773-1778



## **A Program for Developing Locally Food Supervision to Support Sanitary Monitoring Work**

Mahmud Abdullah Jasim Alkhafaji

1779-1783



## **Synthesis and Biological Activities of New 1,2,3,4- Tetrahydroquinoline Derivatives Using Imino Diels-Alder Reaction**

Mariam Abdul Jalil Abdul Razak<sup>1</sup>, Muhammad Abd Kadhum<sup>2</sup>

1784-1789



## **Phenotype Detection of Genetic Enzymes B- Lactamase Isolation of Patients with Urinary Tract Infections Bacteria Escherichia Coli**

Ali Hassoun Hammadi

1790-1796



## **Immunohistochemical Evaluation of Apoptotic Proteins Expression in Liver and Spleen after Treatment of Cystic Echinococcosis: An Experimental Study**

Talib Waseen Hussein<sup>1</sup> , Waheeda Rasheed Ali<sup>1</sup>, Haider F. Ghazi<sup>2</sup>

1797-1802



## **Knowledge of the Mothers toward Sanitation and Hygiene in relation to Childhood Gastroenteritis in Babylon Province**

Sijal Fadhil Farhood Al Joborae<sup>1</sup> , Fadhil Farhood Mekki Al Joborae<sup>2</sup> , Sura Falah Abdul Hadi Al-Jubbawi<sup>3</sup>, Ihab Raad Abbas Abid Ali<sup>4</sup>

1803-1808



## **Expression Studies of the Cancer Drug and Its Effect on Angiogenesis-Promoting Genes in Cancer Cell Lines**

Salam Abbas<sup>1</sup> , Noor Harith AL-Hamid

1809-1815



### **Bacillus cereus in Meat Products: 16S rRNA Phylogenetic Tree analysis and Antimicrobial Investigation of Nisin A, Rosemary Essential Oil and Tetracycline**

Sana MH AL-Shimmary 1, Ghusoon A. Abdulhasan2 , Mais E. Ahmed3

1816-1822



### **Measurement the Chemical Composition and Topography of Copper Nickel Titanium Arch Wires at Dry and Wet conditions (An In-Vitro Study)**

Saba Ammar Mohammed1 , Abeer Basim Mahmood2 , Mohammed Nahidh2

1823-1828



### **Teratogenic Effect of Carbamazepine Drug on the Histological Structure of Testes in the Albino Mouse (Mus musculus)**

Thekra Atta Ibrahim1 , Mohammed Ali Mohammed2, Ibtisam Khalaf Abd ali3 , Mohammed Nsaif Abbs4 , Suaad Abbas Hussien1

1829-1834



### **Continuous Flow Injection Analysis, Turbidimetric and Photometric Determination of Methyldopa Using a New Long Distance Chasing Photometer (NAG-ADF-300-2)**

Nagam S. Turkey1 , Elham N. Mezaal2

1835-1841



### **Assessment of Surface Roughness of Nickel Free Orthodontic Brackets and Archwires (An In Vitro Comparative Study)**

Dina Hamid Obaid1 , Dhiaa J. Nasir AL-Dabagh2

1842-1849



### **Workplace Violence against Health Care Providers in Emergency Departments: an Underrated Problem**

Ali Talib Hameed1 , Amer Abdullah Sachit2, Hussein Abdulmohsin Dabis3

1850-1856





## Effects of Gaseous Ozone (O<sub>3</sub>) on Artificial Population and Aflatoxin B<sub>1</sub> Production by *Aspergillus flavus* in Stored Whole Cinnamon in Iraqi Markets

Diyaa Aljaza

1857-1862



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Afaf K.Shuwaikh<sup>1</sup> , Hiba Resheed Behayaa<sup>2</sup>

1863-1868



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Maha T. Al-Saffar<sup>1</sup> , Abdulsattar Salim Mahmood<sup>2</sup> , Mohammed Salih Sulaiman<sup>3</sup>

1869-1874



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## Pharmacological Study of Weight Losing of Liraglutide Loaded within Layered Double Hydroxide Carrier

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1875-1880



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1881-1887



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1888-1893



PDF

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1894-1899



PDF

## Using Paper Waste and Local Bacterial Isolates to Produce Biomass and Cellulase Enzyme- A Study Waste Recycling

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1900-1906



## Effect of Foliar Application with Nano-Organic Fertilizer and Proline Acid and their Interaction in Leaf Content of Antioxidants and Vitamin D3 for Moringa oleifera

Faten Khalifa Kareem<sup>1</sup> , Mahmood A. Al Shaheen<sup>2</sup>

1907-1913



## Factors Affecting Success of Corneal Grafting : An Observational Study

Saif Al-Shamarti

1914-1916



## Estimation of Some Hormones Levels and Insulin Resistance in Some Infertile Men in Samarra City

Aseel Mokdad Hatam AbdulWahed<sup>1</sup> , Mostafa Ali Abdulrahman<sup>2</sup> , Mahmood I. M. Alkanaani<sup>1</sup> , Atheer N. B. Alhamad<sup>4</sup> , Saif Mohammed Wagaa<sup>4</sup> , Ghanim Talal Ghanim<sup>4</sup>

1917-1919



## Evaluation the Relationship between Oral Contraceptives Containing Drospirenone with Dyslipidemia and Risk of Cardiovascular Diseases among Women in Al-kut City

Haider Hafudh Humaish<sup>1</sup> , Amer Alasadi<sup>2</sup> ,Intithar Aldafae<sup>3</sup>

1920-1926



## Ability of Staphylococcus spp. Isolated from Meningitis Patients to Biofilm Formation

Abbas Hameed Sh. Al-Wandawy<sup>1</sup> , Luma Abdulhady Zwain<sup>2</sup>

1927-1931



## Evaluating the Role of High Molecular Weight Adiponectin in Women with Polycystic Ovary Syndrome Treated with Omega-3 Fatty Acids

Fadhil A. Nasser

1932-1939



## Levels of Leptin and IL-6 With Some Biochemical Parameters in Iraqi Obese Children

Asmehan Adnan AL- naqeeb<sup>1</sup> , Salwa Gahzi Turki<sup>2</sup> , Zahid Jasim Muhamed<sup>2</sup>, Wesam Hameed Ahmed<sup>3</sup>  
1940-1946

 PDF

## Study the Histopathologic Effects of Arsenic Trioxide on Some Parenchymatous Organs of Male Rats and Under Alphalipoic Acid

Khalil H. Aljeboori<sup>1</sup> , Khalil Gzar<sup>2</sup>  
1947-1952

 PDF

## Claudin is an Immunohistochemical Changes Associated with Experimental Infection of White Mice with 0111 – of non – 0157 Shiga Toxin Producing Escherichia Coli

Khalil H. ALjeboori<sup>1</sup> , Mukhald A. Ramadhan<sup>2</sup>  
1953-1960

 PDF

## The Impact of Gold and Silver Nanoparticles on The DNA Yield of Mycobacterium Tuberculosis MTB

Zaid Mohammed Joodi Al- Janabi<sup>1</sup> , Arshed H.Yaseen<sup>2</sup> , Raghad Abdullah Hassan<sup>3</sup>  
1961-1966

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## Molecular Investigation of fliC and blaTEM1 Gene among Clinical Isolates of Salmonella Typhi

Hala Ridha Abbas Al-Fahham  
1967-1972

 PDF

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Maysoon Abdul-zahra Merdaw<sup>1</sup> , Zeina Anwar Jaffar<sup>2</sup> , Zahraa Mohammed Ali<sup>3</sup> , Heba Hashim Khalil<sup>4</sup>  
1973-1978

 PDF

## CD14 C(-260)T Polymorphism and Blood Levels of the Soluble Endotoxin Receptor CD14, their Association with Risk of Ischemic Stroke in Iraqi Populations

Manal M. Kadhim<sup>1</sup> , Ali S. Shakir<sup>2</sup>  
1979-1985

 PDF

## **The Cytotoxicity Effect for the Crude Extract of (Cyperus Esculentus) Tubers on Human Breast Cancer Cell Line (MCF-7) in Vitro**

Zainab Abd Amir Mezher<sup>1</sup> , Liqaa H. Saqban<sup>2</sup>

1986-1993



## **Bacteriological Study and Investigation of Some Virulence Factors of Proteus mirabilis Bacteria Isolated from Urinary Tract Infection Patients in Ramadi City**

Nada Ali Attallah<sup>1</sup> , Muthana Badeea Farhan<sup>2</sup>

1994-2000



## **Molecular and Parasitological Study on Selected Opportunistic Intestinal Parasites in Immunocompromised Patients**

Mahmoud Shehab Al-heety<sup>1</sup> , Sarab Fawzi Al-Ani<sup>2</sup>, Maher Ali Jasem<sup>3</sup>

2001-2008



## **Immunocytochemistry of In vitro produced mucin from HT29- MTX cell line**

Immunocytochemistry of In vitro produced mucin from HT29- MTX cell line

2009-2015



## **Spectrophotometric Determination of Ampicillin with Sulfanilic Acid by Oxidative Coupling Reaction**

Samar Ahmed Darweesh<sup>1</sup> , Hamsa Muneam Yaseen<sup>2</sup> , Ruaa Muayad Mahmood<sup>3</sup> , Rokayia Samir Al-Khalisy<sup>4</sup> , Husam Saleem Khalaf<sup>5</sup>

2016-2021



## **Study of Smoking Effect on Some Liver Functions, Blood Pressure and Some Haematological Parameters in Smokers in Al-Muthanna Province**

Duaa Hamad Hamza<sup>1</sup> , Wafaa Abdulmutalib Naji<sup>2</sup>

2022-2028



## **Awareness of Creativity and Its Relationship to Speed and Accuracy of Futsal Soccer for Female Students**

Mohsen Mohamed Hassan<sup>1</sup> , Nidaa Falih Hassan<sup>2</sup> , Ali Muhammad Jawad Al-Sayegh<sup>3</sup>, Hayder Naji Habash Alshawi<sup>4</sup>

2029-2033

 PDF

### **The Effect Esthetic Factors in Complete Dentures Wearers Acceptances**

Osama A. R. Alheeti 1 , Ghasak H. Jani2 , Jassim A. Al-E'nizy3  
2034-2042

 PDF

### **Genotypic, Phenotypic Identification and Antibioqram Assay of Mrsa Isolated from Healthy Individuals in Wasit Province**

Haneen Saad Farhan1 , Zainab N. Al-Saadi2 , Jassim Fatehi Ali3  
2043-2049

 PDF

### **Study the Elevation of Fibrinogen level in Plasma Patients with Diabetes Mellitus Type 2 in Babylon Governorate**

Khawla A. Shemran  
2050-2053

 PDF

### **Relationship between Echocardiographic Epicardial Fat Thickness and Different Body Fat Distribution**

Noora Ibrahim Mustafa1 , Abbas Fadhil Abd-Alwahab2 , Abdulameer Abdulbari Abdulhame3  
2054-2060

 PDF

### **Biofilm production by E. Coli Isolated from Filter Water System**

Afrah Fahad Abdulkareem1 , Mohammed Khammas Abdul Kareem2  
2061-2065

 PDF

### **Antioxidant Effect of Beta-glucan Extract from Saccharomyces Cerevisiae**

Hiba K. Ibrahim1 , Mayaada S. Mahdi1 , Biologist/ Afnan A.2  
2066-2072

 PDF

### **Survey study of the Factors Determining Culex pipiens Mosquitoes Residing Strategy in Al-Furat Al-Awsat Technical University Campus**

Hamza Ahmed Aziz Al-Aaraji  
2073-2078

 PDF

### **The effect of Cooperative Learning Strategy on Sensory Modeling on the Performance of Some Essential Skills for Female Students' Basketball**

Zahraa Abdul-Jabbar Habib<sup>1</sup> , Hassan Saleh Mahdi Al-Okbi<sup>2</sup>

2079-2084

 PDF

### **The Effect of Special Exercises on the Most Superior Mental Processes and the Performance of Some Offensive Skills for Table Tennis Beginners**

Muhammad Salman Jaber Al-Khuzai<sup>1</sup> , Haider Flayeh Hasan<sup>2</sup>

2085-2090

 PDF

### **The Relationship of Some Psychological Skills to the Leading Personality of Police College Students**

Marwa Tariq Kazem<sup>1</sup> , Shaima Ali Khamis<sup>2</sup>

2091-2095

 PDF

### **Diagnosing the Mutations and the Effect Concentration Chromium Cr (VI) in KRAS gene in Exon1 for lung Cancer Patients in Najaf Governorate**

Farah Khalid H. AL-Mayali <sup>1</sup>, Mohammad Jaber M. AL-Zeyadi <sup>2</sup>

2096-2101

 PDF

### **The Effect of Incorporation Kappa-Carrageenan Powder on the Physical and Mechanical Properties of the Heat-Cured Acrylic-based Soft Denture Lining Material in Clinical Use**

Hiba A. Salih<sup>1</sup> , Faiza M. Abdul-Ameer<sup>2</sup>

2102-2110

 PDF

### **Functional Variables and Enzyme (CPK)to Skill of The main and Alternatives Players in Football (comparative study)**

Majed Sabar Mohammed<sup>1</sup> , Ali Sabar Mohammed<sup>2</sup> , Emad Khadim Abed Al-kaabi<sup>3</sup>

2111-2116

 PDF

### **Glycodeline-A , PIGF Together with ?hCG as Prognostic Markers in First Trimester Pregnancy Loss for Iraqi Women**

Mohammed H Dawood1 , Halla G Mahmood2, Afraa M Nedawi3  
2117-2122



### **Analysis of Single Nucleotide Polymorphism in Fim H Gene from Uropathogenic E.coli**

Sabreen Fleih Neno Al-Fatlawi1, Laith Abdul Hassan M. Jwaid2  
2123-2128



### **The Impact Of Child Abuse and Neglect on Oral Habit among 10-11 Years Old Primary School Students in Baghdad /Iraq**

Wasan Muwafaq Almirhij1 , Ban Sahib Diab2  
2129-2134



### **Genetic Study of the Effect of the Toxoplasma gondii on Somatic Cells of the Bone Marrow in Female Mice and a treatment Attempt by Laurus Nobilis Leaf Extract**

Ghfran Shihab Ahmed Al-badri1, Aysir Salih Mohammed2 , Omar Raheem Khalaf2  
2135-2139



### **Genetic evaluation of rs36513 SNP of UIMC1 and Biochemical Parameters in Early Menopause in Babylon City, Iraq**

Rana A. M. Shaker1 , Maha F. Mohammed2 , Bushra j. Al-Rubaey3  
2140-2146



### **Prevalence of Campylobacter Species in Diarrheal Samples of Children Less than 10 Years**

Ghada N. Khalaf 1 , Thaanaa R. Abdulrahman2  
2146-2153



### **Clinical Evaluation of Recurrent Aphthous Stomatitis and Its Correlation with Helicobacter Pylori**

Shaimaa A. Saeed1 , Taghreed F.Zaidan2  
2154-2159



### **The Effect of An Educational Curriculum According to (K.W.L) Strategy on Some Mental Abilities and Learning Some Basic Football Skills for Students**

Ali Mahdi Hassan<sup>1</sup> , Yousef Abdul Karim Yousef Abu Ghneim<sup>2</sup>, Hayder Naji Habash Alshawi<sup>3</sup>  
2160-2165



### **Molecular and Phenotypic Detection of Some of the Coded Genes for Virulence Factors in Mrsa and Mssa Isolated From Different Clinical Cases**

Noora Hamid Ibrahim<sup>1</sup> , Ahmed Mohammed Turki<sup>2</sup> , Jamal Abd Al-Rahman<sup>3</sup>  
2166-2171



### **Histological Changes Induced by the Fusarium Graminarum Silver Nanoparticles in Some Organs of Male Albino Mice**

Sahar A. H. AL-Sharqi  
2172-2178



### **Seroprevalence of HTLV-type-1 and type-2 among Blood Donors in Some Iraqi Provinces**

Aseel I. Ibrahim<sup>1</sup> , Yakhob A. Al-Musawi<sup>2</sup> , Aruba I. Abdullah<sup>3</sup>  
2179-2184



### **Occurrence of Rodotorula Mucilaginosa among Immunocompromised Patients with Different Infections**

Abbas M. AL-Ammari<sup>1</sup> , Anaam Fuad Hussein<sup>2</sup>  
2185-2189



### **The Impact of Socio-Demographic Characteristics on Quality of Life Among Dental Students in Iraq**

Jenan Othmaan Almaas<sup>1</sup> , Ban Sahib Diab<sup>2</sup>  
2190-2195



### **Investigation Study of Human Spermatozoa Proteome**

Morteta H. Al-Medhtiy<sup>1,2</sup>, Hutheyfa Al-Salih<sup>1</sup> , Hazem Almhanna<sup>1</sup> , Ahmed H. Al-Dabhawi<sup>1</sup>  
2196-2202



### **The Role of CTX-II , Dyslipidemia ,Vitamin D in Polycystic Ovary Syndrome**



Samah Amer Hammood1 , Alaauldeen S. M. AL-Sallami  
2203-2209



### **The Correlation Between Tumor Necrosis Factors-Alpha (TNF- $\alpha$ ) and Resistin in Obese Iraqi Women**

Zinah Abbass Ali1 , Khawla A. Shemran2  
2210-2213



### **Deleted**

Ali Z. Al-Saffar1 , Noora A. Hadi2 , Hadeel Mohamed Khalaf2  
2214-2221



### **The Effect of Multiple Glass Bead Sterilization Cycles On Cyclic Fatigue of AF BLUE S one File**

Bassam Alsheekhly1 Marwah M. Adnan2 Fudhla Sadoon Al- Zubaydi3  
2222-2227



### **Study of Mortality Acanthopagrus Latus Fish in Iraq**

Ali Balash Jebur1 , Rasha Abdulameer Jawad2  
2228-2231



### **Heritability of Ambulatory Blood Pressure in Population of Western Region of Iraq**

Louay M. Alani1 , Huda M. Mahmood2 Bilal J. M. Aldahham3  
2232-2237



### **Correlation Ship between Some Hematological Parameters and Smoking for Students**

Manar Kareem Alquraishy1 , Alia Hussein Al-Mousawi1, Ahmed Adnan AL-Khafagi2  
2238-2243



### **Impact of Digoxin on Endocrine Glands in Male Rats**

Abbas A. Khudhair 1 , Kassim F. Abdulkareem2 , Muhsin S.G. Al\_Mozie'1 3  
2244-2248

 PDF

### **Study Some Biochemical Parameters in Beta Thalassemia Major Patients in Males and Females Adults in Baghdad**

Rusul Yaareb Hameed<sup>1</sup> , Sanad Baqir Mahammad<sup>2</sup> , Bassam Francis Matti<sup>3</sup>  
2249-2253

 PDF

### **Biological Activity of Complexes of Some Amino Acid: Review**

Lekaa K. Abdul Kareem, Israa M. Radhi, Suhad S. Mohammed  
2254-2261

 PDF

### **Measuring of Interleukin -22 and IL -17a Levels in Seropositive and Seronegative Rheumatoid Arthritis Patients**

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2262-2268

 PDF

### **Detection of B1 Gene in Toxoplasma Gondii and the Role of Interleukin 2 in Abortive Women Infected by this Parasite**

Hanan Mohammed Saied Jassam<sup>1</sup> , Thaer Abdulqader Salih<sup>2</sup> 1 Post graduate/Department of Biology/  
College of Science/ University of Anbar/  
2269-2275

 PDF

### **Bacteriocin Production in Bacillus cereus Food Isolates with Molecular Detection of cerA gene**

2276-2282

 PDF

### **The Diagnostic Efficacy of Visually Enhanced Lesion Scope (Velscope) In Identifying Benign , Dysplastic and Cancerous Oral Lesions**

Suroor Ali Jabbar<sup>1</sup> , Fawaz Aswad<sup>2</sup> , Ameena Ryhan. Diajil<sup>3</sup>  
2283-2290

 PDF

### **Test Some New Media for Cultivation of Gram Positive and Gram Negative Bacteria**

Firas Nabeeh Jaafar<sup>1</sup> , Wala'a Shawkat Ali<sup>2</sup> , Nadia Kamil Bashar<sup>1</sup> , Rayim Sabah Abbood<sup>1</sup>  
2291-2296

 PDF

### **Blood Lymphocytes Detection in Iraqi Diabetic Type 2 Patients Infected with Chronic Toxoplasmosis by Using Flow Cytometry**

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2297-2303

 PDF

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2304-2310

 PDF

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Eman H. Altaee1 , Abdulkarim J. Karim1, Mohammed M. Dakheel2  
2311-2318

 PDF

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2319-2325

 PDF

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Raad Al-Taif Jassim1 , Ashwaq Talib Hameed2  
2326-2332

 PDF

### **The effect of Instant Feedback on Performance at the Digital Level on Discus Effectiveness**

Mayasah Abd Ali Kadhim  
2333-2336

 PDF

### **Relationship of Some Anthropometric Measurements With The Explosive Strength of the Upper and Lower Extremities with the Rhythmic Gymnastics of Female Students**

Hala Razzaq Mudalloul  
2337-2341

 PDF

### **Diagnosis of Coronavirus using Biochemical and Hematological Methods for Patients in Anbar Governorate**

Ban Hamid Khalaf1 , Qasim Khlaif Abdullah2 , Oqbah Abdul Halim3  
2342-2348

 PDF

### **Evaluation of Salivary ?- Amylase Enzyme Activity in Smoker and Peptic Ulcer Patients affected by Periodontitis in Relation to Clinical Periodontal Parameters**

Nada Kadhim Imran1 , Omar Husham Ali1 , Maha Abdulaziz Ahmed1  
2349-2355

 PDF

### **Histophysiological Considerations Deals Damaging of Rat's Kidney by Examined Doses of Heroin**

Abdul-Samad Uleiwi Hassan1 , Shatha Atta Abeed2 , Ashwaq Kadhem Obeid3  
2356-2362

 PDF

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Nassreen N. Mzhr 1 : Zaid Raad Abbas2 , Zaid Najji Hassan3  
2363-2368

 PDF

### **Attitude of Prime-Pregnant women toward Normal Vaginal Delivery in Southern of Babylon Governorate**

Maryam Abd Al-Kareem1 , Salma Kadhum2  
2369-2374

 PDF

### **Estimate the Serum Level of IL-17A and TGF- $\beta$ 1 in Iraqi Generalized Vitiligo Patients**

Shatha F. Tariq  
2375-2381

 PDF

### **Pattern of Firearm Discharge Residue on Cotton Cloth Substrate to Determine the Range of Firing by 7.65mm Caliber of Country Made and Standard Firearm: An Original Research Study**

Komal Yadav1 , Prashant Agarwal2 , NB Bardhan3  
2382-2387

 PDF

### **Histological and Physiological Study of the Effects of Exogenous Melatonin on Liver of Female Rats Exposed to Formalin in Drinking Water**

Rana Jafer Abid Khadim AL-Mayahi<sup>1</sup> , Mutashar JaddoaAL-Shewaily<sup>2</sup> , Ali Fayadh Bargooth<sup>2</sup>  
2388-2393

 PDF

### **Effect of Methotrexate on Adenosine Deaminase Activity in Rheumatoid Arthritis Iraqi Patients**

Ammal Esmaeel Ibrahim<sup>1</sup> ; Taha Shawi Morad<sup>2</sup> ; Shatha M. Hasan<sup>1</sup>  
2394-2397

 PDF

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Mohammad Alkubaisi<sup>1</sup>, Mohammed Hakim Shamran <sup>2</sup> , Faisal Al-Obaidi <sup>3</sup> , Azhar Alsalhi <sup>4</sup>, Abdulelah Shdiefat<sup>5</sup> , Ajeel Alajeel<sup>6</sup>, Asem Alchalabi <sup>7</sup>  
2398-2404

 PDF

### **Ctrophotometry Determination of Heavy Metal (Lead) in Cosmetics (Lipsticks) in Commercial Markets**

Adil Mohammed Hashim <sup>1</sup> , Ali Mohammed Abd-Alameer <sup>1</sup> , Ahmed Hameed Rashed<sup>1</sup>  
2405-2409

 PDF

### **Solubility and Dissolution Rate Enhancement of Ketoprofen by Nanoparticles**

Fatimah Mohammed Hussein Wais  
2410-2417

 PDF

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Allaa M. Ahmed  
2418-2424

 PDF

### **Assessment of Psychological Risk Factors for Spontaneous Abortion at Maternity Wards in Baghdad City Hospitals**

Sarah Hashim Mohammed<sup>1</sup> , Rabea Mohsen Ali <sup>2</sup>  
2425-2429

 PDF

### **Assessment of pregnancy - Related Health Problems among Less Than 18 Years Pregnant Women in Al- Nasiriya Maternity Hospitals**

Pregnant women , adolescent , pregnancy related Health, Personal Behavior, Biological Factor  
2430-2434

 PDF

### **The Relationship between Severity of Anemia and Nutritional Factors Determinant at Primary Health Care Centers in Baghdad City**

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2435-2440

 PDF

### **Evaluation The Iron Homeostasis and Erythropoietic Activity in Male Rats with Anemia Related with Induced Chronic Renal Failure**

Afiaa Layth Darweesh1 , Wafaa Kadhim Jasim2  
2441-2446

 PDF

### **Iraqi People Practices toward Adherence of Government Strategies to Control of COVID19**

Thani Asmar Radhi1 , Saad Sabri Shamkh2 , Mustafa Salim Abdulredha3  
2447-2452

 PDF

### **Effectiveness of an Educational Program in Nurses' Knowledge toward Traumatic Head Injury during Golden Hour at Neurosurgical Hospital**

Sabah Abdullah Jaafar1 , Raja Ibrahim Abed 2  
2453-2459

 PDF

### **Evaluation Activity of Boiled Aqueous Extract of Broccoli Flower Plant (Brassica Oleraceae L. Var. Italic) And Albendazole Drug Against the Protoscolices in Vitro**

Nabaa Zahid Sabri Shani Al-Hasnawi 1, Ahmed Khudhair Al-Hamairy2 , Huda Jasim M. Altameme2  
2460-2464

 PDF

### **Effect of compound exercises on Some Kinetic Abilities in Some Bio kinematics Variables and the Characteristics of the Curve (Force - Speed) for hit/spike of High Volleyball for Youth Volleyball**

Ali Naeem sultan1 , Ahmed Adnan Abd1, Asaad Flayyih Hasan 2  
2465-2470



### **Hearing and Children with Autism Spectrum**

Shaima Abd Muhsin AL-Haboobi 1, Sadiq Sahib Hadi Hussain Al-Amin2 , Zainab Abed Muhsin AL-Haboobi3  
2471-2479



### **Antibiotic Resistant Acinetobacter baumannii Isolated from Wounds Infections in Babylon Governorate**

Zahraa A. Al-Ajeeli 1 , Habeeb S. Naher 2  
2480-2483



### **Laparoscopic Cholecystectomy Under Epidural Anesthesia in Patients with Chronic Respiratory Diseases**

Thaer Ali Hussein Al Akam1 , Emad Mohammad Hussein1  
2484-2486



### **A Suggested Vision for Conducting of Electronic Supervision in the General Directorate of Education, Dhi Qar Governorate**

Zuhair Nadhem Khayoon Algabry1 , Ali Abdul Hussein Ali Al-Farjawi 2  
2487-2494



### **The Impact of Over-Learning Exercises in Developing Some of the Optical Vision Capabilities and Shooting Skill from Jumping in Handball Game for Young Players**

Zuhair Nadhem Khayoon Algabry1 , Rashad Tariq Yousef2 , Bahaa Thiyab Faisal3  
2495-2502



### **Effect of COVID-19 Pandemic on Psychological Attitudes' for Nurse Workers at Isolation Hospitals in Baghdad City**

Ahmad Shihab Ahmad  
2503-2507



## **The effect of use of Dexmedetomidine Hydrochloride as an Adjuvant in General Anesthesia on Rate of Awareness**

Nasser M. Meazher<sup>1</sup>, Jaafar Hameed Mahboba<sup>2</sup>, Zuhair Abdul Sada Dhahir<sup>3</sup>  
2408-2514



## **Isolation and Identification of Some Bacterial on the Eggs in Some Regions of Karbala Province**

Hussein Mahdi Kadhimi<sup>1</sup>, Mustafa Abdul Kareem Hameed<sup>2</sup>, Nawars M. Al-Khafaji<sup>3</sup>  
2515-2521



## **Role of Visfatin and Chemerin in Occurrence and Development of Gestational Diabetes in Pregnant Women**

Jaklen Zaea Khoshaba<sup>1</sup>, Rana Hadi Mosa<sup>1</sup>, Samira Sherzad Hussien<sup>1</sup>  
2522-2527



## **Preparation and diagnosis of Xerogel nanocomposites And Studying Their Effect on TNF- $\alpha$ Level before and after Loading Dexamethason in Male White Rats Induced Rheumatoid Arthritis**

Kiaser Abdulsajjad M.Hussain<sup>1</sup>, Ayser Ashour Khalaf<sup>2</sup>, Batool Abbas hussien<sup>1</sup>  
2528-2534



## **Study Effect of Some Pakistan Oil Isolation of Yeast Candida albicans causing oral thrush children Who Visit Women Hospital and Children in Diwaniya**

Nada Ahmed Fairouz<sup>1</sup>, Huda Ahmed Fairouz<sup>2</sup>  
2535-2540



## **The effect of Stylistic Discovery Directed and Programmed Education on teaching some football skills and understand the benefits of physical education and health**

Ahmed Zuhair Kattan<sup>1</sup>, Ahmed Hussein Abd<sup>2</sup>  
2541-2548



## **Effect of Age and the Pretest Score on Critical Thinking and Using Concepts Maps in Learning the Skill Scoring of Football for Students**

Hussein M. Tuama'a<sup>1</sup>, Mohammed Jabbar Munadi<sup>2</sup>, Saif Hadi Minjal<sup>3</sup>, Ayad Rasan Jasim<sup>4</sup>, Heider Sadiq Maki<sup>5</sup>



2549-2555

 PDF

### **Effect of Palliative Style Rhythmic Exercises on Some Physical Abilities**

Haider Sadiq Makki 1 , Ayad Resan Jassim1 , Hasan Abdullah Hnaihien1, Hussein Mhaibes Tuama2

2556-2561

 PDF

### **Psychological and Organizational Contexts for Handball Coaches from the Players' Point of View**

Majid Mohammed Msaed1 , Muhammad Shabram Alwan1

2562-2569

 PDF

### **The Effects of (I.P.A.G.A) Model in Reflection Thinking and Learning Spike Skill in Volleyball for Students**

Muhanad Mohammad Kadhum1, Hayder Salman Mohseen 2, Rafid Saad3

2570-2575

 PDF

### **Contribution Rates of Psychological Skills in Learning Some Basic Handball Skills in Secondary School Students**

Fadhiltailb Sabbar Aljanabi

2576-2582

 PDF

### **Evaluation of Psychological Skills and Effect of an Educational Curriculum with a Model to Accelerate Thinking in the Arbitrary Performance of Students with Handball**

ALaa Ihsan Ali alrikabi1 , Wissam Salah Abdel-Hassan1 , Abbas Abdulhamza Kadhim3

2583-2588

 PDF

### **The Effect of Therapeutic Exercises of Degree and Severity of Tear Tendon Muscle Tendon Rupture and Some Physical and Functional Variables for Athletes**

Ahmed Atshan Abdulradha

2589-2595

 PDF

### **Movement Exercises and their Effect on the Values of Some Physical Abilities for Youth and the Accuracy of Overwhelming Hitting From Position (1) on the Volleyball**

Amjad Abed Kadhim

2596-2602



### **The Effect of Using Some Teaching Methods on Learning the Skill of the Snatch Students**

Ali Sadiq Shaker Al-Sahlawi<sup>1</sup> , Noha Youssef Hashim Al-Amiri<sup>2</sup> , Bashar Abbood Fadel<sup>1</sup>

2603-2608



### **The Reality of Drug Abuse Internationally Prohibited among Bodybuilders in the City of Nasiriyah**

Muhammad Shabram Alwan<sup>1</sup> , Kamal Abd Negm<sup>2</sup> , Haider Mutar Jassim<sup>2</sup>

2609-2613



### **The Effect of Functional Exercises in Some Biomotor Abilities and Metabolism Rate for Volleyball Young Players**

Mahmoud Nasser Radhi<sup>1</sup> , Sawsan Hadood Obaid<sup>2</sup>

2614-2619



### **The Effect of (Vertimax) Exercises on the Molecular Pressure of Oxygen, the Explosive Ability of the Two Legs, and the Accuracy of Scoring for Advanced Football Players**

Bashar Abbood Fadel<sup>1</sup> , A. Muhsin Muhammed Hassan<sup>1</sup> , Ali Sadiq Shaker<sup>1</sup> , Sura Irahim Luabi<sup>2</sup>

2620-2627



### **The Effect of Circular Training According to the Style of Stations (Physical - Skill) in Developing Some Physical Capabilities and Aiming by Jumping in Basketball for Youth**

Rashad Tariq Yousef<sup>1</sup> , Bahaa Dhyab Faisal<sup>2</sup> , Hikmat Abdel Sattar Alwan<sup>3</sup>

2628-2634



### **The Effect of Inverted Learning by the (Edmodo) Platform in Motivation Motor Performance of Some Skills of Artistic Gymnastics for Students**

Khaled Nima Khudair<sup>1</sup> , Ali Abdul Hassan<sup>2</sup> , Mohamed Abdel-Sadah Hassan<sup>3</sup>

2635-2641



## **The Effect of Exercises by Metabolic Conditioning (MetCon) Style in Some Physiological Variables and the Speed Motor Response for Young Volleyball Players**

Mahmoud Nasser Radhi1 , Sawsan Hadood Obaid2

2642-2648



## **The Effect of Both (Ballistic and Plyometric) Methods of Exercises in Protecting from Knee Joint Injury in Terms of the Most Important Muscles Working on it for the Handball Center Player**

Eman Darweesh Jari 1 , Hasim Abdul-Jabar Salih 2 , Ali Hasson Jawad

2649-2655



## **Study the Effect. Extracts and Inhibitory Potency of Plant Capparis Spinosa on Breast Cancer Cells**

Amjed Abbawe Salih1 , Thamer Esmail Ahmed2 , Daa Mahmoud Nigam3

2656-2661



## **Knowledge and Attitude about Sexual Transmitted Disease among Women in Reproductive Age in Al-Nasiriyah Governorate-Iraq**

Fatima K. Naem1 , Suror H, Aneed2 , Amer A. Sachit2

2662-2667



## **Assessment of Patients' Perception of Mechanical Ventilation (Endotracheal Tube) and Intensive Care Unit Post Cardiac Surgery**

Khalida Alwan Mansour 1 , Bassima Amir Naji 2 , Bushra Khairullah Khanjar3

2668-2675



## **Impact of an Educational program on Teachers' Knowledge Toward Chickenpox Prevention at Primary Schools in Al-Najaf Governorate**

Raid Saad Atshan1 , Asmahan Qasim Mohammed2 , Ali Hussien Abbas3

2676-2683



## **Philosophical Foundations of Physical Education and Sport Applied to University Students of Central Area in Iraq According to the Social Understanding**

Haider Abbas Abdul Amir1 , Murtaza Ali Shaalan2

2684-2689

 PDF

### **Activity of Several Purine Metabolic Enzymes in Sera of Pregnant Women**

Noor Khalid Zaidan 1 , Wisam Mahmood Mohammed 1

2690-2695

 PDF

### **Legal knowledge and its relationship to selective attention using the Rehcom system for Al-Rafidain basketball athletes**

Hayder Naji Habash Al-Shawi1 , Shaimaa Farhan Mohammed Al-Jubouri2

2696-2701

 PDF

### **Psychological Safety and its Relationship to the Trends of Achievement Goals for Students of the College of Physical Education and Sports Sciences in Light of the Covid-19 Virus**

Nizar Hussein Al-Nafakh1 , Nadia Jamal Abdul Amir Al-Athari 2, Muhammad Falah Jihad Alathari 3

2702-2707

 PDF

### **Antibacterial activity of Zinc Oxide Nanoparticle (ZnONP) Biosynthesis by Lactobacillus plantarium aganist pathogenic Bacteria**

Zaman S. Hamza

2708-2715

 PDF

### **Fibroblast Growth Factor 21 Association with Renal Function Decline in Type II Diabetes Mellitus**

Zainab A. Hussein 1 , Sura A.Abdulsattar 2 , Essam N.Salman 3

2716-2722

 PDF

### **Morphological and Histological Study on Thyroid Gland Toxicity in Male Rats Exposed to Mercury**

Sawsan A. Ali

2723-2728

 PDF

### **The Effect of Skilful Visual Exercises in the Development of Motor Perception and Preparation Skill in Volleyball**

Hamida Obaid Abdul Amir1 , Baida Abd Ali Kahdim Sebti 2

2729-2734

 PDF

### **The Effect of a Proposed Training Curriculum Using an Electronic Learning Platform According to the Principle of Social Distancing to Improve Some Physical Fitness Elements for Female Futsal Players**

Mohammed Naji Shaker Abugneam<sup>1</sup> , Rajaa Abdel Abbas Mahous<sup>2</sup>

2735-2739

 PDF

### **Relationship between Serum Vitamin D Status and Gestational Diabetes Mellitus**

Bushra Jabbar Hamarashid<sup>1</sup> , Atiya Kareem Mohammed<sup>2</sup>

2740-2748

 PDF

### **Molecular Interactions of Poly (methylmethacrylate), Poly (vinyl alcohol), Poly(ethylene glycol), Chitosan, Cellulose and Starch Polymers with Iodine**

Jaleel Kareem Ahmed<sup>1</sup> , Auda Jabbar Braihi<sup>2</sup> , Duaa Abdul Rida<sup>3</sup>

2749-2757

 PDF

### **Indications and Complications of Tracheostomy in Pediatric Age group in Hilla, Babylon province, Iraq: Cross sectional Study**

Safaa Hussain Alturaihy

2758-2763

 PDF

### **Road Traffic Injury deaths and Correlates in Babylon Province: A Cross- Sectional Study**

Sahar Adham Ali<sup>1</sup> , Hasan Alwan Baiee<sup>2</sup>

2764-2770

 PDF

### **Study the Effect of Adding Iron Salts on the Physiochemical and Sensory Properties of Yoghurt**

Ali Arabi Jasim<sup>1</sup> , Jasim M. S. Al-Saadi<sup>2</sup>

2771-2778

 PDF

### **Prevalence and Correlates of Vitamin D Inadequacy in a Sample of Iraqi People**

Ismael Hasan Jawad<sup>1</sup> , Hasan ALwan Baiee<sup>2</sup> , Issam Salim Ismail<sup>3</sup>

2779-2788

 PDF

### **Study of Type of Bacteria That Found in Ear, Mouth, Nose of Second Stage Nursing Department Student**

Hala Abbas Jasim

2789-2793

 PDF

### **A Comparative Study of Nerve Growth Factor Level in the Follicular Fluid of Polycystic and Non-Polycystic Ovary Syndrome Women Undergoing ICSI: a Cross-sectional Study**

Noor Kareem Alfatlawy 1 , Hanan Abdul Jabbar AL -Tae 2 , Ali Ibrahim Rahim 3

2794-2799

 PDF

### **Anti-Urease Activity of Essential oil and Phenolic compounds of Thymus vulgaris, Melaleuca alternifolia and Betula pendula against Proteus mirabilis isolates**

Mohammed R. Mohammed1 , Ayyad W. Al - Shahwany1

2800-2806

 PDF

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2807-2813

### **Deleted**

2814-2817

### **Application of Infection Control Rules by Iraqi Orthodontics**

Rehab adil Al-Rawi1 , Noor Nourie Abbass Abdulla2 , Fudhla Sadoon Al- Zubaydi3 , Bassam Alsheekhly4

2818-2825

 PDF

### **Deleted**

Mohasmmad Abdul Mohsin Jebur1 , Nabeel Ghazi Hashim2 , R. Hamoudi Al-Obeidi Salim3

2826-2832

 PDF

### **Deleted**

Anwar A. Abdulla

2833-2840

 PDF

### **Sensitive Simultaneous Estimation of Atorvastatin Ca in Pure and Dosage Forms Via Developed CFIA using 1,2 Naphthoquinone-4-Sulfonate as a Suitable Organic Agent**

Bushra B. Qassim<sup>1</sup> , Asmaa A. Zydan<sup>2</sup>

2841-2848

 PDF

### **Evaluating the Effect of Addition of Titanium Dioxide Nanoparticle on Some Physical Properties of Flowable Composite Resin**

Sazgar Srbast Qasim<sup>1</sup> , Diyar Khalid Bakr<sup>2</sup> , Dara H. Rasheed<sup>3</sup>

2855-2861

 PDF

### **Antimicrobial Activities of Green Biosynthesized Iron Oxide Nanoparticles Using F. Carica Fruit Extract**

Nada Khudair Abbas<sup>1</sup> , Azhar A. F. Al- Attraqchi<sup>2</sup> , Jenan Hussien Taha<sup>3</sup>

2862-2868

 PDF

### **Molecular Detection of Antibiotics Resistance Genes in Burkholderia cepacia isolated from Diabetic foot infection**

Maryam Riyadh Al-Muhanna<sup>1</sup> , Mahdi Al-Ammar<sup>2</sup>

2869-2873

 PDF

### **Evaluation the Antimicrobial Effect of Glycerin Magnesia on Some Bacteria, In-Vitro Study**

Maher Saber Owain<sup>1</sup> , Khalid Ismael Oleiwi<sup>2</sup> , Ali Ghazi Atiyah<sup>1</sup> , Mustafa Salah Hasan<sup>2</sup>

2874-2877

 PDF

### **Prevalence of Histopathological Diagnosis of Benign and Malignant Breast Lesions in Al-Muthanna Province for Two Years Duration**

Sarah Ali Abed<sup>1</sup> ; Ali Adnan Jabbar<sup>2</sup> ; Roaa Salih Mahdi<sup>3</sup> ; Ali Mohammed Khaleel<sup>4</sup> ; Suha Suhel Hussein<sup>5</sup> ; Maha Khadum Ghayadh<sup>6</sup>

2878-2884

 PDF

### **A Histological and Histochemical Study on Olfactory Bulbs to Detection Amyloid Protein Depositions by Congo-Red and Routine Staining Techniques**

Rajaa Ali Moheiseen Al- Taei1 , Muna Hussain Al-Aameli2 , Yarub Modhar Al-Qazwini3  
2885-2890



## **The Intraperitoneal-Ketoprofen-Histopathological Induced Alterations in the Wistar Rat Kidneys**

Samer Riyadh Fadhil , Hakim Bahlok Jebur2  
2891-2895



## **A New Method for Dental Cast Analysis**

Jassim Ali Al-E'nizy1 , Zainab Bahjat Mohammed Amin Al-Ani2 , Mohammed Nahidh3  
2896-2900



## **Analysis of Demographic, Socio-Economic and Biological Factors With the Incidence of Leprosy in Bombana Regency Southeast Sulawesi Province**

Fauziah Zahra Fahrunnisa1 , Nur Nasry Noor2 , Muhammad Syafar3 , Andi Zulkifli2 , Ansariadi2 , Syamsuar4  
2901-2907



## **Deleted**

2908-2916

## **Evaluating the Effects of Education on the Understanding and Acceptance of Evidence-based Performance by Nurses**

Hamid Taghinejad1 , Mosayeb Mozafari2 , Hossein Seidkhani3 , Mosstafa Hossini4  
2917-2922



## **Low Carbohydrate High Protein Diet Reduces Levels of Tumour Necrosis Factor-? (TNF-?) in Rats (Rattus norvegicus)**

Hany Aryani1,2, Budi Santoso3 , Bambang Purwanto4  
2923-2928



## **Low Carbohydrate – High Protein Diet Does Not Provide Positive Effect on P38 Mitogen-Activated Protein Kinase (p38MAPK) Expression**

Hany Aryani1 , Budi Santoso2 , Bambang Purwanto3  
2929-2933



 PDF

### **The Influence of Healthy and Productive Female Workers Movement (Gp2sp) on Work Health Behavior on Female Workers**

Heriati<sup>1</sup> , Atjo Wahyu<sup>2</sup> , Masni<sup>3</sup> , Masytha Muis<sup>2</sup> , Furqaan Naiem<sup>2</sup> , Saifuddin Sirajuddin<sup>4</sup>  
2934-2940

 PDF

### **How Possible is it to Recover Semen DNA from Laundered Cotton and Polyester Fabrics?**

Hoda Ahmed Basyoni<sup>1</sup> , Karima Mukhtar Mohamed<sup>1</sup> , Usama Mohamed El-Barrany<sup>1</sup> , Laila Ahmed Rashed<sup>2</sup> , Heba Mohamed Aboubakr<sup>1</sup> , Heba Abdo Abdel Razik<sup>1</sup>  
2941-2948

 PDF

### **Analysis of Manual Sharpening Method Towards the Cutting Edge Index of Sickle Scaler Instrument**

I.Komara<sup>1</sup> , A.Susanto<sup>1</sup> , I.M.Setiapribadi<sup>1</sup> , C.A.Bawono<sup>1</sup>  
2949-2954

 PDF

### **Determination of Complications and Contraception Failure in East Java Province, Indonesia**

Ida Farida<sup>1</sup> , Lutfi Agus Salim<sup>2</sup>  
2955-2960

 PDF

### **Validation of a Simple Extraction Procedure for Stimulants Identification from Human Plasma**

Idha Arfianti Wiraagni<sup>1-4</sup> , Mustafa Ali Mohd<sup>2</sup> , Rusdi Abdul Rashid<sup>3</sup> , Didi Erwandi bin Mohamad Haron<sup>2</sup>  
2961-2968

 PDF

### **Prevalence and Injury Risk Factors based on Sleman Health and Demographic Surveillance System (HDSS) Data**

Idha Arfianti Wiraagni<sup>1</sup> , Beta Ahlam Gizela<sup>1</sup> , Majid<sup>2</sup>  
2969-2974

 PDF

### **Emerging Premarital Sexual Behavior among Adolescent in Indonesia: The impact of Knowledge, Experience, and Media Use to Attitudes**

Ira Nurmalia<sup>1,2</sup> , Citra Ervina Ahiyanasari<sup>2</sup> , Muthmainnah<sup>2</sup> , Anggun Wulandari<sup>2</sup> , Yuli Puspita Devi<sup>2</sup> , Rashmi Pathak<sup>3</sup> , Yashwant V Pathak<sup>4</sup>

2975-2981

 PDF

### **Decreasing and Determining Child Mortality in Indonesia**

IskandarAziz1 , Lutfi Agus Salim2

2982-2987

 PDF

### **Effect of Parkia speciosa Hassk Peels Extract on Total Cholesterol Levels of Hypercholesterolemia Rats**

Joni Tandi1 , Tien Wahyu Handayani1 , Maria Tandebia1 , Jessica Anastasia Wijaya1

2988-2992

 PDF

### **Histopathological Evaluation of Non-Obstructive Azoospermic Males Using Testicular aspirate (TESA) biopsy**

Karbel Hadeel A.1 , Al -Bdairi Adnan A.2 , Khairullah Ahmed R.3 , Al-Humairi Ameer K.4

2993-3000

 PDF

### **Affirmation-Tapping to Reduce Pain Perception and Glutamate Serum Levels of Post-cesarean Patients**

Joko Suwito1 , Nursalam Nursalam2 , Agus Sulistyono3 , I Ketut Suidiana3 , Urged G. A. Suprabawati4 , Ahsan Ahsan5 , Ah. Joseph2

3001-3006

 PDF

### **Prevalence of Cryptosporidium Infection in Calves with Effect on Biochemical Parameters**

Osamah Nasir Wali1 , Ayoub Ibrahim Ali2 , Khalid Ismael Olewi3 , Mustafa Salah Hasan3 , Mohammed Hamid Kareem3 , Marwa Raad Jead4

3007-3010

 PDF

### **Evaluation of Lifestyle among Patients with Irritable Bowel Syndrome in Kirkuk City**

Rebaz Ismeal Ali Wali2 , Assist. Marghoob Hussein Yaas2

3011-3017

 PDF

### **Factors Contributed of Breast Cancer for Women in Iraq**

Marghoob Hussein Yaas1 Rebaz Ismael Ali Al – Brzanji2 , Mohammed Abdual Magead Mohammed3

3018-3024

 PDF

### **Assessment of Family Knowledge toward their Children with Autism Spectrum Disorder at Al-Hilla City, Iraq**

Abdulmahdi A. Hasan

3025-3031

 PDF

### **The Effect of Warm Red Ginger Compress Therapy on the Decrease in Rheumatoid Arthritis Pain in the Elderly at the Social Institution Tresna Werdha Pagar Dewa Bengkulu**

Andri Kusuma Wijaya<sup>1</sup> , Ferasinta<sup>1</sup> , Yandrizal<sup>2</sup>

3040-3045

 PDF

### **Legal Philosophy about the Performance of the Indonesian National Police**

Erlyn Indarti

3046-3052

 PDF

### **Efforts to Prevent Criminal Acts of Money Laundering Using Penal Policy Measures**

Purwoto Purwoto

3053-3057

 PDF

### **Fiduciary Security Execution after Constitutional Court Decision**

R. Suharto<sup>1</sup> , Siti Malikhatun Badriyah<sup>1</sup> , Marjo<sup>1</sup> , Muhammad Shafiyuddin Wafi<sup>1</sup> , Muhammad Haidar Fakhri Allam<sup>1</sup>

3058-3062

 PDF

### **The Anti-Proliferative Activity of Ephedra Aphylla Methanol Extract Against Liver Cancer Cell Line**

Hindreen Saleh Mohammed<sup>1</sup> , Hayder B Sahib<sup>2</sup>

3063-3068

 PDF

### **Histomorphological Studies of the Organs of Malaria Mice Model After Administration Combination of Leaf and Stem Bark Extract of Syzygium cumini with Chloroquine**

Lilik Maslachah1 , Lucia Tri Suwanti2 , Hardany Primarizky3 ,Thomas V Widiyatno4 , Dyah Fitria Ratna Kusuma5 , Ridhofajuri Winanda5 , Ryandika Wahyu Prasetyo5

3069-3075

 PDF

### **Deleted**

3076-3081

### **Ischemic Stroke Caused by Metabolite Factors in Indonesia (GBD Study 2017)**

Lukman Prayitno1 , Mugeni Sugiharto1 , Niniek Lely Pratiwi2

3082-3089

 PDF

### **Genotyping of Exon 2 BRCA1for Breast Cancer in Iraqi Women by Sanger Sequencing**

MahanaOied Hussein1 , Khitam Abdul-Wahhab Ali2 , Najwa S. Ahmed3

3090-3096

 PDF

### **Importance of FoxO3 Level in Progression of Nodal Osteoarthritis Disease**

Mahmood Nazar Mustafa/ Abdullah A.Mohammed

3097-3100

 PDF

### **The Role of Anti-interleukin-4 in BALB/c Mice Infected with Visceral Leishmaniasis**

Manahel H. Mjefy1 , Altamemy A. K. Aakool2

3101-3104

 PDF

### **Predictors of Malaria Incidence in Rural Eastern Indonesia**

Mara Ipa1 , Agung Dwi Laksono2 , Endang Puji Astuti1 , Heni Prasetyowati1 , Lukman Hakim1

3105-3111

 PDF

### **Relationship between Benzene Concentrations with Erythrocyte, Hemoglobin, and Health Complaints of Workers in Surabaya Printing Industry**

Maria Ayu Dwi Bestari1 , Abdul Rohim Tualeka2 , Putri Ayuni Alayyannur3 , Shintia Yunita Arini4 , Syamsiar S Russeng5 , Pudji Rahmawati6 , Indri H Susilowati7

3112-3119

 PDF

### **mRNA Expression of PITX3 gene in Congenital Cataract Caused by Rubella Virus Infection**

Marlyanti Nur Rahmah Akib<sup>1,2</sup> , Budu<sup>3</sup> , Habibah S Muhiddin<sup>4</sup>, Andi Muhammad Ichsan<sup>4</sup>, Ratih Natasha Maharani<sup>2</sup>

3120-3124



### **Determination of Sex Using Humeral Dimensions and Radiographic Measurements of the Humerus in Egyptian Population, Fayoum Governorate**

Marwa Ali Mwaheb<sup>1</sup> , Eman Ali Ali Abd El-Meguid<sup>2</sup> , Mohammed Ahmed Moussa<sup>3</sup> , Enas Mohammed Morsi<sup>1</sup>

3125-3131



### **Study the Effect of Chronic Toxoplasmosis on Serum Testosterone Level in Men**

Masar J.J. Al-Kurdy<sup>1</sup> , Noaman N. A'aiz<sup>2</sup>, Thanaa I. Jawad<sup>3</sup>

3132-3137



### **Curcumin vs Chloroquine in Coronavirus Global Pandemic: Trend analysis Study in Google**

Maslichah Mafruchati

3138-3143



### **Influence of the Work Units' Psychological Ownership and Intention in Establishing the Patient Safety Program in Hospitals in Probolinggo, East Java**

Mirrah Samiyah<sup>1</sup> , Thinni Nurul Rochmah<sup>1</sup> , Widodo J. Pudjihardjo<sup>1</sup> , Usman Hadi<sup>2</sup>

3144-3149



### **The Effect of Estrogen, Progesterone, and Its Combination on The Expression of Brain-Derived Neurotrophic Factor Medula Spinalis in Regeneration Process of Peripheral Nerve**

Mochamad Rizki Yulianto<sup>1</sup> , Agus Turchan<sup>1</sup> , Hari Basuki Notobroto<sup>2</sup>

3150-3155



### **In Silico Study : Phyllanthus Niruri L as Immunomodulator Against Covid-19**

Mohammad Sukmanadi<sup>1</sup> , Emy Koestanti Sabdoningrum<sup>2</sup> aditya Tri Ananda<sup>3</sup>

3156-3161



## **Characterization of Aflatoxin B1 Biomarkers as Preference for Antitoxin Candidates for Biosensors for Halal Food Supply**

Mohammad Sukmanadi<sup>1</sup> , Retno Sri Wahjuni <sup>2</sup>

3162-3167



## **Testing the New Parameters affecting The Outcome of Extracorporeal Shockwave Lithotripsy for Upper Ureteric Stones**

Mohammed B. Ismail<sup>1</sup> , Hasanain F. Hasan Al-Timimi

3168-3174



## **Changes of Anterior Chamber Biometry and Relationship to Intraocular Pressure Changes after Phacoemulsification Emulsification Surgery in Non-Glaucomatous Eyes**

Mohammed Qasim Al Nuwaini<sup>1</sup> ; Giyathaldeen T. Neameh<sup>1</sup> ; Farook M. Albusultan<sup>1</sup> , Alyaa Abood Kareem<sup>2</sup> , MD Mustafa A. Al Zubaidi <sup>3</sup> , MD Zainab Basim<sup>4</sup>

3175-3181



## **Bacterial Contamination between Computers in Computer Labs**

Moroj Ali Fahad<sup>1</sup> , Muna Hameed Ahmed<sup>2</sup> , Shahad Nazar Mustafa<sup>1</sup>

3182-3187



## **Allele Frequencies of 13 Chromosome X STR in Arab Iraqi Population**

Mostafa M. Abdrazik <sup>1,2</sup>, Marrib N. Rasheed <sup>1</sup> , Mohammed Mashni Farhan<sup>2</sup>

3188-3193



## **The Biophysical Efficacy of Smart Phones on the Eyes of Children from 3-12 Years**

Munther Sameen Shuker<sup>1</sup>, Zina Tariq Ali<sup>2</sup>

3194-3200



## **The Effect of 2-Thioxo Imidazolidin-4-Ones (2- Thiohydantion) on Anticancer Activity: An in Vitro Study**

Mutashar Jiheel<sup>1</sup> , Aseel Al -Rekaabi<sup>2</sup> , Nasser Alqurashy<sup>1</sup> , Mahdi Al - Deresawi<sup>1</sup>

3201-3206



## **Determination of Probiotic Potential and Anticancer Activity of Lactobacillus Isolated from Cheese and Yogurt**

Muthanna Hamid Hassan

3207-3212



## **The Protective Effect of Annona Extracts on Renal Histopathology Induced by Ethylene Glycol in Male Rabbits**

Hanaa. Kh. Ismail<sup>1</sup>, Ahmed -Alsaidya<sup>2</sup>, Thaira I. Younis<sup>3</sup>, Shihab A. Al-Bajari<sup>4</sup>

3213-3218



## **The Prevalence of Illicit Drugs and Alcohol in Road Traffic Accident Fatalities in the Eastern Region of Saudi Arabia**

Naglaa F. Mahmoud<sup>1, 2</sup>; Maha K. Al-Mazroua<sup>2</sup>; Mostafa M. Afify<sup>2, 3</sup>

3219-3225



## **Identify and Prioritize Criteria for Efficient Allocation of Donation Resources**

Narges Asadi Janati<sup>1</sup>, Khalil Alimohammadzade<sup>2, 3</sup>, Seyd Mojtaba Hosseini<sup>4</sup>, Ali Maher<sup>5</sup>, Mohammadkarim Bahadori<sup>6</sup>

3226-3232



## **The Utilization of Sugarcane Bagasse to Remove Cr (VI) Heavy Metal Waste with Adsorption**

Narimo<sup>1</sup>, Sajidan<sup>2</sup>, Maskyuri<sup>3</sup>, Maria Theresia Sri Budiastuti<sup>4</sup>

3233-3238



## **Biological Hazard on Multidrug Resistance (MDR) of Escherichia Coli Collected From Cloacal Swab of Broiler Chicken on Wet Markets Surabaya**

Nenny Harijani<sup>1</sup>, Surya Jovinson Tansil Oetama<sup>2</sup>, Koesnoto Soepranianondo<sup>3</sup>, Mustofa Helmi Effendi<sup>1</sup>, Wiwiek Tyasningsih<sup>4</sup>

3239-3244



## **Analysis of Human Decomposition Effect on DNA Quality with Short Tandem Repeats [STRS] Combined Index DNA System [CODIS]**

Nily Sulistyorini, Ahmad Yudianto<sup>1</sup>, Ma'rifatul Ula<sup>2</sup>, Renny Sumino<sup>3</sup>, Fery Setiawan<sup>4</sup>

3245-3251

 PDF

### **Association between The Study of Nerve Conductivity and The Medianus Nerve Cross-Sectional Area in Carpal Tunnel Syndrome**

Ninik Dwiastuti<sup>1</sup> , Meisy Andriana<sup>1</sup> , Mei Wulan<sup>1</sup>  
3252-3259

 PDF

### **Relationships of Service Response with Service Quality in Emergency Department of Rsud Dr. Loekmono Hadi, Kudus, Indonesia**

Nur Hidayat<sup>1</sup> , Ahsan<sup>1</sup> , Masruroh Rahayu<sup>1</sup> , Abdul Rohim Tualeka<sup>2</sup>  
3260-3264

 PDF

### **Study of Adult Injury Fatality in Nigeria**

Obiora Jude Uchendu<sup>1</sup> , Nkadi Francis Nwachokor<sup>2</sup> , Eseroghene Arthur Ijomone<sup>3</sup>  
3265-3271

 PDF

### **Determination and Correlation of Electrolytes and Trace Elements from Vitreous Humour and Synovial Fluid by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for Estimation of Post-Mortem Interval (PMI): Experimental Study in an Animal Model**

Panjai Woharndee<sup>1</sup> , Jutapit Prungkramphun<sup>2</sup> , Sarawut Kumphune<sup>3</sup>  
3272-3280

 PDF

### **Comparison of Surgically Induced Corneal Astigmatism in Cataract Patients after Phacoemulsification Versus Manual Small-Incision Cataract Surgery**

Giyathaldeen T. Neameh<sup>1</sup> , Mohammed Qasim Al Nuwaini<sup>1</sup> , Hayder M. M. Al-Talqani<sup>2</sup> , Mohammed Qasim Al Nuwaini<sup>3</sup>  
3281-3287

 PDF

### **The Improvements in the Prolactin, Thyroid Hormones and Lipid Profiles that are Observed During Three Pregnancy Periods**

Khalid .S. Sahab<sup>1</sup> , Ali. S. Alsaadi<sup>2</sup> , Ammar.M.Kadhim<sup>3</sup>  
3288-3293

 PDF



## **Correlation between Seasons and the Prevalence of Preeclampsia at Tertiary Hospital, Indonesia**

Monika Lijuwardi<sup>1</sup>, Budi Prasetyo<sup>1</sup>, Muhammad Ardian Cahya Laksana<sup>1</sup>, Rizki Pranadyan<sup>1</sup>, Hanifa Erlin Dharmayanti<sup>1</sup>, Pandu Hanindito Habibie<sup>1</sup>, Erni Rosita Dewi<sup>2</sup>

3294-3299



PDF

## **Construction of a New Scale for Biological Estimation of Public Speaking Stress**

Juliet Maarij Al-Assadi<sup>1</sup>, Ali A. Al-fahham<sup>2</sup>, Murtadha kanim Adea Aljebory<sup>3</sup>

3300-3305



PDF

## **Larvicidal Activity of Aedes Aegypti from a Simple Preparation of Cashew (Anacardium occidentale L.) Nut Shell Extract for Community Level Use**

Kowit Suwannahong<sup>1</sup>, Kanittada Thongkao<sup>2</sup>, Pimporn Thongmuang<sup>3</sup>, Torpong Kreetachat<sup>4</sup>, Yuttana Sudjaroen<sup>5\*</sup>

3306-3311

## **Analysis of the Mean Gray Scale and the Mandibular Thickness (Micro-CT) To Mandibular bone Density in Synthetic SCAFFOLD-hADMSC**

Latief Mooduto<sup>1</sup>, Arief Rahman<sup>2</sup>, Fedik A Rantam<sup>3</sup>, Jenny Sunariani<sup>4</sup>, Hendy Jaya Kurniawan<sup>5</sup>

3312-3318



PDF

## **The Effect of Trivalent Influenza Vaccines on Immunoglobulin G and Interleukin 4 in Ferret (Mustela putorius furo)**

Lestari Dewi<sup>1,2</sup>, Erman Tritama<sup>3</sup>, Reviany Vibrianita Nidom<sup>4,5</sup>, Kuncoro Puguh Santoso<sup>6</sup>, Sri Agus Sudjarwo<sup>6</sup>, Harianto Notopuro<sup>7</sup>, Chairul Anwar Nidom<sup>4,6</sup>,

3319-3325



PDF

## **The Possible Nephroprotective Effect of Roflumilast Ameliorating Glycerol -Induced Myoglobinuric Acute Renal Injury in Rabbits Model**

Mukhallad A. Ramadhan<sup>1</sup>; Haider F. Al-Saedi<sup>2</sup>; Ghufran L. Ismaeel<sup>2</sup>; Uday Abdul-Reda Hussein<sup>3</sup>

3326-3332



PDF

## **Diagnosis of Empathic Abilities of Medical Students**

Philipp D. Rasskazov<sup>1</sup>, Marina A. Stavruk<sup>2</sup>, Lubov A. Kushnyr<sup>3</sup>, Anna V. Pichueva<sup>3</sup>, Tatiana S. Tsarskaya<sup>3</sup>

3333-3336



PDF

## **Structures Development of Master of Public Health Program and Doctor of Philosophy Program in Public Health Innovation Research and Development in Thailand**

Phongpisanu Boonda<sup>1</sup> , Sutthisak Surirak<sup>2</sup> , Parinya Jitaram<sup>2</sup>  
3337-3346



## **Hyperbaric Oxygen Exposure Reduces ICAM-1 And HIF1? Expression in Brain Endothelial Cells from Experimental Cerebral Malaria Mice**

Prawesty Diah Utami<sup>1</sup> , Usman Hadi<sup>2</sup> , Yoes Prijatna Dachlan<sup>3</sup> , Guritno Suryokusumo<sup>4</sup> , Loeki Enggar Fitri<sup>5</sup>  
3347-3352



## **Correlation of Safe Benzene Duration (Hours/Day) and Blood Profile (Leukocytes, Hematocrit, Hemoglobin) in the Osowilangun Shoe Home Industry**

Rafiqah Rana Febrianti<sup>1</sup> , Abdul Rohim Tualeka<sup>2</sup> , Juliana Jalaludin<sup>3</sup> , Syamsiar S Russeng<sup>4</sup> , Pudji Rahmawati<sup>5</sup> , Ahsan Ahsan<sup>6</sup> , Indri H Susilowati<sup>7</sup>  
3353-3359



## **A Five Years Retrospective Study of Incidental Findings in Medico-Legal Autopsies**

Raihan Uddin Ahmed<sup>1</sup> , Nitu Kumar Gogoi<sup>2</sup> , Nabajit Barman<sup>3</sup> , Diganta Thakuria<sup>4</sup> , Pradeep Kumar Thakuria<sup>5</sup>  
3360-3364



## **Study the Incidence, Types of Anemia and Associated Risk Factors in Pregnant Women**

Rasha Saadi Abbas  
3365-3372



## **Are Problems During Pregnancy a Predictor of Childbirth in The Hospital?: Determinants Analysis of Hospital Childbirth in Urban Poor Communities in Indonesia**

Ratna Dwi Wulandari<sup>1</sup> , Agung Dwi Laksono<sup>2</sup>  
3373-3378



## **Blood Cells Depletion after Chemotherapy in Iraqi Women with Breast Cancer**

Reem M. Obaid <sup>1</sup>, Farah Tareq Yaseen<sup>2</sup> , Mahmood Yaseen Mukhlif <sup>3</sup>  
3379-3382



## **The Organizational Learning Implementation to Improve Accreditation Performance at the Gayungan Health Center, Surabaya, Indonesia**

Reinaldis Sara<sup>1</sup>, Ratna Dwi Wulandari<sup>2</sup>, Agung Dwi Laksono<sup>3</sup>  
3383-3388

 PDF

## **Relationship between Toluene Concentration, Malondialdehyde (MDA) Level and Health Complaints in Workers of Surabaya Printing Industry**

Pradita Sekar Ayu<sup>1</sup>, Abdul Rohim Tualeka<sup>2</sup>, Shintia Yunita Arini<sup>3</sup>, Syamsiar S Russeng<sup>4</sup>, Pudji Rahmawati<sup>5</sup>, Ahsan Ahsan<sup>6</sup>, Indri H Susilowati<sup>7</sup>  
3389-3395

 PDF

## **The Relationship among Technology Utilization, Obedience to Law and Smoking Culture In Community with Smoking Behavior in Male Adolescents in Semarang Indonesia**

Retno Indarwati<sup>1</sup>, Kristiawati Woespinto<sup>2</sup>, Inas Husnun Hanifah<sup>2</sup>  
3396-3402

 PDF

## **Detection of Multidrug-Resistant (MDR) Escherichia coli Isolated from Raw Milk in East Java Province, Indonesia**

Ribby Ansharieta<sup>1</sup>, Mustofa Helmi Effendi<sup>2,3</sup>, Hani Plumeriastuti<sup>4</sup>  
3403-3407

 PDF

## **First Report of Protein and Fat Level of Alabio Duck (Anas platyrhynchos Borneo) Eggs in Hulu Sungai Utara, Indonesia for Improving Human Health**

Rini Fajarwati<sup>1</sup>, Arif Nur Muhammad Ansori<sup>2</sup>, Sri Pantja Madyawati<sup>3</sup>  
3408-3411

 PDF

## **Anxiety Level Assessment to Primigravidae Women (28-40 week) with Hamilton Anxiety Rating Scale (HARS) Method**

Ruqaiyah<sup>1,2</sup>, Nusratuddin Abdullah<sup>3</sup>, Mochammad Hatta<sup>4</sup>, Nasrudin A Mappeware<sup>5</sup>, Ayatullah Harun<sup>2</sup>, Fatmawati Amir<sup>2</sup>, Alfina Baharuddin<sup>6</sup>  
3412-3417

 PDF

## **Validation of Horizontal Genes Transfer Using Selected Clinical Strain of P. Mirabilis for Swarming Activity**

Rusul Idan Mohsin Ali<sup>1,3</sup>, Muslim Idan Mohsin<sup>2</sup>, Abbas Al-Muhna<sup>3</sup>

3418-3423

 PDF

### **Awareness of Patient Rights and Practice at Fayoum University Hospital**

Safaa Khamis Hassan<sup>1</sup> , Marwa Ali Mwaheb<sup>2</sup>

3424-3431

 PDF

### **Carbon Dioxide Laser in the Treatment of Oral and Craniofacial Soft Tissue Lesions, Pros and Cons**

Saha Abdulkader Ismaeel<sup>1</sup> , Saif Saadedeen Abdulrazaq<sup>2</sup> , Alaaaldeen Abass Alani<sup>3</sup>

3432-3438

 PDF

### **Profile of Multidrug Resistance (MDR) and Methicillin Resistant Staphylococcus aureus (MRSA) on Dairy Farms in East Java Province, Indonesia**

Sancaka Chasyer Ramandinianto<sup>1</sup> , Aswin Rafif Khairullah<sup>2</sup> , Mustofa Helmi Effendi<sup>3, 4</sup> , Eka Pramytha Hestiana

3439-3445

 PDF

### **Questionnaire Study on Knowledge, Attitude and Practice on Factors Associated with Initiation and Cessation of Tobacco Among Patients Using Tobacco Products**

J. Manju<sup>1</sup> , C. Krithika<sup>2</sup> , Mutum Sangeeta Devi<sup>3</sup> , P. Saranya<sup>4</sup> , R. Saravanan BDS<sup>4</sup> , N. Sanjana<sup>4</sup>

3446-3451

 PDF

### **The Perspective of Puritan Moslem on the Family Planning Program: The Case of Salafimovement in Semarang, Central Java, Indonesia**

Saratri Wilonoyudho<sup>1</sup> , Lutfi Agus Salim<sup>2</sup> , Ayis Muhtaram<sup>3</sup>

3452-3456

 PDF

### **Forensic Application of ATP6 Gene Haplogroups in a Cohort of Obese Saudis**

Sayed A. M. Amer<sup>1,2</sup> , Abdulaziz S. Asiri<sup>3</sup> , Ziad F. Al-Shalaan<sup>4</sup> , Ahmed Ch. Kassab<sup>1</sup> , Ahmed M. Refaat<sup>5</sup>

3457-3462

 PDF

### **Bidan Lewu as Local Determinant Factors in Pregnancy and Childbirth Care (Study in the Dayak Ngaju Tribe in Kotawaringin Timur District)**

Sigit Nurfianto<sup>1,2</sup>, Usman Hadi<sup>3</sup>, Windhu Purnomo<sup>4</sup>  
3463-3467



### **The Effects of Prenatal Yoga for Primagravida with Gen Expression mRNA FKBP5 (FK506-binding Protein 51)**

Ruqaiyah<sup>1,2</sup>, Nusratuddin Abdullah<sup>3</sup>, Mochammad Hatta<sup>4</sup>, Nasrudin A Mappeware<sup>5</sup>, Ayatullah Harun<sup>2</sup>, Fatmawati Amir<sup>2</sup>, Alfina Baharuddin<sup>6</sup>  
3468-3473



### **Ecological Analysis of Preeclampsia/Eclampsia Case in Sidoarjo Regency, Indonesia, 2015-2019**

Sesotyaningsih Madiyaning Utami<sup>1</sup>, Farida Handayani<sup>1</sup>, Mam  
3474-3479



### **Deleted**

3480-3485

### **The Relationships of Temperature and Humidity in AirConditioned Room to the Occurrences of Sick Building Syndrome**

Siti Hanifah<sup>1</sup>, Zikri Fathur Rahman<sup>2</sup>, Abdul Rohim Tualeka<sup>3</sup>  
3486-3490



### **Sambiloto (*Andrographis paniculata*) Extract Improves the Performance of Animal Model Infected with *Escherichia coli***

Sri Hidanah<sup>1</sup>, Emy Koestanti Sabdoningrum<sup>1</sup>, Mohammad Anam Al Arif<sup>1</sup>, Arif Nur Muhammad Ansori<sup>2</sup>, Tanika Putri Hasanah<sup>3</sup>, Laurensia Viola Artha Widaya<sup>3</sup>  
3491-3496



### **The Effect of Roasting Temperature on Ferulic Acid Levels of Robusta Coffee Bean with Thin Layer Chromatography (TLC)-Densitometry**

Sugiyanta Sugiyanta<sup>1,2</sup>, Harianto Notopuro<sup>3</sup>, Jusak Nugraha<sup>4</sup>, Soetjipto Soetjipto<sup>3</sup>, Retno Handajani<sup>3</sup>  
3497-3503



### **Antibodies of Double Stranded Deoxyribonucleic Acid and Antinuclear in Patients with Rheumatoid Arthritis: Comparison Study of Seropositive and Seronegative**

Sura A.Abdulsattar<sup>1</sup> , Abdullah Ali Mohammed<sup>2</sup> , Mahmood Nazar Mustafa<sup>3</sup>  
3504-3510



### **Model Mentoring Teachers and Parents as an Efforts for Brushing Teeth Behavior in Preschool Children**

Tedi Purnama<sup>1</sup> , Ngatemi<sup>1</sup> , Indrayati Fadjeri<sup>1</sup> , Rini Widiyastuti<sup>1</sup>  
3511-3516



### **The Relationship between Housing Condition and Pulmonary Tuberculosis Disease in Baraya Village Bontoala Makassar, Indonesia**

Syamsuddin S<sup>1</sup> , Hamsir Ahmad<sup>2</sup> , Wahyuni Sahani<sup>3</sup> , Indah D. Lestari<sup>4</sup> , M. Askar<sup>5</sup>  
3517-3523



### **CYP2D6 (G1934A) Gene Polymorphism in Rice Farmers with Long-term Pesticide Exposure, Suphan Buri, Thailand**

Tanunchanok Pitaksilp<sup>1</sup> , Kampol Ruchiwit<sup>2</sup> , Kowit Suwannahong<sup>3</sup> , Wanida Pongstaporn<sup>4</sup> , Yuttana Sudjaroen<sup>5</sup>  
3524-3530



### **National Health Insurance Participants Satisfaction in General Polyclinic Services at First Level Healthcare Facilities**

Thinni Nurul Rochmah<sup>1</sup> , Djazuly Chalidyanto<sup>1</sup> , Ernawaty<sup>1</sup> , Rachmad Suhandi<sup>2,3</sup>  
3531-3537



### **Identification of DNA Corpse in Ordo Diptera Familia Calliphoridae Larvae in CSF1PO, TH01, and TPOX Locus Using Polymerase Chain Reaction (PCR) Methods**

Tutik Purwanti<sup>1</sup> , Ahmad Yudianto<sup>1</sup> , Mochammad Soekry Erfan Kusuma<sup>1</sup>  
3538-3543



### **Children Death with Child Abuse: A Case Report at Bhayangkara Hospital, Kediri Indonesia**

Mochammad Soekry Erfan Kusuma<sup>1</sup> , Tutik Purwanti<sup>2</sup>  
3544-3549



## **Effectiveness of WhatsApp Group on Breastfeeding Practices**

Wahidah Sukriani 1, Greiny Arisani 1

3550-3555



## **The Effect of the Blood Cupping Therapy on High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) in Hypercholesterol Patients**

Wahyudi Widada1 , Teddy Ontoseno2 , Bambang Purwanto3 , Suhartati4 , Imam Subadi5 , Hari Basuki Notobroto6 , Ahsan7

3556-3562



## **Impact of High Fidelity Simulation Training on the Quality of Cardiopulmonary Resuscitation Performance among Emergency Medical Services (EMS) Professionals in Pune, India**

Anand Hinduja1 , Parag Rishipathak2 , Shrimathy Vijayaraghavan3

3563-3566



## **Relationship of Smartphone Usage and Academic Performance among Undergraduate students – A Systematic Review**

Ancy Albert1 ; Nancy Netto1 ; Neelkant Pawar1 ; Fenilkumar Patel1 , Sharadha Ramesh2 ; Manisha Mistry3

3567-3570



## **A Study to Assess the Effectiveness of Mindful Meditation on Health and Psychological Adjustment Faced by Nursing Students in Selected Colleges of Pune City**

Anu Alexander1 , Ms. Feba Josemon1 , Shreeti Gahare1 , Neha Jadhav1 , Sonopant G Joshi2 , Ranjana Chavan3

3571-3575



## **Health and Lifestyle Practices of Under Graduate Students Studying in Selected Nursing College**

Anagha.M1 , Ritu Gaikwad1 , Ronald Masumba1 , Kraus1 , Asha Varghese1 , Deepak Sethi,2 Merlin Suji3

3576-3581



## **Osteoporosis Risk Analysis in Women Residing in Selected Urban Areas**

Milu Anna Laji 1 , Phale Diksha Ramesh1 , Gumi Riram1 , Sahil Chavan1 , Akash Khade1, Sharadha Ramesh,2 Merlin Suji3

3582-3585

 PDF

### **A Study to Assess the use of Body Mechanics Practices among Nursing Students in Selected Colleges of Nursing in Pune City**

Dipak Sethi<sup>1</sup> , Jasneet Kaur<sup>1</sup> , Jennifer William Anthony<sup>2</sup>  
3586-3589

 PDF

### **A Descriptive Study to Assess the Stress and Coping Mechanism Due to Lockdown among School Going Children**

Dipali Dumbre<sup>1</sup> , Sharadha Ramesh<sup>1</sup> , Ranjana Chavan<sup>1</sup> , Mangesh Jabade<sup>1</sup>  
3590-3597

 PDF

### **Knowledge and Practices on Intra Venous Cannulation among Student Nurses**

Sharadha Ramesh<sup>1</sup> , MerlinSuji<sup>2</sup>  
3598-3602

 PDF

### **Effect of lockdown on Students Studying in Various Colleges of Nursing**

Jennifer W. Anthony, Deepak Sethi<sup>2</sup> , Mangesh Jabade<sup>1</sup> , Poonam Yadav<sup>1</sup> , Stephina Fernandes<sup>1</sup>  
3603-3608

 PDF

### **A Study to Assess the Effectiveness of Pranic Healing Therapy on Backache among Adults At Pranic Healing Centre in Pune City**

Ms Kaguiphullu Sylvia Gangmei<sup>1</sup> , Sheela Upendra<sup>2</sup>  
3609-3613

 PDF

### **Relationship of Environmental Hygiene Practices with Perception of Health among People in Rural Community – A Systematic Review**

Kathri Abhijeet<sup>1</sup> , Kusekar Shweta Sunil<sup>1</sup> , Dalaram<sup>1</sup> , Dhilpe Prachi Daniyal<sup>1</sup> , 1 Hadnalkar Pradya Janati,  
Manisha Aba Borude<sup>1</sup> , Manisha Mistry<sup>2</sup>  
3614-3616

 PDF

### **Attitude & Practices of Hospital Staff Towards Safety In A Tertiary Care Hospital, Pune – A Compilation of Safety Experiments**

Kurapatti Ambika Shrinivas<sup>1</sup> , Neha Ahire<sup>2</sup>



3617-3621

 PDF

### **Students Perception on the Effectiveness of Online Teaching**

Leeja Bonny Thomas<sup>1</sup> , Merlin Suji<sup>2</sup> , Poonam Yadav<sup>1</sup>

3622-3630

 PDF

### **Perception of Counselors Regarding the Contribution to Patients Care by Student Nurses**

Leeja Bonny Thomas<sup>1</sup>, Diana Prasad<sup>2</sup>

3631-3638

 PDF

### **Effectiveness of Instructive Bookleton Knowledge About Organ Donation among GNM Nurses Working at Selected PHCs of Pune**

Mangesh Jabade<sup>1</sup> , Sheela Upendra<sup>2</sup>

3639-3642

 PDF

### **Knowledge Regarding IMNCI among Health Workers**

Mangesh Jabade<sup>1</sup> , Jasneet Kaur<sup>2</sup> ; Ranjana Chavan<sup>2</sup>

3643-3646

 PDF

### **A Study to Assess the Effectiveness of Structured Education on Prevention of Human Papillomavirus Infection among Adolescent Girls in a Selected School at Pune City**

Mangesh Vilinikaran Jabade<sup>1</sup> , Ranjana Chavan<sup>2</sup> , Dipali Dumbre<sup>1</sup> , Jennifer Anthony<sup>1</sup>

3647-3649

 PDF

### **To Evaluate the Knowledge and Practice Regarding Home Care Management among Patient on Hemodialysis with Information Education Communication (IEC) Module in Selected Hospitals of Pune City**

Elangbam Anibala Devi<sup>1</sup> , Manisha Mistry<sup>2</sup> , Mangesh Jabade<sup>3</sup>

3650-3655

 PDF

### **A Descriptive Study to Assess the Preferred Leadership Styles among Nursing Inchrages**

Poonam Yadav<sup>1</sup>, Merlin Suji<sup>1</sup> , Seeta Devi<sup>2</sup> , Manisha Mistry<sup>2</sup>

3656-3661

 PDF

### **Nurses Opinions about the Transformational Leadership in Hospitals of Pune City**

Merlin Suji<sup>1</sup> , Poonam Yadav<sup>1</sup> , Seeta Devi<sup>2</sup>, Manisha Mistry<sup>2</sup>  
3662-3667

 PDF

### **Recalling the Definitions with a Mind Mapping and SelfLearning Technique: A Comparative Study in Undergraduate Students**

Merlin Suji<sup>1</sup> , Sharadha Ramesh<sup>2</sup> , Seeta Devi<sup>3</sup> , Diana Prasad<sup>1</sup> , Leeja Rajan<sup>1</sup>  
3668-3673

 PDF

### **Effectiveness of Structured Teaching Programme on Hazards of Water Pollution**

Monica Nadar<sup>1</sup> , Amol Sirsat<sup>1</sup> , Miracle Joseph<sup>1</sup> , Sharik Khan<sup>1</sup> , Keren Sofia<sup>1</sup>, Prajyot Patel<sup>1</sup> , Leeja Bonny Thomas<sup>2</sup>  
3674-3681

 PDF

### **To Assess the Awareness, Beliefs and Practice Patterns Regarding Oxygen Therapy in Prehospital Management of Respiratory Emergencies amongst EMS Professionals in Pune, India**

Parag Rishipathak<sup>1</sup> , Navnita Sengupta<sup>2</sup> , Anand Hinduja<sup>3</sup>  
3682-3687

 PDF

### **Simulation based Healthcare Education- Barriers in Initiation**

Parag Rishipathak<sup>1</sup> , Monesh Bhandari<sup>2</sup> , Anand Hinduja<sup>3</sup>  
3688-3691

 PDF

### **To Evaluate the Knowledge and Attitude Regarding Management of Acute Poisoning amongst the Emergency Medical Professionals in Pune, India**

Parag Rishipathak<sup>1</sup> , Shrimathy Vijayaraghavan<sup>2</sup> , Anand Hinduja<sup>3</sup>  
3692-3696

 PDF

### **Assessing the Effectiveness of Online Teaching Methodology among Emergency Medical Professionals in Pune, India**

Parag Rishipathak<sup>1</sup>, Monesh Bhandari<sup>2</sup> , Anand Hinduja<sup>3</sup>

3697-3703

 PDF

### **Opportunities and Obstacles Faced During Work From Home in Teaching Faculties**

Poonam Yadav<sup>1</sup> , Sharadha Ramesh, Merlin Suji<sup>1</sup> , Leeja Bonny Thomas<sup>1</sup> , Stephina Fernandis<sup>1</sup>

3704-3711

 PDF

### **Assessment of Selected Practices of Menstrual Hygiene among Rural Women at Pune City**

Prachi Jadhav<sup>1</sup> , Aman Janbandhu<sup>1</sup> , Tushar Thorat<sup>1</sup> , Arunya Rajan<sup>1</sup> , Leeja Bonny Thomas<sup>2</sup> , Sheela<sup>3</sup>

Upendra

3712-3715

 PDF

### **Comprehensive Study on Medication Error to Improve Patient Safety**

Prakash Kalke<sup>1</sup> , Darshana Mundhe<sup>2</sup>

3716-3722

 PDF

### **A study to Assess the Student's Anxiety Level about Examination During Lock Down in Selected Colleges of Pune City**

Priyanka Kadam<sup>1</sup> , Mangesh Jabade<sup>1</sup> , Tejashri Ligade<sup>1</sup>

3723-3725

 PDF

### **Prevalence of Childhood Obesity and its Prevention Systematic Review**

Ranjana Chavan<sup>1</sup> , Mangesh Jabade<sup>2</sup> , Dipali Dumbre<sup>2</sup>

3726-3730

 PDF

### **A Study to Assess the Effectiveness of Information Booklet on Selected Behavioral Problems among School Going Children in Terms of Knowledge of Selected Schools of Primary Schools Teachers of Pune City**

Rutuja Bhagwan Vairat<sup>1</sup> , Nilima Suryakant Shinde<sup>1</sup> , Amol Sanjay Gaikwad<sup>1</sup> , Vishal Rajendra Chabukswar<sup>1</sup> , Jemi V Cheriyan<sup>1</sup> , Dipak Sethi<sup>2</sup> , Merlin Suji<sup>3</sup>

3731-3735

 PDF

### **A Study to Assess the Eating Habits, Physical Activity, and Knowledge Regarding the Prevention of Obesity among School Children in a Selected Residential Area of Pune City**

Shraddha Daf1 , Shital Darekar1 , Dhananjay Kale1 , Gauri Pandey, Anil Jadhav1 , Iken Bole1 ,Seeta Devi2  
3736-3743



### **Assess the Preparedness Regarding Triage Skills Amongst Emergency Medical Professionals in Pune, India**

Shrimathy V 1 , Parag Rishipathak2 , Anand Hinduja3  
3744-3749



### **Challenges Faced by Working Population During Lockdown in Response to Corona Virus Outbreak**

1 Sonopant G Joshi, Sheetal Barde2 , Ranjana Chavan2 , Deepali Dumbre3 , Tejashree Ligade3 , Roma Shinde3 , Saloni Chinmay Desai4  
3750-3756



### **Assess the Knowledge Regarding Food and Vegetable Sanitation among Housewives in Selected Areas of Pune City**

Stephina Fernandes1 , Sharadha Ramesh2 , Jennifer. Anthony1 , Poonam Yadav1  
3757-3762



### **“Social Impact of Pandemic disease (COVID-19) in General Population**

Stephina Fernandes1 , Sharadha Ramesh2 , Merlin Suji1, Poonam Yadav1  
3763-3768



### **Study to Evaluate Level of Job Satisfaction among Health Care Personnel**

Suruchi Pandey1 , Jasneet Kaur2  
3769-3776



### **Deleted**

3777-3782

### **Effectiveness of Selected Yogasanas on Practice of Coping Strategies among Women with Premenopausal Symptoms Residing in Rural area of Pune**

Dhanamma P Pujari1, Seeta Devi2 , Ranjana Chavan2  
3783-3790

 PDF

### **Effectiveness of Video-Assisted Teaching Program Regarding Risk Factors of Cardiovascular Diseases on Knowledge, Attitude, and Practice among IT Professionals**

Kiran E. Jadhav<sup>1</sup> , Seeta Devi<sup>2</sup>

3791-3796

 PDF

### **Impact of Transformational Leadership on Patient Safety & Outcome– A Systematic Review**

Manisha Vikrant Mistry<sup>1</sup> , A. Seeta Devi<sup>1</sup> , Merlin Suji<sup>2</sup> , Poonam Yadav<sup>2</sup>

3797-3800

 PDF

### **Knowledge and Attitude Towards First Aid Management in School Teachers**

Jennifer W. Anthony<sup>1</sup> , Sharadha Ramesh<sup>2</sup> , Mangesh Jabade<sup>13</sup> , Merlin Suji<sup>1</sup>

3801-3806

 PDF

### **Effectiveness of Education Program on Knowledge Regarding Gestational Diabetes among Nurses**

Seeta Devi<sup>1</sup> , Sheela Upendra<sup>2</sup> , Shital Waghmare<sup>1</sup> ,

3807-3811

 PDF

### **Influencing Reasons for Participation and Nonparticipation of Women in Cervical Screening**

Seeta Devi

3812-3820

 PDF

### **Transformational Leadership Educational Bundle for Nursing Supervisors and Nurses in-Charges and its Impact on Clinical Performances of the Nurses in the Labour Room**

Seeta Devi<sup>1</sup> , Manisha Mistry<sup>1</sup> , Poonam Yadav<sup>2</sup> , MerlinSuli<sup>2</sup> , Priya Kamble<sup>2</sup>

3821-3829

 PDF

### **Effectiveness of Structured Teaching Programme on the Level of Knowledge about Psychological First Aid among Psychiatric Nurses Working in Selected Psychiatric Nursing Homes in Pune City**

Sheetal Barde<sup>1</sup> , Sheela Upendra<sup>2</sup>

3830-3832

 PDF

### **Effectiveness of antenatal IEC Package On Knowledge and Attitude of Antenatal Mother's Spouses**

Chandbibi Pathan1 , Seeta Devi2 , Tejashri Ligade3  
3833-3837

 PDF

### **Smartphone Usage and Its Addiction among Undergraduate Nursing Students**

Chaitrali Khatgaonkar1 ; Wasiya Sayyad1 ; Dhananjay Mali1, Sharadha Ramesh2 ; Manisha Mistry3  
3838-3843

 PDF

### **A Study to Assess the Usage of Personal Protective Equipment among Health Care Providers Working in Covid-19 Wards in Pune City, Maharashtra**

Dipak Sethi1 , Sharadha Ramesh1 , Tejashree1  
3844-3846

 PDF

### **A Study to Assess the Menstrual Hygiene Practices among the Women of Rural Community of Pune City**

Samiksha Pote1 , Geetanjali Yadav1 , Vishal Thopte1 , Sheela Upendra2 , Leeja Rajan3  
3847-3851

 PDF

### **Impact of Information Booklet on Harmful Effect of Use of Plastics in Relation to Practices among Urban Resident of Pune**

Greety Paul1 , Nakabuye Sharifah1 , Rupali Bhujbal1 , Shruti Thorat1 , Sonopant.G Joshi2 , Ranjana Chavan3  
3852-3857

 PDF

### **A Study to Assess the Effectiveness of Information, Education and communication on Knowledge and Practice regarding MR Vaccination among the Mothers in Selected Slum Areas of Pune City**

Shaikh Salim Karim1, Jasneet Kaur2 , Mangesh Jabade3  
3858-3864

 PDF

### **A Study to Assess the Awareness and Attitude Towards maintaining Social Distancing during Corona virus Disease (COVID 19) Pandemic among Residents of Selected Area of Pune City**

Jasneet Kaur<sup>1</sup> , Sheetal Barde<sup>1</sup> , Shital Waghmare<sup>1</sup> , Sheela Upendra<sup>2</sup> , Priyanka Kadam<sup>3</sup> , Suresh Ray<sup>4</sup>  
3865-3869



### **Hurdles and Possible Solutions in Development and Implementation of Online Learning in Nursing Education- an Integrative Review**

Jasneet Kaur<sup>1</sup> , Deepak Sethi<sup>1</sup> , Sheetal Barde<sup>1</sup>  
3870-3874



### **Effectiveness of Community Education Programme on Knowledge and Attitude Regarding Prevention and Management of Selected Non-Communicable Diseases among Urban Adults in Pune City**

Najama Wahid Beg<sup>1</sup> , Sonopant Joshi<sup>2</sup> , Shital Waghmare<sup>3</sup>  
3875-3880



### **Impact of Information Booklet on Harmful Effect of Use of Plastics in Relation to Knowledge among Urban Resident of Pune**

Hesham Abdulrab Homaid<sup>1</sup> , Rushikesh Savant<sup>1</sup> , Anjali Chauhan<sup>1</sup> , Sonopant.G Joshi<sup>2</sup> , Ranjana Chavan<sup>3</sup>  
3881-3886



### **A Study to Assess the Knowledge Regarding Air Pollution and Its Prevention People Residing in Selected Urban Areas of Pune City**

Siyona Chavan<sup>1</sup> , Akshra Sharon Bansod<sup>1</sup> , Rishal Reji<sup>1</sup> , Laya K1 , Jasneet Kaur<sup>2</sup> , Mangesh Jabade<sup>3</sup>  
3887-3891



### **A Study to Assess the Knowledge Regarding Air Quality Index among People Residing in Selected Urban Areas of Pune City**

Goyir Ori<sup>1</sup> , Jumyir Rime<sup>2</sup> , Suraj Jhadhav<sup>3</sup> , Jasneet Kaur<sup>4</sup> , Mangesh Jabade<sup>5</sup>  
3892-3896



### **Knowledge and Attitude on Hazards of Passive Smoking among Women of Pune City**

Manisha Mistry  
3897-3902



## **COVID – 19 Awareness among Adolescents in Pune City: A Survey Study**

Priya Kamble<sup>1</sup> , Sheela Upendra<sup>2</sup>

3903-3906



## **A Descriptive Study to Assess on Knowledge and Attitude of Nurses Regarding Prevention of Pressure Ulcers in Selected Hospitals**

Priya Kamble<sup>1</sup> , Dipali Dumbre<sup>2</sup>

3907-3909



## **A Study to Assess the Impact of Social Media on Psychological Health During Lock Down among Adolescents in the Selected Colleges of Pune City**

Priyanka Kadam<sup>1</sup> , Mangesh Jabade<sup>1</sup> , Tejashri Ligade<sup>1</sup> , Jasneet Kaur<sup>1</sup> , Dipali Dumbre<sup>1</sup>

3910-3912



## **Association of Knowledge on Environmental Hygiene With Demographic Variables of Rural Population in the Community**

Dhole Prutha Chandrakant<sup>1</sup> ; Dongare Reshma Vilas<sup>1</sup> ; Gaikwad Jyoti Raju<sup>1</sup> ; Gujar Abhijeet Kailas<sup>1</sup> ; Japtap Sandesh Peter<sup>1</sup> ; Manisha Mistry<sup>2</sup> ; Sheela Upendra<sup>3</sup>

3913-3915



## **A study to Assess the Effectiveness of Mindful Meditation on Health and Academic Adjustment Problem Faced by Nursing Students in Selected Colleges of Pune City**

Arsha Sarangm<sup>1</sup> , Laxmi Kharatm<sup>1</sup> , Shubham Talot<sup>1</sup> , Sonopant Joshi<sup>2</sup> , Ranjana Chavan<sup>3</sup>

3916-3920



## **A study to Assess Anxiety and Coping Strategies Adopted by the Students Regarding Education During COVID-19 Lockdown**

Roma Shinde<sup>1</sup> , Sharadha Ramesh<sup>2</sup> , Jasneet Kaur<sup>3</sup>

3921-3923



## **A Study to Assess the Knowledge Regarding Corona Virus and Its Management among the People Residing in Pune City**

Roma Shinde, Shital Waghmare,

3924-3926



 PDF

### **A Descriptive study on Knowledge of Environmental Hygiene Among Rural Residents in Rural Community**

Ewho Ruth Omoteboloko<sup>1</sup> ; Arti Chand<sup>1</sup> ; Madhu Bhatia<sup>1</sup>; Vrushali Kanade<sup>1</sup> ; Mokashi Reen<sup>51</sup>; Sheela Upendra<sup>2</sup> ; Mrs. Manisha Mistry<sup>3</sup>  
3927-3930

 PDF

### **Correlational Study to Assess the Musculoskeletal Quandry with the Bag Carried by Children Studying at Selected School of Pune**

Ms. Komal Jain<sup>1</sup> , Sharadha Ramesh<sup>2</sup> , Jasneet Kaur  
3931-3934

 PDF

### **A Study to Assess The Incidence of Suspect Cases of Leprosy and Tuberculosis at Selected Urban Slum of Pune City**

Sharadha Ramesh<sup>1</sup> , Jasneet Kaur<sup>2</sup> , Deepak Sethi<sup>2</sup> , Leeja Thomas<sup>3</sup> , Priyanka Kadam<sup>3</sup>  
3935-3938

 PDF

### **A Phenomenological Study of Pregnant Women's Experience : COVID-19 Lockdown Period**

Sheela Upendra<sup>1</sup> , Seeta Devi<sup>2</sup> , Jasneet Kaur<sup>2</sup> , Shital Waghmare<sup>2</sup> , Sheetal Barde<sup>2</sup>  
3939-3942

 PDF

### **Awareness on Immunity Boosting Measures for Self-care among Residents of Urban Community of Pune City**

Sheela Upendra  
3943-3948

 PDF

### **Effectiveness of Video Teaching Programme on Knowledge Regarding Impact of Smartphone Dependence on Physical Health, Academics and Behavior among Adolescents Studying in Selected in Junior Colleges**

Bipasha Sharma<sup>1</sup>, Sheetal Barde<sup>2</sup>, Merlin Suji<sup>3</sup>  
3949-3954

 PDF

## **Nursing Clinical Preceptorship: Knowledge and Attitude of Nurse Educators Working in Teaching Institutes in India**

Sheetal Barde<sup>1</sup>, Sheela Upendra<sup>2</sup>, Jasneet Kaur<sup>1</sup>, Shital Waghmare<sup>1</sup>

3955-3962



## **Effect of Group Therapy Promotion on of Psychological wellbeing among Elderly at Selected Old Age Home**

Sheetal Barde

3963-3966



## **Deleted**

3967-3970

## **Deleted**

3971-3975

## **Deleted**

3976-3978

## **Quality of Sleep among the Patients in High Dependency Unit (Hdu)- A Descriptive Study**

Shruti Dabhade,<sup>1</sup> Manisha Mistry,<sup>2</sup> Dipali Dumbre<sup>3</sup>

3979-3982



## **Nurses' Preparedness regarding Emerging Infectious Diseases (EID) in Selected Hospitals of Maharashtra (India)**

Joshi Sonopant G

3983-3988



## **A Study to Assess Knowledge and Attitude Towards HIV among Students from Pune City**

Soumiksha<sup>1</sup>, Jomol<sup>1</sup>, Anushri<sup>1</sup>, Madhura<sup>1</sup>, Shefali<sup>1</sup>, Sharadha Ramesh<sup>2</sup>, Dipak Sethi<sup>3</sup>

3989-3992



## **A Study to Assess the Effectiveness of Video Assisted Teaching (VAT) on Knowledge of Environmental Hygiene in Rural Community**

Anjumole Varghese<sup>1</sup> , Babli Shama<sup>1</sup> ; Dalvi Vinaya Anil<sup>1</sup> , Bhosale Pooja Bhosale<sup>1</sup> ; Kangude Shivdas<sup>1</sup> ; Sheela Upendra<sup>2</sup> ; Manisha Mistry

3993-3996



### **Systematic Literature Review: “Effect of Hypoglycemia on Quality of Life among Patient with Diabetes mellitus**

Dipali Dumber<sup>1</sup> , Sheela Upendra<sup>2</sup>

3997-3999



### **A Study to Assess the Effectiveness of Structured Teaching Programme on Knowledge Regarding Updated National Immunization Schedule among the Postnatal Mothers Visiting Health Centre**

Tejashri Ligade<sup>1</sup> , Sagar Kulkarni<sup>2</sup>

4000-4004



### **A Study to Assess the Impact of Lockdown on Physical and Psychosocial Health among Senior Citizen in the Selected Areas of Pune City**

Tejashri Ligade , Deepak Sethi, Priyanka Kadam<sup>3</sup>

4005-4010



### **Quality of Life in Children with Type 1 Diabetic – A Systematic Review**

Merlin Suji<sup>1</sup> , Anita Y. Nawale<sup>2</sup>

4011-4017



### **An implementation study on Hazard Identification and Risk Assessment (HIRA) technique in the Critical Care Unit of a Tertiary Care Hospital**

Rajvinder Kour<sup>1</sup> , Ankit Singh<sup>2</sup>, Neha Ahire<sup>2</sup>

4018-4026



### **A study to Assess the Awareness And Practice of Fast Food Consumption and Hazards on Health among Adolescents in Urban Community Area of Pune City**

Ranjana Chavan<sup>1</sup> , Sharadha Ramesh<sup>2</sup> , Mangesh Jabade<sup>3</sup>

4027-4030



## **An Exploratory Study on Opinion of Tracking System For Patient With Dementia among Mental Health Team Member of Pune City**

Pravesh Shukla<sup>1</sup> , Sheela Upendra<sup>2</sup> , Sharadha Ramesh<sup>3</sup>

4031-4034

 PDF

## **Knowledge Assessment on Hazards of Plastic Use among Residents of Selected Urban Community of Pune City**

Sheela Upendra<sup>1</sup>, Shital Waghmare<sup>2</sup> , Mangesh Jabade<sup>3</sup> , Diana Prasad<sup>3</sup> , Jennifer W Anthony<sup>3</sup>, Stephina Fernandes<sup>3</sup>

4035-4039

 PDF

## **Molecular Mechanism of Caffeine-Aspirin Interaction in Kopi Balur 1 as Anti-Inflammatory Agent: A Computational Study**

Viol Dhea Kharisma<sup>1,2</sup>, Arif Nur Muhammad Ansori<sup>2,3</sup>, Amaq Fadholly<sup>3</sup> , Teguh Hari Sucipto<sup>4</sup>

4040-4046

 PDF

## **Literacy Level on Dental and Oral Health in Health Students (Non Dentistry)**

Widya Cahyati Puteri<sup>1</sup> , Ayu Nur Aisyah Ruata<sup>1</sup> , Ghinalya Chalbi Ananda<sup>1</sup> , Mochamad Ghoza Satur Panama<sup>1</sup> , Martining Shoffa Puspitaningrum<sup>1</sup> , Fuad Adhi Waskita<sup>1</sup> , Retno Palupi<sup>2</sup>

4047-4051

 PDF

## **Assessments Self –Care of Sickle- Cell Patients at Baghdad Teaching Hospitals**

Saud. Jassim.Mohammed

4052-4057

 PDF

## **Adverse Effects of Mercury Exposure in DDW Strain Mice during Organogenesis**

Win Darmanto<sup>1</sup> , Saikhu Akhmad Husen<sup>2</sup> , Arif Abu Hasan<sup>3</sup> , Raden Joko Kuncoroningrat Susilo<sup>4</sup>, Suhailah Hayaza<sup>4</sup> , Hunsa Punnapayak<sup>5</sup>

4058-4064

 PDF

## **Bioremediation of Crude Oil-Contaminated Water and Sediments from the Shatt Al-Arab River (SAR)**

Wisam Abdul-Ameer Farid<sup>1</sup> , Wasen Abdul-Ameer Ali<sup>1</sup> , Aseel Nadum Al-Salman<sup>2</sup>

4065-4072

 PDF

### **Contamination of Polynuclear Aromatic Hydrocarbons- (PNAH) in Sediments: Identification And Distribution in the River of Shatt Al-Arab-(ROSA)**

Wisam Abdul-Ameer Farid<sup>1</sup> , Wasen Abdul-Ameer Ali<sup>1</sup> , Aseel Nadum Al-Salman<sup>2</sup>  
4073-4079

 PDF

### **Community Awareness and Participation in Handling Covid-19 in Surabaya, Indonesia**

Wiwi Wikanta<sup>1</sup> , Sukadiono<sup>2</sup> , Abdul Aziz Alimul Hidayat<sup>3</sup> , Sujnah<sup>4</sup> , dan Hetty Murdiyani<sup>5</sup>  
4080-4086

 PDF

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Wiwiek Tyasningsih<sup>1</sup> , Lingga Pulung Wijanarka<sup>2</sup> , Indra Raja Syahputra<sup>2</sup> , Mustofa Helmi Effendi<sup>3</sup>  
4087-4092

 PDF

### **Spatial Analysis of Pneumonia in Toddlers Based on Environmental, Individual, and Behavior Factors in Pelalawan Regency, Riau Province**

Yessi Harnani<sup>1</sup> , Rasoel Hamidy<sup>1</sup> , Sukendi<sup>1</sup> , Dedi Afandi<sup>1</sup>  
4093-4099

 PDF

### **Forensic Medicine in Indonesia: The Application of Visum ET Repertum in Case Resolution**

Yogi Prasetyo<sup>1</sup> , Ferry Irawan Febriansyah<sup>2</sup> , Indiantoro<sup>3</sup> , Absori<sup>4</sup> , Chrisna Bagus Edhita Praja<sup>5</sup>  
4100-4105

 PDF

### **A Root Cause Analysis of Prolonged Waiting Times for Compounded Prescriptions at an Indonesian Private Hospital**

Yuniar Sunarko<sup>1,2</sup> , Mulyatim Koeswo<sup>3</sup>  
4106-4110

 PDF

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Yusuf Saefudin<sup>1</sup> , Hartiwiningsih<sup>2</sup> , Isharyanto<sup>3</sup>  
4111-4115

 PDF

### **Effect of Acridine Orange on Pathogenicity of E. coli Isolated from Urinary Tract Infection Patients**

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4116-4123

 PDF

### **Evaluation of HE4 and IGFBPs as Novel Biomarkers of Systemic Lupus Erythematosus with Lupus Nephritis**

NoorAlhuda Kh. Ibrahim<sup>1</sup> , Kadhim K. Ghudhaib<sup>2</sup> , Ali Abdulmajid Dyab Allawi<sup>3</sup> , Faten Abdulghani

Hammoudi<sup>4</sup>

4124-4130

 PDF

### **Antimicrobial Resistance on Escherichia coli from Poultry Production on Blitar, Indonesia**

Freshinta Jellia Wibisono<sup>1</sup> , Bambang Sumiarto<sup>2</sup> , Tri Untari<sup>3</sup> , Mustofa Helmi Effendi<sup>4,5</sup> , Dian Ayu

Permatasari<sup>5</sup> , Adiana Mutamsari Witaningrum<sup>5</sup>

4131-4136

 PDF

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4137-4142

### **Forensic Odontology-A Review**

Prabhu Manickam Natarajan, Asok Mathew<sup>2</sup> , Vijay Bhavrao Desai<sup>3</sup> , Shibu Thomas<sup>4</sup> , Eyas Abuhijleh<sup>5</sup> , S.

Srinivasan<sup>6</sup>

4143-4147

 PDF

### **Study of Violent Asphyxial Deaths in Raichur District of Karnataka**

Sidramappa Gouda<sup>1</sup> , Suraj<sup>2</sup> , Gopinath M<sup>3</sup>

4148-4152

 PDF

### **Deleted**

Citra Manela<sup>1</sup> , Biomechy Oktomali Putri<sup>2</sup> , Majesty Anita Imran<sup>3</sup>

4153-4159

 PDF

## **The Profile of Laryngopharyngeal Reflux Patients at Dr. Soetomo Teaching Hospital, Surabaya Indonesia**

Ami Pratami Munifah1 , Rizka Fathoni Perdana1 , Sri Herawati Juniati1, Muhtarum Yusuf1 , Erni Rosita Dewi2  
4160-4166



## **The Association between Sex, Knowledge, and Education Level of Evidence-Based Dentistry Behavior among Dentistry Student**

Oki Fadhila1 , Aryo Dwipo Kusumo2  
4167-4171



## **Knowledge, Attitude and Practices Regarding Epilepsy among Nurses in a Tertiary Care Hospital in South India**

Arvind N. Prabhu, Pai Divya Venkatesh2,Ajo Paul3 , Akhilesh Pandey4, Rajib Mandal5 , Kamath Madhusudhana6  
4172-4179



## **Pre -treatment implant CBCT Analysis of Anterior Mental Loop Length, Lingual Concavity and Inclination of Alveolar Ridge in the Mandible - A UAE Study**

Asok Mathew1 , Raghad Mohammed Sadiq2 , Suleiman Ergieg3 , Maher al Shayeb4 , Eyas Abuhijleh5 , Sudhir Rama Varma6 , Sam Thomas7  
4180-4188



## **A Multicentral Quasi experimental Study on Impact of Neuromuscular Taping over Neck Pain**

Yu Chye Wah1 , Kshtrashal Singh2 , Mahesh Hegde3 , Komala Bakrishnan4 , Mallisah Kaur5, Kamaraj B6 , Rahul Krishnan Kutty7  
4189-4195



## **A Multicentral Quasi experimental Study on Impact of Neuromuscular Taping over Low Back Pain**

Yu Chye Wah1 , Kshtrashal Singh2 , Susmitha Govind3 , Kasmalina binti Mohd4 , Siva Sangary Murthy5 , Kamaraj B6 , Rahul Krishnan Kutty7  
4196-4202



## **The Effect of Cytokines and CD Markers in Patients Infected with COVID- 19**

Ali Anok Njum, Jabbar Afate ALwane2

4203-4207

 PDF

## **A Case Report on an Estimation of Financial Damage Occurring at A Rural University, South Africa**

B Meel

4208-4211

 PDF

## **Estimation of Shortfall in Salary from 1996 to 2018 from Health and University, South Africa**

B Meel

4212-4218

 PDF

## **Mandibular Second Molar Protostylid: A Rare Case Report**

Anupama Nayak P1 , Ashwin Rao2 , Karuna YM3 , Suprabha BS4 , Sowmya Sridhar5

4219-4223

 PDF

## **Protein Supplement Drinks, the Modern Killer that Induces Oxidative Stress in Mice Liver**

Rasha Shaker Nima1 , Dhifaf Zeki Aziz2 , Rasha Amer Noori Al-Tufaili1

4224-4228

 PDF

## **Medical Negligence Dispute Settlement in Indonesia**

Deri Mulyadi1 , Elwi Danil2 , Wila Chandrawila3 , Kurnia Warman2

4229-4233

 PDF

## **Protective Strategies for the Ownership of Traditional Medicine Knowledge**

Zainul Daulay1 , Kurnia Warman2

4234-4239

 PDF

## **Effectiveness of Online Oral Health Education During the Covid-19 Pandemic**

Nila Kasuma1 , M. Biomed1 , Murniwati2 , Dedi Sumantri3 , Reni Nofika4 , Surya Nelis1 , Susi2, Aria Fransiska3

4240-4248

 PDF



## **The Implementation of Honesty Principles in Therapeutic Agreements based on the Health Law Perspective in Indonesia**

Isdian Anggraeny<sup>1</sup> , Isdiyana Kusuma Ayu<sup>2</sup>

4249-4253



## **Study of Lipid Profiles levels in Iraqi children with GH Deficiency Before and after 6 Months GH Replacement Therapy**

Mithal R Alkubaisi<sup>1</sup> , Hameed Hussein Ali<sup>2</sup> , Fakhri J. Al-Dalla Ali<sup>1</sup> , Abbas M. Rahmah<sup>3</sup>

4254-4260



## **The Association between The Thickness of the Endometrium at the Day of Human Chorionic Gonadotrophin Injection and the Outcome Intracytoplasmic Sperm Injection**

Zainab Alkhafajy<sup>1</sup> , Zainab Abd alkathem fatnan<sup>2</sup> , Hayfaa Dinar Jaber AL Habeeb<sup>3</sup>

4261-4266



## **Effects of Dance and Movement Therapy (DMT) versus Progressive Muscle Relaxation(PMR) on Depression among Elderly Residing in Selected Old Age Homes of Haryana: A Quasi Experimental Study**

Prakash Jha<sup>1</sup> , Srinivasan.P<sup>2</sup> , Uma J Deaver<sup>3</sup> , Jyoti Sarin<sup>4</sup>

4267-4273



## **Assess the Caregiver's Knowledge on the Adverse Effect of Antipsychotics in the Selected Psychiatric unit of South India**

Joyce Fernandes<sup>1</sup> , Sukesh Shetty<sup>2</sup> , Nalini.M<sup>3</sup>

4274-4278



## **The Analysis Effect Prenatal Yoga and the Expression gen MRHR-CRHR1 and mRNA FKBP5 with Real Time-PCR**

Ruqaiyah<sup>1,2</sup> , Nusratuddin Abdullah<sup>3</sup> , Mochammad Hatta<sup>4</sup> , Syarfianty Arifuddin<sup>5</sup> , Saidah Syamsuddin<sup>5</sup> , Irfan Idris<sup>6</sup> , Agusalim Bukhari<sup>5</sup>

4279-4285



## **Oral Health Care Guidelines for Patients with Diabetes Mellitus: A Review**

Prasanna Kumar Rao<sup>1</sup> , Dharnappa Poojary<sup>2</sup> , Shailesh Kudva<sup>3</sup> , Ashok Kumar Jeppu<sup>4</sup> , Kavitha Ashok Kumar<sup>5</sup> , Alok Dubey<sup>6</sup>

4292-4301

 PDF

### **The Effect of Escherichia coli on Pro-Inflammatory Mediators Level and Kidney and Liver Function of Sepsis in Rattus novergicus**

Dian Samudra<sup>1,2</sup>, Sumarno<sup>3</sup>, Sanarto Santoso<sup>3</sup>, Aswoco A. Asmoro<sup>4</sup>, Husnul Khotimah<sup>5</sup>, Arif N. M. Ansori<sup>6</sup>, Emy K. Sabdoningrum<sup>6,7</sup>

4302-4305

 PDF

### **Stress Related Temporomandibular Joint Disorders - A Comparative Study between Preclinical and Clinical Undergraduate Students of Dentistry**

Munazza Sharfuddin<sup>1</sup>, Ayshwarya Muralidharan<sup>2</sup>, Premalatha Shetty<sup>3</sup>, Aditya Nandan<sup>4</sup>, Animesh Jain<sup>5</sup>

4306-4310

 PDF

### **Deleted**

4311-4317

### **Verbal Aggressive Behaviours of Elementary School Age Children**

Pipit Festi Wiliyanarti<sup>1</sup>, Rabiatal Awaliyah<sup>1</sup>, Hariyono<sup>2</sup>

4318-4324

 PDF

### **The Relationship between Obesity and the Incidence of Hypertension in Young Adults (18-40 Years) in Tomohon City in 2019**

Jonesius Eden Manoppo<sup>1</sup>, Andi Zulkifli Abdullah<sup>2</sup>, Citra Kesumasari<sup>2</sup>, Ridwan Mochtar Thaha<sup>2</sup>, Sri Achadi Nugrahaeni<sup>3</sup>, Stang Abdul Rahman<sup>2</sup>, Ridwan Amiruddin<sup>2</sup>

4325-4330

 PDF

### **The Art and the Science of Manuscript Publication: Tips and Tricks for Health Science Students and Professionals**

Waqar M. Naqvi<sup>1</sup>, Chanan V. Goyal<sup>2</sup>, Arti Sahu<sup>3</sup>

4331-4339

 PDF

### **Knowledge, Attitude and Practice of Undergraduate Students Regarding Toothbrush Contamination and Disinfection**

Swapna Bettanapalya Venkatesh<sup>1</sup>, Vignesh Kamath<sup>2</sup>, Shibani Shetty<sup>3</sup>, Smitha Sammith Shetty<sup>4\*</sup>

4340-4345

 PDF

### **Clinical Evaluation of Local Drug Delivery of Chlorhexidine and Ornidazole in the Treatment of Gingivitis as an Adjunct to Scaling and Root Planing**

Roopalakshmi Narayanan<sup>1</sup> , Munivenkatappa Lakshmaiah Venkatesh Prabhuji<sup>2</sup> , Shaeesta Khaleelahmed Bhavikatti<sup>3</sup> , Shahabe A. Saquib<sup>4</sup> , Rashmi Paramashivaiah, Mohmed Isaqali Karobari<sup>6</sup> , Farheen Ustad<sup>7</sup>

4346-4353

 PDF

### **Postmortem Cardiac Troponin-T levels in Heart Blood as a Marker to Diagnose Acute Myocardial Infarction**

P. Vinod Kumar<sup>1</sup> , Amudhasaharan, Magendran J<sup>2</sup>

4354-4360

 PDF

### **Biological Effects of Tumor Necrosis Factor Alpha (TNF-?) in Systemic Inflammation. Running title: TNF-? for systemic inflammation**

Masriadi<sup>1</sup> , Hasta Handayani Idrus<sup>2</sup> , Sukmawati<sup>3</sup>

4361-4367

 PDF

### **Experiencing the Ancient Indian Healthcare Science of Ayurveda Through the Immersive Experiential Prism of a Scholar from the Global North West Visiting an Ayurveda Centre in Coastal Karnataka**

Basavaraj S Hadapad<sup>1</sup> , Anupama V Nayak<sup>2</sup> , Chaitanya Sanjay Mishra<sup>3</sup>

4368-4372

 PDF

### **An Autopsy Study of Burn Injuries in Elderly Women**

Asma Kausar<sup>1</sup> , Dayananda R<sup>1</sup> , Varghese P S<sup>2</sup>

4373-4377

 PDF

### **Sudden Deaths in Mthatha Area of South Africa**

Bl Meel

4378-4382

 PDF

## **An Opinion based on the Autopsy Report on the Manner of Death by a Gunshot Injury: Concealed Murder or Accidental**

B Meel

4383-4385



## **Deleted**

4386-4389

## **Deleted**

4390-4395

## **Prevalence of Myths and False Beliefs Regarding HIV/AIDS among Xhosa Community in the Transkei Region of South Africa**

BL Meel

4396-4401



## **Metformin : as First-Line Treatment for Type 2 Diabetes Prevention**

Ranjita V. Gaur<sup>1</sup> , Dimple Patel<sup>2</sup> , Rohit Zariwala<sup>3</sup>

4402-4406



## **The Spiritual Care Competence Scale: A Confirmatory Study of the Malay Language Version**

Ali H. Abusafia<sup>1</sup> , Zakira Mamat<sup>2</sup> , Nur Syahmina Rasudin<sup>3</sup> , MujahidAli H. Abusafia<sup>1</sup> , Zakira Mamat<sup>2</sup> , Nur Syahmina Rasudin<sup>3</sup> , Mujahid Bakar<sup>4</sup> And Rohani Ismail<sup>4</sup> Bakar<sup>4</sup> And Rohani Ismail<sup>4</sup>

4407-4414



## **Bovine Polyclonal Anti-eCG and eCG Combined Vaginal Sponges Progesterone to Induce Estrus and Pregnancy of Etawa Crossbred Goats**

Herry Agoes Hermadi<sup>1</sup> , Sunaryo Hadi Warsito<sup>2</sup>

4415-4419



## **Compensation for Ex-Mineworkers in the Mthatha Region of South Africa: A Long Road to Travel**

BL Meel<sup>1-2</sup>

4420-4423

 PDF

### **Nurses – Midwives Knowledge Concerning Polycystic Ovary Syndrome in Baghdad Hospitals**

Aqdas Dawood Salman

4424-4430

 PDF

### **The Effect of Repetition Simulation after Debrief Method on Learning Satisfaction and Learning Achievement in Midwifery Students at Universitas Airlangga, Indonesia**

Budi Prasetyo<sup>1,2</sup>, Wahyul Anis<sup>2</sup>, Rize Budi Amalia<sup>2</sup>, Dwi Izzati<sup>2</sup>, Woro Setia Ningtyas<sup>2</sup>, Ratna Dwi Jayanti<sup>2</sup>, Farida Fitriana<sup>2</sup>

4431-4435

 PDF

### **The Effectiveness Meaning in Life and Antiretroviral Therapy (Metart) Book as an to Promotion Adherence Antiretroviral Therapy among New People Living with HIV/AIDS Asesment Pre Intervention**

Yafi Sabila Rosyad<sup>1</sup>, Firmina Theresia Kora<sup>2</sup>, Rika Monika<sup>3</sup>, Salis Miftahul Khoeriyah<sup>4</sup>, I Putu Juni Andika<sup>5</sup>

4436-4441

 PDF

### **Through the Life of their Spouses- Coping Strategies of Wives of Male Alcoholics**

Chinnu Sebastian<sup>1</sup>, Suja M.K<sup>2</sup>

4442-4446

 PDF

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Saikiran Pendem

4447-4451

 PDF

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Saleena. M<sup>1</sup>, Suja M.K<sup>2</sup>

4452-4456

 PDF

### **Effects of Smokers and Second Hand Smoke by Gender and Age on DALYs Stroke in Indonesia**

Mugeni Sugiharto<sup>1</sup>, Lukman Prayitno<sup>2</sup>

4457-4463



## **Depression and Suicidal Ideation as a Consequence of Academic Stress among Adolescent Students**

Veereshkumar S.Nandagaon<sup>1</sup>, Sudha A. Raddi<sup>2</sup>

4464-4468



## **Epidemiological Pattern of Head Injuries in Road Traffic Accident Victims Presenting to a Tertiary Care Hospital in South India**

Shilpa Pateria, Rajesh Kamath<sup>2</sup>, Vikram Palimar<sup>3</sup>, Harshith L.K<sup>4</sup>, Biju Soman<sup>5</sup>, Vishnu Sagar<sup>4</sup>

4469-4475



## **Clinical, Laboratory and Radiologic Evaluations in Patients with Malignant Tuberculous Spondylitis**

Bambang Prijambodo<sup>1</sup>, Udria Satya Pratama<sup>2</sup>

4476-4481



## **International Human Rights Instruments and Indonesian Legal Protection For Persons With Disabilities**

Elfia Farida<sup>1</sup>, Adya Paramita Prabandari<sup>1</sup>, Rahayu<sup>1</sup>

4482-4486



## **Deleted**

4487-4490

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4491-4496

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4497-4500

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4501-4504

## **Deleted**

4505-4514

### **Emerging and Re-Emerging Virus**

Dharsan.R1 , R.V.Geetha2 , Lakshmi.T3  
4515-4521



### **Matrix Vesicle Mediated Mineralization - A Review**

Devika. B1 , K. R. Don2 , V. Vishnu Priya3 ,  
4522-4528



### **Brain Distinguish Speech From Music - A Review**

Praneeksha1 , R. Gayatri Devi2  
4529-4533



### **Animal Model Used in Dentistry-A Review**

R.Kushali 1, Subhabrata Maiti2 , R.V.Geetha3 , Jessy4  
4534-4543



### **Lasers in Endodontics- A Review of Literature**

P.A.Vasishta1 , K Anjaneyalu2 , Vinay Sivaswamy3 , K Anjaneyalu4  
4544-4550



### **Role of Dermal Fillers in Aesthetic Dentistry**

Kayal Vennila Mahendran1 , Jayalakshmi Somasundaram2, Vinay Sivaswamy3  
4551-4559



### **Nano Glass Ionomers - A Review**

S.Padmaja1 , Jayalakshmi Somasundaram2 , Vinay Sivaswamy3  
4560-4566



### **Shade Matching in Fixed Partial Denture Patients**

P. Chithambara Shathviha<sup>1</sup> , Dhanraj Ganapathy<sup>2</sup> , Mebin Mathew<sup>3</sup>  
4567-4572



### **Prevalence of Fluorosis among Patients Visiting a Private College and Hospital in Chennai, India**

Neha Sharma M<sup>1</sup> , Pratibha Ramani<sup>2</sup> , L. Leelavathi<sup>3</sup>  
4573-4582



### **Correlation of Clinically Desquamative Gingivitis and Histopathology at a Private College Hospital in Chennai, India - A Retrospective Study**

Shivani. N<sup>1</sup> , Pratibha Ramani<sup>2</sup> , Leelavathi.L<sup>3</sup>  
4583-4591



### **Prevalence of Odontogenic Tumours in a Dental Hospital in Southern India**

Sathvika K<sup>1</sup> , Gifrina Jayaraj<sup>2</sup> , Leelavathi L<sup>3</sup>  
4592-4603



### **Determination of Sex Using Mastoid Process among South Indian Population - A Review**

A. Aksha Sharen, Karthik Ganesh Mohanraj  
4604-4610



### **DNA Profiling in Forensic Dentistry**

M. Laksita<sup>1</sup> , K. R. Don<sup>2</sup> , Jothi Priya A<sup>3</sup>  
4611-4617



### **Effects of Temperature on Mechanical Properties of Dental Restoration - A Review**

Sasipriya G<sup>1</sup> , Balaji Ganesh S<sup>2</sup> , A .Jothi Priya<sup>3</sup>  
4618-4623



### **Therapeutic Potential of Stem Cells From Human Exfoliated Deciduous Teeth(Shed)- A Review**

Obuli Ganesh Kishore.S<sup>1</sup> , K. R. Don<sup>2</sup> , Jothi Priya.A<sup>3</sup> ,  
4624-4629



 PDF

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Taanya Imtiaz<sup>1</sup> , Dinesh Premavathy, Kavitha.S<sup>2</sup>  
4630-4638

 PDF

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Shruthi Devi<sup>1</sup> , K. R. Don<sup>2</sup> , Karthik Ganesh Mohanraj<sup>3</sup>  
4639-4646

 PDF

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Akash<sup>1</sup> , Geetha R V<sup>2</sup> , Jayalakshmi Somasundaram<sup>3</sup>  
4647-4651

 PDF

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 PDF

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K. Helen Reshma<sup>1</sup> , K. R. Don<sup>2</sup>  
4658-4665

 PDF

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S.Barani Shankar<sup>1</sup> , R.V.Geetha<sup>2</sup> , Jayalakshmi Somasundaram<sup>3</sup>  
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 PDF

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Hariprasath T.K<sup>1</sup>, Balaji Ganesh S<sup>2</sup>, R.Gayatri Devi<sup>3</sup>  
4673-4678

 PDF

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Keerthiga Nagarajan

4679-4686

 PDF

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Saishree Anchana Rajeswaran1 , Jayalakshmi Somasundaram2 , Smiline Girija AS3

4687-4695

 PDF

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Keerthana B1 , Keerthi Sasanka2 , R Gayathri3 , Visalakshi Ramanathan4

4696-4700

 PDF

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Ritya Mary Jibu1 , R. V. Geetha2 , Lakshmi.T3

4701-4705

 PDF

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Immadi Laxmi Sujith Kumar1 , Pradeep S2 , Deepak Selvam3

4706-4713

 PDF

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Yazhlini P1, Anjaneyulu K2

4714-4721

 PDF

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Manali Deb Barma1 , L Leelavathi, Jayashri Prabakar3

4722-4730

 PDF

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V. T. Thamarai Selvi1 , Jayalakshmi2 , M.P. Brundha3

4731-4745

 PDF

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Lalitha Rani Chellappa<sup>1</sup> , L.Leelavathi<sup>2</sup> ,  
4746-4754

 PDF

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Keerthana T<sup>1</sup> , Mahalakshmi. J<sup>2</sup> , Deepak. S<sup>3</sup>  
4755-4762

 PDF

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4763-4768

 PDF

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4769-4776

 PDF

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4777-4785

 PDF

### **Most Commonly Associated Primary Tooth in Mandibular Arch with Space Infection or Abscess - A Retrospective Study**

Abigail Ranasinghe<sup>1</sup>, Geo Mani<sup>2</sup> , Raghu Sandhya<sup>3</sup>  
4786-4793

 PDF

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Priyadharshini Suresh Babu<sup>1</sup> , Geo Mani<sup>2</sup>  
4794-4802

 PDF

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Meghana Reddy J1 , Deepa Gurunathan2 , Deepika Rajendran3  
4803-4811

 PDF

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Nivethitha.R, Dhanraj Ganapathy2 , S.P.Saravana Dinesh3  
4812-4819

 PDF

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Amit Singh1 , Jayashri Prabakar2 , Revathi Duraisamy3  
4820-4827

 PDF

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Reethega.L1 Lakshmi.T2, RV Geetha3  
4828-4833

 PDF

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Danisca Uthayasankar1 , L.Keerthi Sasanka2 , R. Gayathri3 , Visalakshi Ramanathan4 , Dhanraj Ganapathy5  
4834-4840

 PDF

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4841-4846

 PDF

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4847-4857

 PDF

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Vidyashri. S1 , Jayalakshmi Somasundaram2

4858-4865



## **Novel Trends in Drug Delivery and Application of Curcumin in Dentistry**

Kaviya.L1 , Anitha Roy2 , Jayalakshmi Somasundaram3 ,

4866-4872



## **Prevalence of Reticular Oral Lichen Planus: A Retrospective Study**

Sneha Sree.S1 , Jayanth Kumar Vadivel2

4873-4879



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Rinki Susan George1 , Anitha Roy2 , Lakshmi.T3

4880-4886



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Harini B1 , Anitha Roy2 , Lakshmi.T2

4887-4891



## **Oncogenes Associated with Oral Cancer - An Update**

Nishanthana Murali1 , Vijayashree Priyadharsini J2 , Anitha Roy3

4892-4897



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L.Sai Charan1 , Balaji Ganesh S2 , V. Vishnu Priya3

4898-4905



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J. Dhayanithi1 , M.P. Brundha2 ,

4906-4911

 PDF

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4912-4926

 PDF

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4927-4932

 PDF

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4933-4940

 PDF

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 PDF

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Nirubama.K<sup>1</sup>, M. P.Brundha<sup>2</sup> , Ezhilarasan<sup>3</sup>  
4948-4954

 PDF

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Anu Iswarya Jaisankar, Jayalakshmi Somasundaram, D. Ezhilarasan,  
4955-4961

 PDF

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Aditya Jain<sup>1</sup> , Geo Mani<sup>2</sup> , Pradeep Kumar. R<sup>3</sup>  
4962-4970

 PDF

## **Assessment of Partial Edentulism Based on Kennedy's Class IV Classification- Retrospective Study**

Cibikkarthik T1 , Revathi Duraisamy2 , Jessy P3

4971-4977



## **Comparison of the Prevalence of Space Maintainers among Different Age Groups and Genders in Children**

S.Vignesh, Vignesh Ravindran, Arvina Rajasekar

4978-4984



## **An Estimation of Blood Glucose Level in Children with Early Childhood Caries- A Retrospective Study**

Prabhav Kumar Iyer1 , Geo Mani2 , Sarvana Dinesh S.P3

4985-4993



## **Caries of First Primary Molars Requiring Glass Ionomer Cement Versus Composites as Restorative Materials**

Niharika Kotian, EMG Subramanian, Ganesh Jeevanandan, Mebin George Mathew

4994-4998



## **Knowledge and Awareness on Infant Oral Health among Parents**

Kaviaselvi Gurumurthy1 , K. R. Don2

4999-5011



## **Analysis of Pulp Capping Procedures Done in Permanent Molars in A University Setting - A Retrospective Study**

Fahmida Binti Abd Rahman1 , M.S. Nivedhitha2, T.N.Uma Maheshwari3

5012-5019



## **Awareness on the Methods to Overcome Depression among Undergraduate Students in An University Hospital Setting**

Hiranya.S1 , V.Vishnu Priya2 , R.Gayathri3

5020-5026



## **Correlation between Hard and Soft Tissue Lower Anterior Facial Height of Different Skeletal Pattern on South Indian Population**

Seerab Husain<sup>1</sup> , Naveen Kumar<sup>2</sup> , Sri Rengalakshmi<sup>2</sup>  
5027-5035

 PDF

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5036-5044

 PDF

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5045-5052

 PDF

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Umayal.S<sup>1</sup> , A.S.Smiline Girija<sup>2</sup>  
5053-5058

 PDF

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Lasya Genji<sup>1</sup> , Geo Mani<sup>2</sup> , Raghu Sandhya<sup>3</sup>  
5059-5066

 PDF

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G.Nivedhita<sup>1</sup> , M.P.Brundha<sup>2</sup>  
5067-5073

 PDF

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Tasleem Abitha S<sup>1</sup> , Dhanraj Ganapathy<sup>2</sup> , Geo Mani<sup>3</sup>  
5074-5080

 PDF



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Ankita Komal Labh<sup>1</sup> , Pratibha Ramani<sup>2</sup>

5081-5092



## **Role of Vitamin C in Immune Function of Human Body**

V.Nancy Selvamary<sup>1</sup> , M.P.Brundha<sup>2</sup> , A.S.Smiline Girija<sup>3</sup>

5093-5099



## **A Survey on Knowledge about Oxidative Stress and Inflammatory Disease among Dental Students**

S.M.Sowbaraniya<sup>1</sup> , Anitha Roy<sup>2</sup> , N.P.Muralidharan<sup>3</sup>

5100-5107



## **Awareness on Application of Antacids among Undergraduate Students**

Ranjana V<sup>1</sup> , Keerthi Sasanka<sup>2</sup> , R Gayathri<sup>3</sup> , Visalakshi Ramanadhan<sup>4</sup>

5108-5118



## **Osteosynthetic Materials Used in Open Reduction and Internal Fixation of Facial Fractures in University Hospital Setting- A Retrospective Study**

M.Ashwin Shravan Kumar<sup>1</sup> , Madhulaxmi Marimuthu<sup>2</sup> , Revathi Duraisamy<sup>3</sup>

5119-5125



## **IgG4 Related Lung Disorder -A Review**

S.Balamithra<sup>1</sup> , Brundha M.P.<sup>2</sup> , Smiline Girija AS<sup>3</sup>

5126-5132



## **Artificial Intelligence in Dentistry - A Survey**

Rieshy. V<sup>1</sup> , L.Keerthi Sasanka<sup>2</sup> , R. Gayatri Devi<sup>3</sup> , Visalakshi Ramanadhan<sup>4</sup> , Dhanraj Ganapathy<sup>5</sup>

5133-5139



## **Knowledge and Awareness about Importance of Aesthetics in Dentistry**

S Sneka<sup>1</sup> , Keerthi Sasanka<sup>2</sup> , Gayatri Devi R<sup>3</sup> , Visalakshi Ramanadhan<sup>4</sup> ,  
5140-5150



### **Properties of Denture Liners - A Review**

Nivedha .M<sup>1</sup> , Balaji ganesh S<sup>2</sup> , K. R. Don<sup>3</sup>  
5151-5157



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Shreya Kothari<sup>1</sup> , Sivakumar M<sup>2</sup> , Balaji Ganesh S,<sup>3</sup> ,  
5158-5167



### **A Retrospective Analysis of the Most Frequently Root Canal Treated Tooth in Pediatric Patients Visiting a University Hospital In Chennai**

Shruthi Manivannan<sup>1</sup> , Geo Mani<sup>2</sup> , Murugan Thamaraiselvan<sup>3</sup>  
5168-5177



### **Survey on Recent Advances in Root Canal Disinfection**

Arokia Rajkumar Shancy Merlin<sup>1</sup> , S.Haripriya<sup>2</sup> , Anitha Roy<sup>3</sup>  
5178-5187



### **Survey on Management of White Spot Lesions**

N.E.Kaviya<sup>1</sup> , S.Haripriya<sup>2</sup> , Anitha Roy<sup>3</sup>  
5188-5196



### **Recent Advances in Apex Locators- A Review**

Akshaya.R<sup>1</sup> , Anjaneyulu K<sup>2</sup> , A.K.Anjali<sup>3</sup>  
5197-5203



### **Immune Boosting Drugs -A Myth or Reality - A Review**

Allour Vaishnavi<sup>1</sup> , Anitha Roy<sup>2</sup> , A.K.Anjali<sup>3</sup>  
5204-5211

 PDF

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Deepika. R1, Anitha Roy2 , A.K. Anjali3  
5212-5221

 PDF

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Arun Kishore RN1 , L. Keerthi Sasanka2 , Visalakshi Ramanadhan3 , Dhanraj Ganapathy4  
5222-5229

 PDF

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M.Kavya Shree1 , R.V Geetha2 , Leslie Rani.S3, Lakshmi.T4  
5230-5237

 PDF

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Pushpaanjali.G1 , R.V.Geetha2 , Leslie Rani.S3 , Lakshmi.T4  
5238-5243

 PDF

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Karishma Desai1, Subhabrata Maiti2 , Leslie Rani. S3  
5244-5250

 PDF

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Purva Verma1 , Naveen Kumar.M2  
5251-5259

 PDF

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R.Pon Preeja1 , RV Geetha2 , Lakshminarayanan Arivarasu3, Lakshmi.T4  
5260-5265

 PDF

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K.Sree Kala Priyadharsini1 , R.V.Geetha1 , Lakshminarayanan Arivarasu3  
5266-5272



### **Relationship of Tooth Mobility with Systemic Diseases among Patients Reporting Private Dental College in Chennai**

S.Sushanthi1, L.Leelavathi2 , Arthi balasubramaniam3  
5273-5281



### **Evaluation of Clinical Marginal Adaptations of the Copings Fabricated in Cad Cam - Retrospective Study**

Rithanya.P1 , Subhashree.R2 ,  
5282-5287



### **Prevalence of Ridge Defects According To Sieberts Classification in Patients Undergoing Fixed Partial Denture - A Retrospective Analysis**

Inchara. R1 , Subhashree. R2  
5288-5293



### **A Smile Analysis in Completely Edentulous Patients Treated by Graduate Students**

Ditty J Mary1 , Suresh Venugopalan2 , Nashra Kareem3  
5294-5301



### **Hemoglobin Levels and the Associated Factors in the Patients Visiting A University Hospital in Chennai, India**

Sadhvi.B1 , Suresh V2  
5302-5309



### **Prevalence of Complications after Gingival Recession Coverage Procedure - A Retrospective Cohort Study**

Revathi.B1 , Kiran Kumar Pandurangan2  
5310-5318



### **Liquid Biopsies in Cancer Diagnosis**

R.Sharmila<sup>1</sup> , Anitha Roy<sup>2</sup> , K. Anjaneyulu<sup>3</sup> , Lakshmi.T<sup>4</sup>  
5319-5327

 PDF

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Kowshihan P<sup>1</sup> , Anitha Roy<sup>2</sup> , Jayalakshmi Somasundaram<sup>3</sup> , Lakshmi.T<sup>4</sup>  
5328-5335

 PDF

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Krishna Doshi<sup>1</sup> , S.Delphine Priscilla Antony<sup>2</sup>  
5336-5343

 PDF

### **Prevalence and Incidence of Desquamative Gingivitis among Patients Visiting a Private Teaching Hospital in Chennai, India**

A.Shamaa Anjum<sup>1</sup> , Pratiba Ramani<sup>2</sup> , Delphine Priscilla Antony S<sup>3</sup>  
5344-5356

 PDF

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Anubhav Das<sup>1</sup> , Revathi Duraisamy<sup>2</sup> , Ravindra Kumar Jain<sup>3</sup>  
5357-5363

 PDF

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Preethi Shankar<sup>1</sup> , Dinesh Premavathy<sup>2</sup> , Preetha S  
5364-5371

 PDF

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5372-5379

 PDF

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Baala vignesh.A<sup>1</sup> , Revathi Duraisamy<sup>2</sup> , Ravindra Kumar Jain.M<sup>3</sup>  
5380-5387

 PDF

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Shalini Sathiyamoorthy<sup>1</sup> , Revathi Duraisamy<sup>2</sup>

5388-5394

 PDF

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5395-5405

 PDF

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Nashwah Hinaz, Pradeep D<sup>2</sup> , Ashok V<sup>3</sup>

5406-5413

 PDF

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B.Sushma<sup>1</sup> , K. R. Don<sup>2</sup> ,

5414-5420

 PDF

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Thiviya Raaj<sup>1</sup> , Vignesh Ravindran<sup>2</sup> , Sri Sakthi D<sup>3</sup>

5421-5426

 PDF

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5427-5436

 PDF

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G L Gokul<sup>1</sup> , Balaji Ganesh S<sup>2</sup> , K. R. Don<sup>3</sup>

5437-5442

 PDF

## **Awareness about Face Bow and its Types among Dental Professionals- A Questionnaire Based Survey**

Venkatesh Kommi<sup>1</sup> , Reethega L<sup>2</sup> , Keerthi Sasanka.L<sup>3</sup> , Venkatesh Kommi<sup>4</sup> , Geetha.R.V<sup>5</sup> , Dhanraj Ganapathy<sup>6</sup>  
5443-5450

 PDF

## **Prevalence and Assessment of Periapical Lesions in Subjects Reporting to a Private Teaching Hospital, Chennai,India : A Retrospective Study**

Chris Noel Timothy<sup>1</sup>, Pratibha Ramani  
5451-5461

 PDF

## **Assessment and Study of Types of Finish Lines in Tooth Preparation - An Observational Study**

Vigneshwaran Ravichandran<sup>1</sup> , Manjari Chaudhary<sup>2</sup>  
5462-5468

 PDF

## **Trends in Prosthodontics - A Review**

Pratheebha C<sup>1</sup> , Keerthi Sasanka<sup>2</sup> , Gifrina Jayaraj<sup>3</sup> , Visalakshi Ramanadhan<sup>4</sup> , Dhanraj Ganapathy<sup>5</sup>  
5469-5476

 PDF

## **Review on Caries Preventive Effect of Fluoride Toothpaste**

Aldrin Joshua A<sup>1</sup> , Keerthi Sasanka L<sup>2</sup> , Gifrina Jayaraj<sup>3</sup> , Dhanraj Ganapathy<sup>4</sup>  
5477-5485

 PDF

## **Orthodontics and Smile Correction in Teenagers-A Survey**

Shifa Jawahar Ali<sup>1</sup> , L. Keerthi Sasanka<sup>2</sup> , Visalakshi Ramanadhan<sup>3</sup> , Dhanraj Ganapathy<sup>4</sup>  
5486-5493

 PDF

## **Awareness of Oral Hygiene among Dental Students-A Survey**

Pravalika<sup>1</sup> , Keerthi Sasanka<sup>2</sup> , Gifrina Jayaraj<sup>3</sup> , Dhanraj Ganapathy<sup>4</sup>  
5494-5502

 PDF

## **Awareness on Alzheimer's Diseases among College Students**

Lekha.D1 , Vishnu Priya V2 , Hannah.R3 , Gayathri R4  
5503-5510



### **Knowledge on Emergency Management of Avulsed Tooth among Dental Students**

P. S. Thana Lakshme1 , K. R. Don2 , V. Vishnu Priya3  
5511-5521



### **Retrospective Study on Patient Compliance Following Tooth Extraction**

Rachel Paul1, Pradeep D2 , Deepa Gurunathan3  
5522-5529



### **Awareness on Oral Hygiene and Practices among General Population**

Vignesh.S1 , Archana Santhanam2  
5530-5537



### **Prevalence of Space Maintainers as A Treatment Modality in Maxillary Arch among Children between the Age Group of 6 to 12 Years- A Retrospective Study**

Cinthura.C1 , Ganesh Jeevanandam2 , Arvina Rajasekar3 ,  
5538-5545



### **Incidence of Non Habit Associated Oral Squamous Cell Carcinoma among Patients in a Private College Hospital - A Retrospective Study**

M. Sagana1 , Pratibha Ramani2 , M. Jeevitha3  
5546-5552



### **Prevalence of Dental Plaque among Adults Attending Private Dental College in Chennai City- A Descriptive Study**

Swetaa.A1 , Jayashri Prabakar2, Jessy P  
5553-5560



### **Prevalence and Distribution of Unerupted/Impacted Teeth Among Individuals 18-25 Years of Age Visiting Outpatient Department of Private Dental College and Hospital in Chennai, India**



C.S. Yuvashree1 , Pratiba Ramani2

5561-5569



### **Prevalence of Angular Bone Defects in Chronic Periodontitis Patients with and without Systemic Diseases**

Niveda Rajeshwaran1 , Arvina Rajasekar2

5570-5576



### **Assessment of Postoperative Obturation Quality of Retreatment Cases - A Retrospective Study**

Jerry Jose1 , Subash Sharma2 , Surendar Sugumaran

5577-5584



### **Prevalence of Kennedy Classification in Partially Edentulous Patients - A Retrospective Study**

Tulsani Minal Gopal, Subhashree. R

5585-5591



### **Prevalence of Vitality in Fixed Partial Denture Done by the Undergraduates - A Retrospective Study**

Tulsani Minal Gopal1 , Subhashree. R2

5592-5600



### **Dentists' Preference of Pulp Capping Agent for Indirect Pulp Capping in Primary and Permanent Molars - An Observational Study**

Maria Anthonet Sruthi1 , EMG Subramanian2 , Vignesh Ravindran

5601-5610



### **Awareness about Common Disinfection Procedures among Undergraduate Students in Clinics**

S.Santhosh Bala1 , N.P Muralidharan2 , Vivek Narayan3

5611-5618



### **Evaluation of Pain in Relation to Root Canal Treated Teeth Having Instrument Separation-A Retrospective Analysis**

Nur Qistina Binti Ahmad Fauzi<sup>1</sup> , Raghu Sandhya<sup>2</sup> , Gurunathan Deepa<sup>3</sup>  
5619-5625

 PDF

### **Prevalence of Cardiovascular Diseases among Complete Denture Patients**

Chaitanya Shree.P<sup>1</sup> , V. Rakshagan<sup>2</sup> , Subhashree.R<sup>2</sup>  
5626-5632

 PDF

### **Prevalence of Attrition, Abrasion, Erosion and Abfraction Among Patients Visiting A Private College Hospital in Chennai - A Retrospective Study**

Bharathi R<sup>1</sup> , Senthil Murugan P<sup>2</sup> , Senthil Murugan P<sup>3</sup>  
5633-5642

 PDF

### **Prevalence and Age Related Risk of Three Clinical Variants of Aphthous Stomatitis: a Retrospective Study**

Kethiswar Raj<sup>1</sup> , Jayanth Kumar Vadivel<sup>2</sup> , Vinay Sivaswamy<sup>3</sup>  
5643-5649

 PDF

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Fahmida Binti Abd Rahman<sup>1</sup> , Mahesh<sup>2</sup> , T.N.Uma Maheshwari<sup>3</sup>  
5650-5657

 PDF

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P. Keshaav Krishnaa<sup>1</sup> , Manjari Chaudhary<sup>2</sup>  
5658-5666

 PDF

### **Prevalence of Vesiculobullous Lesions: A Hospital Based Retrospective Study**

Pranati T<sup>1</sup> , Manjari Chaudhary<sup>2</sup> , Adimulapu Hima Sandeep<sup>3</sup>  
5667-5678

 PDF

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Ramvihari Thota<sup>1</sup> , Senthilnathan Periasamy<sup>2</sup> , Mahathi N<sup>3</sup>

5679-5684

 PDF

### **An Assessment of Complete Denture Changes from Try in to Clinical Insertion- A Retrospective Study**

Sai Sandhya.T1 , Mahathi Neralla2 , Suresh Venugopalan3

5685-5690

 PDF

### **Incidence of Pocket Depth More than 3 MM in Maxillary Premolars and Molars with a Class II Restoration**

Md Sohaib Shahzan, Adimulapu Hima Sandeep

5691-5698

 PDF

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Monica Antony1, Dhanraj Ganapathy2, Leelavathi3

5699-5705

 PDF

### **Soft Tissue Upper Lip Thickness in Different Skeletal Malocclusion Subjects -A Comparative Cephalometric Study**

S.Abirami, Ravindra Kumar Jain

5706-5712

### **Evaluation of the Lip-Tooth Relation in Various Growth Patterns- A Cephalometric Study**

Akriti Tiwari1 , Ravindra Kumar Jain2

5713-5718

 PDF

### **Analysis of Root Stumps in Middle Aged Group**

Sarojini Ramya Pillay1 , Dhanraj Ganapathy2 ,

5719-5726

 PDF

### **Analysis of Class III Skeletal Malocclusion that Require Orthognathic Surgery for Correction as Compared to Camouflage Treatment - A Retrospective Study**

Swapna Sreenivasagan1 , Aravind S Kumar2 , SriRengalakshmi3

5727-5734

 PDF

### **Assessment of Most Common Furcation Site among Smokers with Chronic Periodontitis**

Srijan Sunar<sup>1</sup> , Balaji Ganesh S<sup>2</sup> , SS Raj<sup>3</sup>  
5735-5743

 PDF

### **Evaluation of the Knowledge Attitude and Practice of Management Skills in Laboratory Set Up among Undergraduate Dental Students**

Deepa Viswasini. R<sup>1</sup> , Hannah. R<sup>2</sup> , Abilasha R<sup>3</sup> , Pratibha Ramani<sup>4</sup>, Gheena S<sup>3</sup>  
5744-5751

 PDF

### **Assessment of Knowledge and Awareness on Various Precautionary Measures one Must Follow While Treating Patients During Coronavirus Pandemic among Dental Practitioners - A Questionnaire based Survey**

Shunmugam Kumar Mangal.C<sup>1</sup> , Anjaneyulu K<sup>2</sup> , Vignesh Ravindran<sup>3</sup>  
5752-5759

 PDF

### **Analysis of Prevalence of Oral Squamous Cell Carcinoma in Patients with History of Chronic Irritation of Oral Tissues - A Retrospective Study**

Suvarna K<sup>1</sup> , Abilasha.R<sup>2</sup> , Gheena.S<sup>3</sup> , Pratibha Ramani<sup>4</sup>  
5760-5768

 PDF

### **Association of Retn (Rs3219175) Polymorphism with Ossc Progression and Development - An In Silico Study**

Monica.K<sup>1</sup> , J. Vijayashree Priyadharsini<sup>2</sup> , Gheena. S<sup>3</sup> , Pratibha Ramani<sup>4</sup> , Abhilasha. R<sup>5</sup> ,  
5769-5776

 PDF

### **Prevalence of Posterior Cross Bite in Children Visiting a University Hospital in Chennai - A Retrospective Study**

Srinisha Marimuthu<sup>1</sup> , Geo Mani<sup>2</sup> , Hemavathy Muralidoss<sup>3</sup>  
5777-5785

 PDF

### **Awareness on the Use of Hand Sanitizer and Face Mask in Disease Spread- A Survey among College Students**

John Francis<sup>1</sup> , V.Vishnu Priya<sup>2</sup> , Lavanya Prathap<sup>3</sup>  
5786-5793



### **Knowledge and Awareness of Various Dental Biomaterials Used In Pediatric Patients - A Survey**

Aleena Alex<sup>1</sup>, Balaji Ganesh S<sup>2</sup> , Lavanya Prathap<sup>3</sup>  
5794-5802



### **Assessment of Knowledge, Attitude and Practice based Survey on the Use of Ultrasonics in Endodontics among Practitioners**

Mulumoodi Rama Sowmya<sup>1</sup> , Pradeep.S<sup>2</sup> , Adimulapu Hima Sandeep<sup>3</sup>  
5803-5812



### **Association of Age And Gender Distribution of Patients Reported with Open Apex in Maxillary Central Incisor**

Balaji V<sup>1</sup> , Surendar Sugumaran<sup>2</sup>  
5813-5820



### **Choice of Initial Alignment Archwires in Moderate to Severe Crowding in Patients Treated with Damon and MBT Systems: A Retrospective Study**

Anjali Anna Thomas<sup>1</sup> , Remmya Mary Varghese<sup>2</sup> , Remmya Mary Varghese<sup>3</sup>  
5821-5825



### **Oral Hygiene in Patients with Fixed Partial Dentures**

A.Alagu Rathi Bharathi<sup>1</sup> , M.SivaKumar<sup>2</sup>  
5826-5831



### **Analysis Into Extra Oral Features of Completely Edentulous Patients Pre and Post Complete Denture Treatment**

Ashwin Kumar.S.P<sup>1</sup> , Suresh Venugopalan<sup>2</sup> , Sreedevi Dharman<sup>3</sup>  
5832-5838



### **Hypertension and Complete Denture Therapy - A Retrospective Study**

Chithralekha B1 , Revathi Duraisamy2 , Madhulaxmi M3  
5839-5847

 PDF

### **Association of Clinical Appearance with Histopathological Features in Oral Squamous Cell Carcinoma**

Varusha Sharon Christopher1 , Hannah R2, Manish Ranjan3  
5848-5855

 PDF

### **Prevalence of HIV and Hepatitis-B in the Out Patient Department of a Private Dental College Hospital Chennai, India**

Ramya G1 , Pratibha Ramani2 , Jeevitha M3  
5856-5863

 PDF

### **Utilization of Sealants and Conservative Adhesive Resin Restoration for Caries Prevention by Dental Students**

Lakshmi Lakshmanan1 , Vignesh Ravindran2 , Vignesh Ravindran3, EMG Subramanian4  
5864-5870

 PDF

### **Patients Reporting on Appointment Date After Access Opening For Further Treatment to Dental Hospital - A Cross Sectional Study**

KrishnaKanth Jaju1 , Iffat Nasim2 , J.Mahalakshmi3  
5871-5878

 PDF

### **A Questionnaire based Evaluation of the Awareness among Dental Practitioners on Minimally Invasive Approach for Superficial Enamel Stains**

Rukhsaar Gulzar, Subash Sharma2 , Mahalakshmi3, Rukhsaar Gulzar, Subash Sharma2 , Mahalakshmi3  
5879-5887

 PDF

### **Toxic Biological Effects Associated with Materials Used in Prosthodontics- A Survey**

G.Swetha1 , Venkatesh Kommi2 , Keerthi Sasanka.L3 , Leslie Rani. S4  
5888-5898

 PDF

### **Assessment of Partial Edentulism Based on Kennedy's Class III Classification**

Palak Mayur Shah<sup>1</sup> , Revathi Duraisamy<sup>2</sup> , Manjari Chaudhary  
5899-5904

 PDF

### **Evaluation of Mandibular Third Molar Extractions Performed By Intra Alveolar Method as Opposed to Transalveolar Method**

Aniruddh Menon<sup>1</sup> , Balakrishna.R.N<sup>2</sup> , Jayanth Kumar Vadivel<sup>3</sup>  
5905-5913

 PDF

### **Analysis of the Association between Fast Food on Oral Health and Hygiene - A Survey**

Baala vignesh. A<sup>1</sup> , Karthik Ganesh Mohanraj<sup>2</sup> , Ravindra Kumar Jain<sup>3</sup>  
5914-5921

 PDF

### **Transalveolar Extractions in Different Age Groups**

Vivek.D.Menon<sup>1</sup> , M R Muthusekhar<sup>2</sup> , Dinesh Prabu<sup>3</sup>  
5922-5928

 PDF

### **Incidence of Pathologies Associated with Impacted Mandibular Premolars and Third Molars**

Yandeti Srinivasulu<sup>1</sup> , Abdul Wahab<sup>2</sup> , Senthil Murugan.<sup>3</sup>  
5929-5937

 PDF

### **Prevalence of Oral Lichen Planus and Assessment of Factors Associated with It- A Retrospective Study**

Sanjana Devi<sup>1</sup> , Revathi Duraisamy<sup>2</sup>  
5938-5946

 PDF

### **Prevalence of Angular Cheilitis and Assessment of Factors Associated with It - A Retrospective Study**

Sanjana Devi<sup>1</sup> , Revathi Duraisamy<sup>2</sup>  
5947-5954

 PDF

### **Reliability of Profile Photography for Determining Growth Pattern and Sagittal Jaw Relationship in Different Classes of Malocclusions**

Prasanna T.R1 , Navaneethan.R2 , Sri Rengalakshmi3 , Prasanna Arvind T.R.4  
5955-5963



### **Analysis of the Cost Associated with the Treatment of Early Childhood Caries in Children Aged 2-6 Years**

Pavithiraa Sankar1 , Deepa Gurunathan2 , Vignesh Ravindran3  
5964-5972



### **Evaluation of Primary Central Incisors Requiring Restoration and Pulpectomy in 2 to 6 Year Old Children-An Observational Study**

Pavithiraa Sankar, Vignesh Ravindran  
5973-5980



### **Association between Composite Restoration in Anterior Teeth and Secondary Caries**

Nurul Syamimi binti Mohd Azlan Sunil1 , Iffat Nasim2 , Revathi Duraisamy3  
5981-5988



### **Evaluation of Direct/Indirect Pulp Capping Procedures Requiring Root Canal Therapy**

Nurul Syamimi binti Mohd Azlan Sunil1 , Revathi Duraisamy2 , Iffat Nasim  
5989-5996



### **Gingival Health Status in Children with and without Cleft Lip and Palate: A Case Control Study**

Kausalyah Krisna Malay1 , Vignesh Ravindran2, Jayanth Kumar3  
5997-6003



### **Association of the Depth of Invasion with Lymph Node Metastasis in Oral Squamous Cell Carcinoma Patients - A Retrospective Study**

Aklesha Behera1 , Hannah.R2 , Abilasha Ramasubramanian3 , Pratibha Ramani4 ,  
6004-6011



### **Correlation Between Highly Sensitive C-Reactive Protein Level in Cases of Preeclampsia with or without Intrauterine-Growth Restriction**



Alaa Mohammed Sadiq<sup>1</sup> , Canary Muhamed Hussein<sup>2</sup> , MaithmGhaly Yousif<sup>3</sup> and RausamJassim Mohammed<sup>4</sup>

6012-6016



### **Renal Function Tests in Women with Preeclampsia with and without Intrauterine Growth Restriction**

Alaa M. Sadeq<sup>1</sup>, Fawz A. Mohammed<sup>2</sup> , Canary Muhammed Hussein<sup>3</sup> , Maitham G. Yousif<sup>4</sup>

6017-6024



### **5-Years Prevalence Study of HBV and HCV infections in Babylon Province: Forensic Helping Data**

Kadhim Jawad Mohammed<sup>1</sup> , Hussein O.M. Al-Dahmoshi<sup>2</sup>

6025-6032



### **Cytotoxic Effects of Al-Yohedia Municipal Waste Water**

Wassan M. A. Al-Morshidy<sup>1</sup> , Hussein O.M. Al-Dahmoshi<sup>2</sup> , Ayad M. J. Al-Mammori<sup>3</sup>

6033-6041



### **Antibacterial Effect of Virgin and Refined Coconut Oils on Pathogenic Bacteria: A Review**

Manar Saad Hussain<sup>1</sup> , Farah Tareq Al-Alaq<sup>2</sup> , Noor Salman Al-Khafaji<sup>2</sup> , Hussein O. M. Al-Dahmoshi<sup>2</sup>

6042-6048



### **Recent Advancements in Pulp Testing Devices**

Akansha Kishen<sup>1</sup> , Anjaneyulu<sup>2</sup> , N. P. Muralidharan<sup>3</sup>

6049-6059



### **Knowledge and Awareness of Basic Life Support (BLS) among Dental Students**

Yoshita Guntupalli<sup>1</sup> , K. R. Don<sup>2</sup> , Krthik Ganesh Mohanraj<sup>3</sup>

6060-6070



### **Prevalence of Anemia among Women Patients Visiting A Dental Hospital**

Jagadheeswari Ramamoorthy<sup>1</sup> , Gifrina Jayaraj<sup>2</sup> , Geo Mani<sup>3</sup>

6071-6079

 PDF

### **An Estimation of Haemoglobin Levels in Children with Early Childhood Caries- A Retrospective Study**

Ranjith Raj VPRB1 , Geo Mani2

6080-6088

 PDF

### **Novel Gene Mutations Associated with Crouzon Syndrome**

D.Angel Fastina Mary

6089-6094

 PDF

### **Knowledge, Attitude, Practice and Barriers Towards Research Among Dental Undergraduates: A Questionnaire Based Survey**

Sudarsan R1 , Vignesh Ravindran2 , Anjaneyulu K3

6095-6103

 PDF

### **An Evaluation of Marginal Fit and Aesthetics in FPD Is Achieved During Graduate Training**

M. P. Induja1 , Suresh V2 , Surendar S3

6104-6111

 PDF

### **Awareness on Ebola Virus among Undergraduate Dental Students in a Private Dental Institution, Chennai - A Questionnaire Survey**

S.Divyashri1 , V. Vishnu Priya2 , R.Gayathri3

6112-6119

 PDF

### **Retrospective Study of Pattern of Mandibular Third Molar Impaction in South Indian Population**

Gayathri Karthikeyan1 , Pradeep Dhasarathan2, Jaiganesh Ramamurthy3

6120-6130

 PDF

### **Incidence of Dry Socket after Extraction Done in Private College and Hospital-A Retrospective Study**

J.S Thaslima Nandhini1 , Pratibha Ramani2

6131-6137



### **The Assessment of Longevity and Efficacy of Different Types of Splints Used in Periodontal Therapy: A Single Center Epidemiological Retrospective Study**

Siddharth Narayan<sup>1</sup> , Priya Lochana Gajendran<sup>2</sup> , Arvina R<sup>3</sup>

6138-6146



### **Incidence of Impacted Canines in Maxillary Arch**

Hanshika Ravi<sup>1</sup> , Dinesh Prabu<sup>2</sup>

6147-6153



### **Most Frequently Extracted Primary Tooth in Maxillary Arch of Children Visiting A University Hospital In Chennai: A Retrospective Study**

Azima Hanin S.M<sup>1</sup> , Geo Mani<sup>2</sup> , Hemavathy Muralidoss<sup>3</sup>

6154-6161



### **Deleted**

6162-6168

### **Semiological Discourse Analysis of the Editorial Cartoons of International Newspapers on COVID-19**

Mutaz Mohammad Alkhresheh

6169-6179



### **Deleted**

6180-6186

### **Deleted**

6187-6195

### **Deleted**

6196-6202

## Deleted

6203-6210

### **The Relationship between Believing in Resurrection (Ma'ad in Islam) and Self-efficacy in Mothers of Children with Thalassemia**

Elham Hesari<sup>1</sup> , Fatemeh SHeikhy<sup>2</sup> , Nadia Kolbadinezhad<sup>3</sup> , Hamid Hojati<sup>4</sup>

6211-6217



### **Surgery for Gastric Adenocarcinoma with Hospital Stay: A Prospective Study**

Avinash Rinait<sup>1</sup> , Yashwant R. Lamture<sup>2</sup> , Pulavarty Prateek<sup>3</sup> , Dilip Gode<sup>4</sup>

6211-6216



### **Study between Fracture Fixation of Patella with Cerclage and Tension Band Wiring Technique**

Aakash Sharma<sup>1</sup> , Nitin Samal<sup>2</sup> , Kunal Saoji<sup>3</sup> , Vasant Gawande<sup>4</sup>

6217-6221



### **Critical Review of Asthikshaya and its Management: Ayurvedic Perspective**

Shilpa M. Gabhane<sup>1</sup> , Rajiv J. Mundane<sup>2</sup> , Geeta V. Sathavane<sup>3</sup> , Vasant Gawande<sup>4</sup>

6222-6227



### **A Self-Controlled Prospective Study between Methylprednisolone and Dexamethasone Inter-Laminar Epidural Steroid Injection in Radicular Pain for Prolapse Intervertebral Disc in Lumbar Vertebrae**

Ashutosh Parate<sup>1</sup> , Nitin Shamal<sup>2</sup> , Vasant Gawande<sup>3</sup> , Kunal Saoji<sup>4</sup> , Ankit Mittal<sup>5</sup>

6228-6232



### **A Rare Case Report of Isolated Tuberculous Caecal Perforation Presented as Acute Appendicitis**

Lamture Y.R.<sup>1</sup> , Gode Dilip<sup>2</sup> , Aditya Mundada<sup>3</sup>

6233-6236



### **A Rare Presentation of Systemic Lupus Erythematosus with Juvenile Dermatomyositis**

Ashish Varma<sup>1</sup> , Sneh Kumar<sup>2</sup> , Rupali Salve<sup>3</sup> , Anjali Kher<sup>4</sup> , Jayant Vagha<sup>4</sup> , Sachin Damke<sup>5</sup>

6237-6240

 PDF

### **A Prospective Study of Etiology and Clinical Presentation of Gastric Adenocarcinoma**

Avinash Rinait1 , Yashwant R. Lamture2 , Pulavarty Prateek3 , Dilip Gode4  
6241-6245

 PDF

### **Study of Functional Outcome of Calcaneal Fractures with Percutaneous Cancellous Screws Fixation in Central Rural India**

Vasant Gawande1 , Ashwin Chauhan2 , Kunal K. Saoji3 , Ankit Mittal4 , Suvarn Gupta5  
6246-6252

 PDF

### **Principles of Agnikarma and its Advances for Shonitsthapana (Hemostasis)**

Fadanvis Prafulla1 , Jaiswal Reena2 , Sharma Harshala3 , Vasant Gawande4  
6253-6258

 PDF

### **Cardiovascular Risk Assessment and Profiling among Employees of Tertiary Care Rural Hospital: Central India**

Anjalee Chiwhane1 , Gajendra Agrawal2 , Joel Joy Thomas3 , Saurbh Hadke4  
6259-6265

 PDF

### **To Determine the Role of Clonidine as an Adjuvant to Intrathecal Bupivacaine in Patients Undergoing Lower Abdominal Surgery**

Priyanka Deshmukh1 , Pratibha Deshmukh2 , Sohal Parate3 , Aruna Chandak4  
6266-6272

 PDF

### **Psychosocial Impact of Lock Down among College Students in Nagpur**

Vaishali D. Tendolkar1 , Shaini Suraj2 , Harshala Rajurkar3  
6273-6278

 PDF

### **Significance of Hematological Parameters in Uncomplicated Diabetes Mellitus**

Neha Bhatt1 , Pratibha Dawande2 , Obaid Noman3 , Sweta Bahadure3 , Arvind Bhake4  
6279-6285

 PDF

## **Intubating Conditions Comparison, After Administration of Propofol with Muscle Relaxant and Profol without Muscle Relaxant-An Observational Study**

Parag Sabale<sup>1</sup> , Pratibha Deshmukh<sup>2</sup> , Swapnil Meshram<sup>3</sup> , Nandkishor Bankar<sup>4</sup> , Ashok Choudhary<sup>5</sup>  
6286-6291



## **A Study of Semen Parameter Contributing Infertility in Vidarbha Region**

Akash More<sup>1</sup> , Brij Raj Singh<sup>2</sup> , Deepti Shrivastava<sup>3</sup> , Ujwal Gajbe<sup>4</sup> , Nandkishor Bankar<sup>5</sup>  
6292-6296



## **Outcome of Infero Temporal Suture Less and Glue Free Conjunctival Autograft in Patients with Pterygium at a Tertiary Care Hospital of Vidarbha Region**

Chetan Saoji<sup>1</sup> , Sonal Muley<sup>2</sup> , Sachin Daigavane<sup>3</sup>  
6297-6302



## **Incidence of Post Dural Puncture Headache in Our Set Up with Quincke Spinal Needle: An Observational Crossectional Study**

Swapnil Meshram<sup>1</sup> , Pratibha Deshmukh<sup>2</sup> , Parag Sabale<sup>1</sup> , Nandkishor Bankar<sup>3</sup> , Vijay C. Chandak  
6303-6309



## **Effectiveness of Mind Body Intervention on Subfertile Females: An Approach to Accesses Oocyte Quality**

Anupama Sawal<sup>1</sup> , Ujwal Gajbe<sup>2</sup> , Brij raj Singh<sup>3</sup> , Akash More<sup>4</sup> , Nandkishor Bankar<sup>5</sup>  
6310-6314



## **Relevance of MCV and Knuckle Pigmentation in Early Diagnosis of Vitamin B12 Deficiency in Children**

Rohini Gulhane<sup>1</sup> , Swarnika Barde<sup>2</sup> , Swati Maldhure<sup>3</sup> , Neelam Sukhsohale<sup>4</sup> , Amit Gulhane  
6315-6319



## **A Prospective Clinical Study to Evaluate Recurrence and Infection in Laparoscopic Hernia in a Tribal Area**

Gode Dilip<sup>1</sup> , Minaxi Yeola<sup>2</sup> , Lamture Y.R.<sup>3</sup>  
6320-6325



## **Glimpses of Eugenics in Ayurveda WSR to Preconceptional and Antenatal Remedies**

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6326-6330



PDF

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6331-6335



PDF

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6336-6339



PDF

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6340-6346



PDF

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6347-6350



PDF

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6351-6357



PDF

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6358-6363



PDF

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6364-6368

 PDF

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Ujwal Gajbe<sup>1</sup> , Brij Raj Singh<sup>2</sup> , Nandkishor Bankar<sup>3</sup>  
6369-6373

 PDF

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Anuja Nagrare<sup>1</sup> , Sonali Wairagade<sup>2</sup> , Tanvi Wairagade<sup>3</sup> , Praful Patil<sup>4</sup>  
6374-6380

 PDF

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Swati Tikale<sup>1</sup> , Krutika Umate<sup>2</sup> , Madhulika Tiwari<sup>3</sup> , C. Mahakalkar<sup>4</sup>  
6381-6385

 PDF

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Archana Dhok<sup>1</sup> , Ranjit Sidram Ambad<sup>2</sup> , Sanjay Deshpande<sup>3</sup> , Ninad Nagrale<sup>4</sup>  
6386-6390

 PDF

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6391-6395

 PDF

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Dhruba Hari Chandi<sup>1</sup> , Praful Patil<sup>1</sup> , Nandkishor Bankar<sup>2</sup> , Smita Damke<sup>3</sup> , Karan Jain<sup>4</sup>  
6396-6401

 PDF

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6402-6407

 PDF

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6408-6411

 PDF

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6412-6415

 PDF

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6416-6419

 PDF

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6420-6425

 PDF

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Pranjali Parikshit Muley<sup>1</sup> , Parikshit Ashok Muley<sup>2</sup> , Komal Ajay Meshram<sup>3</sup>

6426-6429

 PDF

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6430-6435

 PDF

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6436-6439

 PDF

## **Role of Vrishyashatavarighrit on Sukrakshaya (Oligospermia) by Studying Siddhant**

Snehal V. Kukade1, Bhokardankar Prashant S 2, Milind Rampurkar3, Abhishek Joshi4

6440-6445



## **Review on Effect of Sarshap Taila and Til Taila in Prevention of Atiyog of Karnendriya with Special Reference to Karnanaad**

Vivek V. Dhongdi1 , Premkumar Badvaik2 , Shailesh Nagpure3

6446-6449



## **Clinical Outcome of Supracondylar Femoral Fractures Managed Locking Plate Osteosynthesis in a Rural Hospital**

Shashank Jain1 , Sunil Nikose2 , Sohael Khan3 , Suvarn Gupta3 , Ankush Mohabey4

6450-6456



## **Investigate the Effect of Role Play on the Skill among 2nd Basic B.Sc. Students if SRMMCON Regarding Selected Community Procedure**

Charlotte Paul1, Nikita Bhokare2, Swanand Pathak3

6457-6460



## **Domestic Violence: A Silent Panendemic on the Rise During Covid-19**

Shaini Suraj1 , Vaishali D. Tendolkar2 , Rupali Sarode3

6461-6463



## **Analysis of Nidan (Etiological Factors) Of Metabolic Syndrome.**

Sneha Chandankar1 , Vaishali Kuchewar2

6464-6470



## **A Review of Advance Directives As Per Mental Health Care Act 2017**

Sharjeel Khan1 , Shadma Quazi2 , Ranjit Ambad3 , Ragini Patil4 , Ajinkya S. Ghogare3

6471-6475



**Deleted**

6476-6481

### **Efficacy of Median Nerve Mobilization Versus Upper Trapezius Muscle Stretching on Extensibility of Median Nerve in Young Healthy Individuals: A Randomized Controlled Trial**

Shiril R. Nagarkar<sup>1</sup>, Varsha S. Nagarkar<sup>2</sup>, Divya V. Bagaria<sup>3</sup>, Vasant Gawande<sup>4</sup>

6482-6487



### **Simultaneous Ipsilateral Distal Interphalangeal and Metacarpophalangeal Dislocation of the Fifth Finger: A Rare Case Report**

Suvarn Gupta<sup>1</sup>, Vasant Gawande<sup>2</sup>, Ankush Mohabey<sup>3</sup>, Kunal Saoji<sup>3</sup>, Ashwin Chavan<sup>4</sup>

6488-6491



### **Current Treatment Approaches for Pandemic Corona Virus Disease 2019 (Covid 19)**

Jagdish Nikose<sup>1</sup>, Swati Gaikwad<sup>2</sup>, Sanjana Gaikwad<sup>2</sup>, Ravi Kalsait<sup>3</sup>, Abhishek Joshi<sup>4</sup>

6492-6495



### **Selfcare Management and Strategies of Drug Development for COVID 19**

Sanjana Gaikwad<sup>1</sup>, Swati Gaikwad<sup>1</sup>, Mayuri Deshmukh<sup>1</sup>, Jagdish Nikose<sup>1</sup>, Ravi Kalsait<sup>1</sup>, Praful Patil

6496-6500



### **Physiological Effect of Yogic Procedures on Pranvahastrotas (Respiratory System) in Healthy Individuals**

Namrata B. Chouragade<sup>1</sup>, Bhushan Mhaiskar<sup>2</sup>, Snehal Kukade<sup>3</sup>, Swapnil S. Bhirange<sup>4</sup>

6501-6507



### **The Association Between Flat Feet and Body Mass Index in Various Age Groups**

Milind Giridhar Kahile<sup>1</sup>, Neha Arun Deshmukh<sup>2</sup>, Parikshit Muley<sup>3</sup>, Mihika Suryawanshi<sup>4</sup>

6508-6513



### **Experimental Evaluation of Talisadi Suspension for Anti-Histaminic and Anti-Inflammatory Activities in Animal Models**

Poonam Madan<sup>1</sup>, Bharat Rathi<sup>2</sup>, Shailesh Nagpure<sup>3</sup>, Raunak Kotecha

6514-6518

 PDF

## **Attendance and Academic Performance of First BAMS Students in Subject Rachana Sharir: A Retrospective Cohort Study**

Sanjivani Jadhao<sup>1</sup> , Pooja Jadhao<sup>2</sup> , Anita Wanjari<sup>3</sup> , Madhulika Tiwari<sup>4</sup> , Abhishek Joshi<sup>5</sup>  
6519-6525

 PDF

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6526-6530

 PDF

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6531-6535

 PDF

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6536-6540

 PDF

## **A Clinical Study of Perinatal Outcome and its Relation to Oligohydramnios in Pregnant Women at Term**

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6541-6545

 PDF

## **A Prospective Comparative Study to Assess-Post Operative Analgesic Effect of Intraperitoneal Instillation of Bupivacaine with Dexmedetomidine to Bupivacaine with Tramadol in Patients Undergoing Laparoscopic Cholecystectomy**

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6546-6551

 PDF

## **Review Article on Etiopathogenesis (Samprapti) of Tamak Shwasa W.S.R. Kriyakala**

Geeta Vishwanath Sathavane<sup>1</sup>, Shilpa M. Gabhane<sup>2</sup>, Seema Thakre<sup>3</sup>, Dhiraj A. Zade<sup>4</sup>, Prashant Bhokardankar<sup>5</sup>, Samarth Shukla<sup>6</sup>

6552-6559

 PDF

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6560-6564

 PDF

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6565-6571

 PDF

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6572-6578

 PDF

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6579-6584

 PDF

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6585-6588

 PDF

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Koshish B. Gabhane<sup>1</sup> , Arshiya M. Khan<sup>1</sup> , Manish P. Desmukh<sup>2</sup> , Ashish B. Budhrani<sup>3</sup>

6589-6592

 PDF

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Ayushi Jain<sup>1</sup> , Dhruv Talwar<sup>2</sup> , Sunil Kumar<sup>3</sup>  
6593-6599



### **COVID-19 Pandemic: Inducing Pan-Hysteria and Panic Disorder; An Overview**

Arpita Aswinikumar Sahu  
6600-6604



### **COVID-19 Pandemic: A Positive Influence on Lifestyle, Economy, Administration & Environment; An Overview**

Kunal Dwari  
6605-6610



### **Gastric Outlet Obstruction: A Case Report**

Deepali Ghunrud<sup>1</sup> , Vaishali Tembhare<sup>2</sup> , Sheetal Sakharkar<sup>3</sup>, Manoj Patil<sup>4</sup>  
6611-6614



### **Thalassemia in Children: A Case Report**

Julie Vanlalsawmi<sup>1</sup> , Deeplata Mendhe<sup>2</sup> , Manoj Patil<sup>3</sup>  
6615-6617



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Pratiksha Thakare<sup>1</sup> , Ruchira Ankar<sup>2</sup> , Sonali Wavare<sup>3</sup> , Manoj Patil<sup>4</sup>  
6618-6622



### **Case Report on Systemic Lupus Erythematosus**

Hina Y. Rodge<sup>1</sup> , Bibin Kurian<sup>2</sup> , Manoj Patil<sup>3</sup>  
6623-6626



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Amruta V. Taksande<sup>1</sup> , Vaishali Taksande<sup>2</sup> , Manoj Patil<sup>3</sup>  
6627-6635

 PDF

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Vishal Bawankar<sup>1</sup> , Gaurav Sawarkar<sup>2</sup>

6636-6640

 PDF

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Deepata M. Mendhe<sup>1</sup> , Arti Lohakare<sup>2</sup> , Tejaswee Lohakare<sup>2</sup> , Komal Madankar<sup>2</sup> , Alweena Makasare<sup>2</sup> , Rohini Mathankar<sup>2</sup> , Manoj Patil<sup>3</sup>

6641-6645

 PDF

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6646-6652

 PDF

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Keshav Vijan<sup>1</sup> , Kiran Saoji<sup>2</sup>

6653-6657

 PDF

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Pooja R. Kasturkar<sup>1</sup> , Prajakta Nandeshwar<sup>2</sup> , Neha Mahabudhe<sup>2</sup> , Priyanka Mohite<sup>2</sup> , Priyanka More<sup>2</sup> , Sadhana Naik<sup>2</sup>

6658-6664

 PDF

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Sirjan Singh<sup>1</sup> , Samarth Shukla<sup>2</sup> , Sourya Acharya<sup>3</sup> , Neema Acharya

6665-6668

 PDF

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6669-6674

 PDF

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6675-6679

 PDF

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 PDF

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Sahebrao Kadam<sup>1</sup> , Shweta Parwe<sup>2</sup> , Milind Nisargandha<sup>3</sup>

6685-6690

 PDF

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 PDF

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 PDF

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6705-6708

 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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6722-6724

 PDF

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6725-6728

 PDF

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6729-6731

 PDF

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6732-6736

 PDF

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6737-6742

 PDF

## **Role of Frozen Section in Neck Dissection of Oral Cancer Patients**

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 PDF

## **A Study on Designing, Development and Testing of an Innovative External Jig for the Percutaneous Screw Fixation of the Acetabular Column Fractures**

Aditya Kekatpure<sup>1</sup>, Sandeep Shrivastava<sup>2</sup>, Aashay Kekatpure<sup>3</sup>, Sunil Nikose<sup>4</sup>, Kiran Saoji<sup>5</sup>, Ashish Bhagat<sup>6</sup>, Ulhas Dudhekar<sup>7</sup>

6749-6753

 PDF

## **Exploring the Etiological Evidences at Avascular Necrosis of Femoral Head by Investigative Approaches of Histopathology, Clinical Assessment and Radiology**

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6754-6758

 PDF

## **Role of Anion Gap as a Prognostic Indicator in Acute Coronary Syndrome (ACS)**

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6759-6763

 PDF

## **Comparative Evaluation of Effective Route of Administration of MASAM Oil in Vishwachi (Cervical Radiculopathy): A Study Protocol**

Shweta Parwe<sup>1</sup>, Vaishali Kuchewar<sup>2</sup>, Milind Nisargandha<sup>3</sup>, Manoj Patil<sup>4</sup>

6764-6768

 PDF

## **A Concise Review of Gingival Depigmentation Techniques and Re-Pigmentation**

Chitrika Subhadarsanee<sup>1</sup>, Prasad V. Dhadse<sup>2</sup>

6769-6775

 PDF

## Artificial Intelligence: A Dental Odyssey.

Gargi Nimbulkar<sup>1</sup> , Heena Keshwani<sup>2</sup> , Taniya Katakwar<sup>2</sup> , Kumar Gaurav Chhabra<sup>3</sup> , Shravani Deolia<sup>4</sup> , Amit Reche<sup>1</sup>

6776-6781



## Review on Covid-19 Disease: Its Outbreak and Current Status

Pankaj Wagh<sup>1</sup> , Saood Ali<sup>1</sup> , Ulhas Jadhav<sup>2</sup>, Babaji Ghewade<sup>3</sup> , Deepanshu Chawla<sup>4</sup>

6782-6788



## Role of Ayurveda with Multi modal Therapy in Prevention and Management of Autism Spectrum Disorders (ASD): The Whole System Approach

Renu Rathi<sup>1</sup> , Bharat Rathi<sup>2</sup>

6789-6795



## Nanotechnology in Periodontal Regeneration: A Review

Gargi Nimbulkar<sup>1</sup> , Priyanka Multani<sup>2</sup> , Khushbu Balsara<sup>2</sup> , Kumar Gaurav Chhabra<sup>3</sup> , Shravani Deolia<sup>4</sup> , Amit Reche<sup>1</sup>

6796-6801



## Tongue Prints: A Forensic Review

Gargi Nimbulkar<sup>1</sup> , Ruchita Patil<sup>2</sup> , Simran Nathani<sup>2</sup>, Sakshi Salve<sup>2</sup> , Kumar Gaurav Chhabra<sup>3</sup> , Shravani Deolia<sup>4</sup> Amit Reche<sup>1</sup>

6802-6806



## Impact of Covid 19 Pandemic on Tuerculosis

Deepanshu Chawla<sup>1</sup> , Pankaj Wagh<sup>2</sup> , SaoodAli<sup>2</sup>, Ulhas Jadhav<sup>3</sup> , Dr Babaji Ghewade<sup>4</sup>

6807-6810



## Case Report on Acute Lymphatic Leukemia

Nilam Raut<sup>1</sup> , Archana Mourya<sup>2</sup> , Manoj Patil<sup>3</sup>

6811-6814



## Case Report on Thalassemia in Children

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6815-6818

 PDF

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6819-6824

 PDF

### **The Effects of Multi Vitamins Supplementation in the Prevention of Preeclampsia**

Ranjit S. Ambad1 , Nandkishor Bankar2 , Lata Kanyal Butola3 , Minal Kalambe4 , Brij Raj Singh5

6825-6830

 PDF

### **Prevalence of Depression in College Students During Covid-19 Pandemic**

Samiksha Sathe1 , Kirti Thodge2 , Abhishek Joshi3

6831-6835

 PDF

### **Combination of Misoprostol with Transcervical Foley's Catheter Compared to Misoprostol Alone for Cervical Ripening at Term and Labour Induction in Tertiary Care Hospital: A Randomized Trial**

Minal Kalambe (Akare)1 , Mugdha L. Jungari2 , Priya Pratapan Nair2 , Dipti Shrivastava3

6836-6840

 PDF

### **Online Teaching in the Time of Covid 19-Effectiveness and Challenges**

Rucha R. Lohi1 , Nikita Dhahikar2 , Ranjit Ambad3 , Dhruva Hari Chandi4

6841-6844

 PDF

### **Estimation of stature from foot anthropometry in Central Indian population**

Karan Jain1 , Ninad Nagrale2 , Ranjit Ambad3 , Nandkishor Bankar4 , Swapnil Patond5

6845-6848

 PDF

### **Radiological Evaluation of Innominate Bone for Age Estimation: A Cross Sectional Study from Central India**

Ninad Nagrale<sup>1</sup> , Swapnil Patond<sup>2</sup> , Ranjit Ambad<sup>3</sup> , Nandkishor Bankar<sup>3</sup> , Karan Jain<sup>4</sup>  
6849-6852



### **Age Estimation among Adolescent Individuals from Fusion of Tri-Radiate Cartilage: A Radiological Study from Central India**

Ninad Nagrale<sup>1</sup> , Swapnil Patond<sup>2</sup> , Ranjit Ambad<sup>3</sup> , Nandkishor Bankar<sup>3</sup> , Karan Jain<sup>4</sup>  
6853-6856



### **Comparison between Immediate Effects of MET and Passive Stretching Techniques on Hamstring Flexibility in Patients with Hamstring Tightness: An Experimental Study**

Samiksha Sanjiv Sathe<sup>1</sup> , Tejal Rajandekar<sup>2</sup> , Kirti Thodge<sup>3</sup> , Vasant Gawande<sup>4</sup>  
6857-6862



### **Role of Prolactin and $\beta$ -hCG in Female Infertility**

Mahendra Dhabe<sup>1</sup> , Ranjit S. Ambad<sup>2</sup> , Archana Dhok<sup>3</sup>  
6863-6867



### **Chondroid Chordoma: An Uncommon Bone Neoplasm**

Anita B. Sajnar<sup>1</sup> , Neha Bhatt<sup>2</sup> , Obaid Noman<sup>3</sup> , Pratibha Dawande<sup>4</sup>  
6868-6870



### **Comparison between Immediate Effects of Post Isometric Relaxation and Reciprocal Inhibition Techniques on Hamstring Flexibility in Patients with Hamstring Tightness: An Experimental Study**

Samiksha Sanjiv Sathe<sup>1</sup> , Tejal Rajandekar<sup>2</sup> , Kirti Thodge<sup>3</sup> , Ashwin Chavhan<sup>4</sup>  
6871-6875



### **Prospective Analytical Study of Burn Deaths in Infant and Children Below 6 Year**

Tikendra Dewangan<sup>1</sup> , Rajesh Babulalji Ramteke<sup>1</sup> , Kalpesh C. Patani<sup>2</sup> , Ninad Nagrale<sup>3</sup> , Shailesh Nagpure<sup>4</sup>  
6876-6879



### **Effect of Exposure of Smartphone Use on Visually Evoked Potential (VEP) in Healthy Adults**

Swapnil Bhirange<sup>1</sup> , Alka Rawekar<sup>2</sup> , Avinash Taksande<sup>3</sup> , Komal Meshram<sup>4</sup>  
6880-6886



### **Metabolic and Cardiovascular Responses to External Weights During Treadmill Walk in Overweight Young Adults**

Swapnil Bhirange<sup>1</sup>, Dalia Biswas<sup>2</sup> , Avinash Taksande<sup>3</sup> , Prerana Agarwal<sup>1</sup>  
6887-6892



### **Assessment of the Financial Status in Adult Diabetic Patients Attending the Diabetic Clinic in Bankura Sammilani Medical College, Bankura**

Indu Padmey<sup>1</sup> , Karan Jain<sup>2</sup> , Arindam De<sup>3</sup> , Abhishek Joshi<sup>4</sup>  
6893-6899



### **Assessment and Comparison of Knowledge and Awareness about Hand Hygiene among Health Care Workers from PHC and Medical College**

Amit Pasari<sup>1</sup> , Pratibha Deshmukh<sup>2</sup> , Sweety Agrawal<sup>3</sup>  
6900-6904



### **Myositis Ossificans Traumatica of the Wrist in Elderly: A Description of an Unusual Case**

Suvarn Gupta, Pratik Kawde, Aashay Kekatpure, Sonal Karmore, Romil Rathi  
6905-6907



### **Prevalence of Different Resistance Pattern in Staphylococcal Aureus Isolates From Tertiary Care Hospital**

Dhruba Hari Chandi<sup>1</sup> , Dr. Nandkishor Bankar<sup>2</sup> , Ranjit Ambad<sup>3</sup> , Brij Raj Singh<sup>4</sup> , Karan Jain<sup>5</sup>  
6908-6912



### **A Unusual Case of Charcot's Arthropathy of Elbow Caused by Syringomyelia and Chiari Malformation Complicated with Scoliosis**

Suvarn Gupta<sup>1</sup> , Vasant Gawande<sup>2</sup> , Ankush Mohabey<sup>3</sup> , Kunal Saoji<sup>4</sup> , Ashwin Chauhan<sup>4</sup> , Rohit Bhandari<sup>5</sup>  
6913-6917



**Deleted**

### **Molecular study of antibiotic resistance Mycobacterium tuberculosis isolated from clinical samples in Baghdad city**

Heba Firas Saeed<sup>1</sup> , Muhammad Fulayyih Tarif<sup>2</sup>

6925-6932



### **Assessment of Risk Factors of Asthma in Health Institutions in Maysan Governorate, Iraq**

Ali Abdulwahed Abdul Jabbar<sup>1</sup> , Buthaina Ateyah Rashid<sup>2</sup>

6933-6938



### **Atherogenic Index of Plasma Levels in Iraqi Diabetic and Diabetic Nephropathy Patients**

Sulaiman M. Hasan

6939-6942



### **Evaluation of Serum Level of Serum Catalase and SOD as a Predictive Factors in Preterm Labor Patients**

Sura Zeki Salih<sup>1</sup> , Mutaz S. Ahmied<sup>2</sup> , Israa H. Abdulkareem<sup>2</sup>

6943-6948



### **Comparative Clinical Study of Role of Saraswatchurna in Apasmar**

Sonali Dilip Wairagade<sup>1</sup> , Tanvi Wairagade<sup>2</sup> , Anuja Vasant Nagrare<sup>3</sup> , Abhishek Joshi<sup>4</sup>

6949-6956



### **Critical Appraisal of Communicable Diseases in the Perspective of Ayurveda**

Punam Sawarkar<sup>1</sup> , Gaurav Sawarkar<sup>2</sup>

6957-6962



### **Study of Fresh Versus Frozen Embryo Transfer with Reference to Early Pregnancy Loss**

Deepti Shrivastava

6963-6967



## **Review on Dhatryadi Churna: An Ayurvedic Antipyretic Polyherbal Formulation**

Sanket Shelke<sup>1</sup>, Bharat Rathi<sup>2</sup>

6968-6972



## **Case Report on Oligohydramnios**

Lalhriatpuii<sup>1</sup>, Manjusha Mahakalkar<sup>2</sup>, Manoj Patil<sup>3</sup>

6973-6975



## **Case Report on Full Term Normal Delivery**

Pradnya Gavhale<sup>1</sup>, Shalini Moon<sup>2</sup>, Manoj Patil<sup>3</sup>

6976-6978



## **Case Report on Obsessive-Compulsive Disorder**

Samuel Vanlalpeka<sup>1</sup>, Jaya Gawai<sup>2</sup>, Manoj Patil<sup>3</sup>

6979-6981



## **COVID 19–Laboratory Scenario**

Anmol Taneja<sup>1</sup>, Sunita Vagha<sup>2</sup>

6982-6985



## **A Study of Lipid Profile and Estradiol for Evaluation of Cardiovascular Risk in Pre- and Post-Menopausal Women**

Prajakta Warjekar<sup>1</sup>, Rakesh Kumar Jha<sup>2</sup>, Prakash Kute<sup>3</sup>

6986-6991



## **Comparison of Quality Enhancing Method of Modern & Ayurvedic on Tulasi (Ocimum Sanctum)**

Dhiraj A. Zade<sup>1</sup>, Prakash Tathed<sup>2</sup>, Sambhaji Tike<sup>3</sup>, M.K. Rehman<sup>4</sup>, Swanand Pathak

6992-6996



## **Impact of Outcome Based Education (OBE) on Teaching Effectiveness of Faculty Members of Professional Program**



Vaishali Rahate<sup>1</sup>, Sujit Metre<sup>2</sup>, Ranjit Ambad<sup>3</sup>, Swapnil Bhirange<sup>4</sup>  
6997-7004



### **A to Z Activities for Engaging School Going Children During Lockdown**

Archana Dhok<sup>1</sup>, Devendra Punewar<sup>2</sup>, Ranjit Ambad<sup>3</sup>, Lata Butola<sup>4</sup>  
7005-7010



### **Yoga Helps to Improve Immunity & Fight Against Infection During Covid19**

Pravin Pande<sup>1</sup>, Vaishali Tendolkar<sup>2</sup>, Shaini Suraj<sup>3</sup>, Ashish Anjankar<sup>4</sup>  
7011-7014



### **Supplementation of Vitamin D in Diabetic Patients**

Rakesh Kumar Jha<sup>1</sup>, Ankita Kondhalkar<sup>2</sup>, Pradip Jain<sup>3</sup> Mrs. Lata Kanyal Butola<sup>4</sup>  
7015-7017



### **Rasaushadhi (Herbomineral Formulations) Induced Nephrotoxicity in Children: Safety Concern Case Series**

Rekha V Shinde<sup>1</sup>, Ranjit Ambad<sup>2</sup>, Pradip Adhav,<sup>3</sup> Praful Fadanvis<sup>4</sup>, Swarupa Chakole<sup>5</sup>  
7018-7022



### **A Resistant Grave's Disease in Pregnancy: Case Report and Review of the Literature**

Minal Kalambe (Akare)<sup>1</sup>, Mugdha L. Jungari<sup>2</sup>, Dipti Shrivastava<sup>3</sup>  
7023-7025



### **Observational Study of Patients Presenting with Bleeding in First Trimester**

Sonal Nandekar<sup>1</sup>, Mugdha Jungari<sup>2</sup>, Deepika Dewani<sup>3</sup>, Mrunal Nakade<sup>4</sup>  
7026-7029



### **The Effect of Eye-Hand Coordination Device on Subjects with Hemiparesis**

Muzahid K. Sheikh<sup>1</sup>, Neha Chaudhary<sup>2</sup>, Parikshit Muley<sup>3</sup>  
7030-7034

 PDF

### **Evaluation of the Effect of Local Application of Kalpit Kasisadi Yogain the Management of Ksharasutra Induced Hyper Granulation at External Opening Offistula-in-Ano: A Randomized Controlled Trial**

Prafulla Fadanvis<sup>1</sup>, Rashmi Gurao<sup>2</sup>, Dhiraj Zade<sup>3</sup>, Sonali Wairagade<sup>3</sup>, Vasant Gawande<sup>4</sup>, Pradip Adhav<sup>5</sup>  
7035-7041

 PDF

### **Anesthetic Management of Patients with Sickle Cell Disease Posted for Bipolar Prosthesis**

Dr. Sweety Agrawal, Dr. Pratibha Deshmukh, Dr Priyanka Deshmukh, Dr Anjali Modak,  
7042-7045

 PDF

### **Triple Drug Therapy with Proton Pump Inhibitor a Better Option for Helicobacter Pylori Eradication**

Lamture Y.R.1, Thavendra Jaglal Dihare<sup>2</sup>, Varsha Gajbhiye<sup>3</sup>, Gode Dilip<sup>4</sup>  
7046-7050

 PDF

### **Correlation of Helicobacter Pylori Infection with Acid Peptic Disorders**

Thavendra Jaglal Dihare<sup>1</sup>, Lamture Yashwant R.<sup>2</sup>, Varsha Gajbhiye<sup>3</sup>, Gode Dilip<sup>4</sup>  
7051-7055

 PDF

### **Increased Paediatric Screen Time During Pandemic: A Cause of Concern to Child Health**

Yogita Shrivastava<sup>1</sup>, Sujata Shamkuwar<sup>2</sup>, Radha Andhare<sup>2</sup>, Ashish Varma<sup>3</sup>  
7056-7059

 PDF

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7060-7064

 PDF

### **Assessment of age in female population by radiographic examination of Carpal bones**

Pawan A. Wankhade<sup>1</sup>, Rajesh B. Ramteke<sup>2</sup>, B.H. Tirpude<sup>3</sup>, Ninad Nagrale<sup>4</sup>, Shailesh Nagpure<sup>5</sup>  
7065-7069

 PDF

## **Yash Scoring System in the Diagnosis of Acute Appendicitis**

Yeshwant Lamture<sup>1</sup>, Rahul Y. Netragaonkar<sup>2</sup>, Varsha Gajbhiye<sup>3</sup>, Ranjeet Ambad<sup>4</sup>  
7070-7076



## **Tzanakis Scoring System in Acute Appendicitis Concern to Indian Population**

Yeshwant Lamture<sup>1</sup>, Rahul Y. Netragaonkar<sup>2</sup>, Varsha Gajbhiye<sup>3</sup>, Ranjeet Ambad<sup>4</sup>  
7077-7083



## **Comparison between the Tzanakis Scoring System and Yash Scoring System in the Diagnosis of Acute Appendicitis**

Aditya Mundada<sup>1</sup>, Yeshwant Lamture<sup>2</sup>, Varsha Gajbhiye<sup>3</sup>, Ranjeet Ambad<sup>4</sup>  
7084-7093



## **Nerve Conduction Study in Early Diagnosed Cases of Hypothyroidism in Central India**

Komal Meshram<sup>1</sup>, Alka Rawekar<sup>2</sup>, Ajay Meshram<sup>3</sup>, Harshwardhan Meshram<sup>4</sup>, Anjali Vaggha<sup>5</sup>, Shilpa Ingle<sup>6</sup>  
7094-7100



## **Work Related Musculoskeletal Disorders Assessment in Cab drivers**

Samreen Fatima<sup>1</sup>, Ambreen Fatima<sup>2</sup>, Utsav Raj<sup>3</sup>, Mangalam Kumari<sup>4</sup>, Akshay Anand<sup>5</sup>, Neha Chauhan<sup>6</sup>,  
Sakshi Arora<sup>6</sup>  
7101-7105



## **Immediate Effect of Buteyko Breathing and Bhramari Pranayama on Blood Pressure, Heart Rate and Oxygen Saturation in Hypertensive Patients: A Comparative Study**

Samiksha Sanjiv Sathe<sup>1</sup>, Tejal Rajandekar<sup>2</sup>, Kirti Thodge<sup>3</sup>, Amol Bhawane<sup>4</sup>, Utkarsh Thatere<sup>5</sup>  
7106-7111



## **The Normal Response to Slump and Straight Leg Raising Test (SLR) in an Asymptomatic Subjects**

Shweta A. Panchbudhe<sup>1</sup>, Kiran Janbandhu<sup>2</sup>, Shilpa Hatewar<sup>3</sup>, Vasant Gawande<sup>4</sup>  
7112-7116



## **Forensic Age Estimation from Proximal End of Femur: A Radiological Study in Living Individuals**

Ninad Nagrale<sup>1</sup>, Swapnil Patond<sup>2</sup>, Ranjit Ambad<sup>3</sup>, Nandkishor Bankar<sup>4</sup>, Karan Jain<sup>5</sup>  
7117-7120

 PDF

## **A Prospective Observational Case Series of Liver Injury in Paediatric Patients Secondary to Consumption of Ayurvedic Herbomineral Formulations.**

Rekha V. Shinde<sup>1</sup>, Ranjit Ambad<sup>2</sup>, Sunanda C. Patil<sup>3</sup>, Parag Aradhey<sup>4</sup>  
7121-7125

 PDF

## **Study of Non-Stress Test as a Screening Tool in Low Risk Pregnancies at Term Gestation**

Snehal Deshmukh<sup>1</sup>, Amruta Choudhary<sup>2</sup>, Mugdha Jungari<sup>3</sup>, Arpita Jaiswal<sup>4</sup>  
7126-7131

 PDF

## **To Assess the Serum Vitamin D in Vitiligo Patients: SMHRC and AVBR Hospital of Vidarbha Region**

Ranjit S. Ambad<sup>1</sup>, Mihika Suryawanshi<sup>2</sup>, Rakesh Kumar Jha<sup>3</sup>, Pankaj D. Mulchandani<sup>2</sup>, Sudhir Singh<sup>4</sup>  
7132-7135

 PDF

## **Immediate Effect of Buteyko Breathing in Hypertensive Patients: An Experimental Prospective Study**

Samiksha Sanjiv Sathe<sup>1</sup>, Tejal Rajandekar<sup>2</sup>, Saurabh Hadake<sup>3</sup>, Vinayak Shegaonkar<sup>4</sup>  
7136-7141

 PDF

## **Osteometric Evaluation of Human Skull for Sex Determination: A Comparative Study**

Karan Jain<sup>1</sup>, Ninad Nagrale<sup>2</sup>, Ranjit Ambad<sup>3</sup>, Nandkishor Bankar<sup>4</sup>, Swapnil Patond<sup>5</sup>  
7142-7146

 PDF

## **Review on Utility of Antitoxic Preparations in Non-Poisonous conditions w.s.r. to Agadtantra**

Anuja Vasant Nagrare<sup>1</sup>, Sonali Wairagade<sup>2</sup>, Tanvi Wairagade<sup>3</sup>, Anjali Chiwhane<sup>4</sup>  
7147-7151

 PDF

## **Perception of Medical Professionals and Pharmacists towards Generic and Branded Drugs**

Shrikrishna Dhale1, Ankit Singh1

7152-7156

 PDF

### **Drivers of Home Health Nursing Profession**

Ankit Singh1, Vivek Chaudhari1

7157-7162

 PDF

### **Effectiveness of an Educational Program on Critical Care Nurses' Practices Regarding Endotracheal Suctioning of Adult Patients Who are Mechanically Ventilated in Hospitals at AL-Najaf, Iraq**

Jihad Jawad Kadhim1, Fakhria Gaber Mhabes2

7163-7171

 PDF

### **Nurse Educators Examine the Applicability of Health as Expanding Consciousness Theory: A Qualitative Review Study**

Jihad Jawad Kadhim1, Mansour Abdullah Falah2, Iman Qasim Kteo Al-hussein3

7172-7178

 PDF

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Neha Ahire1, Parag Rishipathak2

7179-7183

 PDF

### **A Study to Assess the Knowledge, Attitude and Practice about Management of Acute Exacerbation of Chronic Obstructive Pulmonary Disease amongst EMS Professionals in Pune, India**

Parag Rishipathak1, Anand Hinduja2, Shrimathy V.3, Navnita Sengupta4

7184-7188

 PDF

### **Effect of Facilitated Tucking on Physiological and Behavioural Responses to Pain among Infants Receiving Vaccination**

Mercy Joseph1, Reshma K. Sasi1, Alphonsa Michael2, Amrutha C.2

7189-7193

 PDF

## **A Cross-Sectional Study to Estimate the Presence of Anxiety in Injured Cricketers.**

Bindya Sharma<sup>1</sup>, Yashasree Patil<sup>2</sup>

7194-7198

 PDF

## **Incomplete Kawasaki Disease: A Case Report**

Kuldeep Dhanker<sup>1</sup>, Swati Sharma<sup>2</sup>, Navin Anand Ingle<sup>3</sup>, Alankrita Chaudhary<sup>2</sup>, Sahil Thakar<sup>1</sup>

7199-7203

 PDF

## **The Prevalence and Structure of Arterial Hypertension and the Role of Apolipoproteins in its Development**

Kadomtseva L.V.<sup>1</sup>, Babadjanov A.S.<sup>2</sup>, Pulatova S.Sh.<sup>3</sup>, Polikarpova N.V.<sup>4</sup>

7204-7208

 PDF

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7209-7217

 PDF

## **Analysis of the Interrelation of Speed and High-Speed Endurance in 14-17 Years of Football Players**

Shokhrukh Sh. Erkinov

7218-7223

 PDF

## **The Role of Proinflammatory Cytokines in the Development of Clinical Picture of Myoma and Adenomyosis**

G.S. Babadjanova<sup>1</sup>, S.I. Abdurakhmanova<sup>2</sup>, Muratova N.D.<sup>3</sup>, Suleymanova N.Zh<sup>4</sup>

7224-7228

 PDF

## **Pyeloplasty in Children: Advantages of External Trans-Anastomotic Drainage**

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7229-7232

 PDF

## **Pathogenesis Specifics of Allergic Reactivity in Hypertensive Conditions of Pregnants**

Mukhamedkhanova Sh. T.1, Yuldasheva D.S.2, Mirzaev N.B.3, Ruzieva N.Kh.4, Aripova F.S.5

7233-7238



PDF

## **Testing Techniques in the Evaluation of Severity of HIV-Associated Neuro-Cognitive Disorder in Children**

Saida N. Saidkhodjaeva1, Yokutkhon N. Majidova2, Asal N. Abduvakhitova3, Umida U. Abdullaeva4

7239-7245



PDF

## **Conservative Treatment of Juvenile and Adolescent Idiopathic Scoliosis**

Marat Iskandarov1, Gayrat Kariev2, Fatkhulla Umarkhodjaev3

7246-7252



PDF

## **Comparative Clinical Signs of Herpetic Infection Coupled with HIV Infection**

Malika Daminova1, Gulchekhra Makhkamova2, Shakhnoza Abzalova3, Abdusamat Valiyev4, Surayyo Ikramova5

7253-7257



PDF

## **Early Detection and Prevention of Suicidal Behavior in Adolescents**

Sabir Nurkhodjaev1, Sayora Babarakhimova2, Vasila Abdullaeva3

7258-7263



PDF

## **Certain Indicators of Diclofenac Sodium Pharmacokinetics in Patients with Rheumatoid Arthritis Considering Comorbide States**

Nigora Tukhtaeva1, Marif Karimov2, Marina Sibirkina3, Mavluda Azimova4, Layli Khudoyberganova5, Bekhzod Abdullaev6

7264-7269



PDF

## **Repeated Reconstructive Surgeries for Postoperative Complications of Anorectal Malformations in Children**

Abdurashid Z. Khamraev1, Dilshod B. Rakhmonov2

7270-7278



PDF

## Lower Extremity Deformities in Children with Vertebromedullary Anomalies

Nargiza Ergasheva<sup>1</sup>, Akhrorbek M. Djuraev<sup>2</sup>, Bekzod N. Ergashev<sup>3</sup>

7279-7283



## Diagnostic and Therapeutic Preventive Features of Sagittal Dentofacial Anomalies in Children

Sur'at Gulyamov<sup>1</sup>, Dilshod Karimov<sup>2</sup>

7284-7292



## A Study of Mental Health in Children with Vitiligo Using Projective Method

Kakhramon Khayitov<sup>1</sup>, Khusan Akhrrarov<sup>2</sup>, Nigora Bababekova<sup>3</sup>

7293-7298



## Methemoglobin Role in the Subdural and Epidural Hemorrhage Recentness Evaluation in Hot Climate

B.M. Daljanov<sup>1</sup>, O.I. Khvan<sup>2</sup>

7299-7305



## Management of Nursing in Preschool Institutions of Ex-Soviet Countries

Feruza Azizova<sup>1</sup>, Feruza Salomova<sup>2</sup>, Zilolakhon Adilova<sup>3</sup>, Ilmira Uruzaliev<sup>4</sup>, Guloyim Avezova<sup>5</sup>

7306-7311



## Development and Validation of UV-Spectrophotometric Method for Quantitative Assessment of Metronidazole in the «Metromed® Neo» Solution for Infusion

Khilola Ganieva<sup>1</sup>, Akhmad Yunuskhodjaev<sup>2</sup>

7312-7318



## Features of Manifestation of Optokinetic Nystagmus in Meniere'S Disease

Makhzuna Tahsinovna Nasretdinova<sup>1</sup>, Ozod Rakhmatullaevich Nabiev<sup>1</sup>

7319-7321



## Some Features of the Bacteria of the Family Enterobacteriaceae Allocated from Small Cattle at Different Pathological Processes



Ruslan Gayrabekov<sup>1</sup>, Raisa Gayrabekova<sup>1</sup>, Luiza Molocheva<sup>1</sup>  
7322-7327



### **The Communicative Skills Correction Technology of Children with Autistic Spectrum Disorder in the Crimea**

Svetlana Motornaya<sup>1</sup>, Margarita Belenkova<sup>1</sup>  
7328-7334



### **Indices Changes of Central Hemodynamics at Carry Out Anesthesia with Keeping Independent Breathing at Children**

Gulchekhra Ashurova<sup>1</sup>, Nigora Zokirova<sup>2</sup>, Talat Agzamkhodjayev<sup>3</sup>, Anvar Yusupov<sup>4</sup>, Ikhtiyor Mamatqulov<sup>5</sup>  
7335-7340



### **Current View of the Problem: A New Approach to Covid-19 Treatment**

Jasur A. Rizaev<sup>1</sup>, Ezozbek A. Rizaev<sup>2</sup>, N.N. Akhmadaliev<sup>3</sup>  
7341-7347



### **Morphological Characteristics of Intestinal Vessels of Animals with an Experimental Model of Diabetes Mellitus Type 2 Complicated by Microangiopathy**

Alisher Okhunov<sup>1</sup>, Rajabbay Israilov<sup>1</sup>, Shukhrat Razzakov<sup>1</sup>, Pakiza Azizova<sup>1</sup>, Alisher Mominov<sup>1</sup>  
7348-7353



### **Clinical and Biological Importance of Micro RNA in the Formation of Women Reproductive Losses**

Kamola A. Sattarova<sup>1</sup>, Farida M. Ayupova<sup>2</sup>, Dilnoza D. Saidjalalova<sup>3</sup>, Dildora A. Ayupova<sup>4</sup>, Irina S. Akperbekova<sup>5</sup>, Firuza B. Mirodilova<sup>6</sup>  
7354-7360



### **The Value of Neurospecific Protein S-100 and Lipoproteins in the Development of Multiple Sclerosis**

Markhamat M. Yakubova<sup>1</sup>, Mukaddas A. Mukhiddinova<sup>2</sup>  
7361-7363



## **Epidemic Process of Salmonellosis in Tashkent**

Nargiza S. Saidkasimova<sup>1</sup>, Omon M. Mirtazaev<sup>2</sup>

7364-7367

 PDF

## **Effectiveness of Integrated Treatment Strategy for Diabetic Retinopathy in Patients with Diabetes Mellitus: A Case Report**

Nargiza Normatova

7368-7371

 PDF

## **Organ-saving Operations in Comprehensive Treatment of Lower-Ampullary Rectal Cancer**

Uktam Kurbankulov<sup>1</sup>, Abrorbek Yusupbekov<sup>2</sup>, Bekhzod Abdullaev<sup>3</sup>

7372-7383

 PDF

## **Microhemodynamic Parameters of Cortical Substance of Kidneys at Experimental Hydronephrosis**

Bakhtiyar Iriskulov<sup>1</sup>, Ra'no Tadjibayeva<sup>2</sup>

7384-7387

 PDF

## **Health Quality Assessment of Patients with Toxic-Diffuse Goiter**

Saydiganikhodja I. Ismailov<sup>1</sup>, Komila Kh. Khayitboeva<sup>2</sup>, Rashid Yu. Ruzibaev<sup>3</sup>, Duschan Sh. Sapaev<sup>4</sup>

7388-7394

 PDF

## **Prognosis Innovative Approaches in Relation to Results and Complications of Coronary Remedial Surgical Procedure in Case of Acute Coronary Syndrome**

A.S. Babadjanov<sup>1</sup>, S.A. Babadjanov<sup>2</sup>, M.M. Zufarov<sup>3</sup>

7395-7402

 PDF

## **Characteristics of Cerebral Hemodynamics and Vascular Responsiveness of Brain Arteries among HIV-Infected Patients**

Satima R. Kuranbaeva<sup>1</sup>, Gulnara Rakhimbaeva<sup>2</sup>, S.H. Kalandarova<sup>3</sup>

7403-7408

 PDF

## **Cardiovascular Development Abnormalities in Young Athletes**

Adiba Usmanhodjaeva<sup>1</sup>, Fotima Rakhmatova<sup>2</sup>, T.A. Taraleva<sup>3</sup>  
7409-7412



### **The Form of Erythrocytes and Laser Doppler Flowmetry of Skin with ACNE and Use of Medication FarGALS in its Comprehensive Treatment**

Khashimov F.F.<sup>1</sup>, Baibekov I.M.<sup>1</sup>  
7413-7417



### **Social and Demographic Characteristics of Elderly and their Lifestyle in Developing Countries: On the Example of Uzbekistan**

Sherzodbek A. Inakov<sup>1</sup>, Bakhramjon B. Mamatkulov<sup>2</sup>, Khilola Kosimova<sup>3</sup>, Shoirra Saidalikhujeva<sup>4</sup>, Khadichakhon B. Shoyusupova<sup>5</sup>  
7418-7425



### **Efficient Means of Development of Speed Abilities Based on Analysis between Morphofunctional Indicators with Speed Qualities for Footballers on the Stage of Advanced Specialization**

Shoxrux Erkinov  
7426-7432



### **Modern Trends in the Prevention of Liver Echinococcosis**

M.M. Akbarov<sup>1</sup>, R.Yu. Ruzibaev<sup>2</sup>, D.Sh. Sapaev<sup>3</sup>, P. Yu. Ruzmatov<sup>4</sup>, F.R. Yakubov<sup>5</sup>  
7433-7437



### **Disturbance of Coagulative Hemostasis among Patients with Henoch-Schonlein Purpura**

Sh.A. Babadjanova<sup>1</sup>, Z.S. Islamova<sup>2</sup>, Z.Ch. Kurbanova<sup>3</sup>, Khurshida S. Yusufkhojaeva<sup>4</sup>, Feruza Kh.Inoyatova<sup>5</sup>  
7438-7442



### **Some Features of Hemorrhagic Syndrome in Chronic Diffuse Liver Diseases**

Zumrad Ch. Kurbonova<sup>1</sup>, Shaira A. Babadjanova<sup>2</sup>, Zulfiya S. Islamova<sup>3</sup>, Abdumurat S. Babadjanov<sup>4</sup>, Nargiza B. Musayeva<sup>5</sup>  
7443-7448



## **The Possibilities of Ultrasound in the Diagnosis of Congenital Dysplasia of the Hip Joint**

Nozima Salieva<sup>1</sup>, Umida Rustamova<sup>2</sup>, Sarvinoz Rakhmanova<sup>3</sup>

7449-7453



## **Clinical and Instrumental Evaluation of the Effectiveness of Surgical Treatment of Chronic Generalized Periodontitis Using RANK-RANKL-OPG Biomarkers**

Elbek A. Gaybullaev<sup>1</sup>, Jasur A. Rizaev<sup>1</sup>, Bekhzod Sh. Abdullaev<sup>1</sup>

7454-7456



## **Comparative Analysis of Clinical and Laboratory Parameters and Assessment of Antiretroviral Therapy Effectiveness in HIV-Associated Cryptococcal Meningoencephalitis in the Case of Immune Inflammation Syndrome**

D.A. Mustafaeva<sup>1</sup>, L.N. Tuychiev<sup>2</sup>, E.I. Musabaev<sup>3</sup>, T.S. Zaytseva<sup>1</sup>, K.S. Mukhamedov<sup>4</sup>

7457-7464



## **Treatment of Acute Lung Abscesses Considering their Non-Respiratory Function in Patients with Diabetes**

Okhunov A.O<sup>1.</sup>, Israilov R.I.<sup>1</sup>, Khamdamov Sh.A.<sup>1</sup>, Azizova P.X.<sup>1</sup>, Anvarov K.D.<sup>1</sup>

7465-7469



## **Increasing the Effectiveness of Public Information on Risk Factors and Self-Control of Hypertensive Disease in Primary Link**

Shakhzoda Gulyamova<sup>1</sup>, Abdigaffar Gadaev<sup>2</sup>, Rustam Zakirkhodjaev<sup>3</sup>, Dilmurad Kalandarov<sup>4</sup>, Dilrabo Sodikova<sup>5</sup>

7470-7477



## **Peculiarities of the Pregnancy in Women with Hepatobiliary System Pathology**

Guljakhan S. Babadjanova<sup>1</sup>, Mukhayo D. Abdurazakova<sup>2</sup>, Gulnara M. Nigmatova<sup>3</sup>, Dildora A. Ayupova<sup>4</sup>, Dilufar N. Khodjaeva<sup>5</sup>

7478-7490



## **Achilles Tendinopathy and Associated Factors among Recreational Ballet Dancers: A Cross Sectional Study**

Suvinlal Stalin Russel<sup>1</sup>, Tan Xin Yi<sup>2</sup>, Rahul Krishnan Kutty<sup>1</sup>, Syed Abudaheer Kajamohideen<sup>1</sup>, Arunkumar Nedunchezhiyan<sup>1</sup>, Mahendran Jayaraman<sup>1</sup>

7491-7496

 PDF

### **Multidisciplinary Approaches in Surgical Treatment and Rehabilitation of Patients with Defects of Trachea Anterior Wall**

Rustem Hayaliev<sup>1</sup>, Shukhrat Khudaybergenov<sup>2</sup>, Otabek Eshonkhodjaev<sup>3</sup>, Abdurashit Kayumhodjaev<sup>4</sup>, Nazira Aripova<sup>5</sup>

7497-7505

 PDF

### **Barrier Factors In Maintaining Breastmilk Volume**

Imelda Iskandar<sup>1</sup>, Suryani As'ad<sup>2</sup>, Nasrudin Andi Mappaware<sup>3</sup>, EmaAlasiry<sup>4</sup>, AzniahSyam<sup>5</sup>, Suradi Efendi<sup>6</sup>

7506-7514

 PDF

### **Effectiveness of Septic Tanks Floating in Reducing COD, TSS, Temperature and pH of Waste Water Black**

Marhama<sup>1</sup>, Agus Bintara Birawida<sup>1</sup>, Syamsuddin Toaha<sup>2</sup>

7515-7518

 PDF

### **Maternal and Perinatal Outcome on Pregnancy with Covid-19 Infection at Dr. Wahidin Sudirohusodo Hospital Makassar During the Period of April-July 2020**

Fadlia Pratiwi Suyuthi<sup>1</sup>, Maisuri T. Chalid<sup>1</sup>, A. Nursanty Padjalangi<sup>1</sup>, Irawaty Djaharuddin<sup>2</sup>, Muh. Nasrum Massi<sup>3</sup>

7519-7522

 PDF

### **The Effect of Pravastatin Provision on Endotelin-1 Levels in Preeclapmsia's High Risk Patients**

Rizky A Ramadhani<sup>1</sup>, Deviana SorayaRiu<sup>1</sup>, Irnawati Bahar<sup>1</sup>, Isharyah Sunarno<sup>1</sup>, Retno B. Farid<sup>1</sup>, Eddy Hartono<sup>1</sup>

7523-7529

 PDF

### **A Model of Cigarette Advertisement Policy in Preventing Children Smoking Habits in Palu City, Indonesia: A Systematic Review**

Muhammad Ryman Napirah<sup>1,2</sup>, Ridwan Amiruddin<sup>3</sup>, Sukri Palutturi<sup>4</sup>, Stang<sup>5</sup>, Vidyanto<sup>6</sup>, Rosmala Nur<sup>7</sup>, Muhammad Basir<sup>8</sup>

7530-7534

 PDF

## **A Transtheoretical Model in Controlling Smoking Behavior in Junior High School Students in Palu City, Indonesia: A Systematic Review**

Muhammad Ryman Napirah<sup>1,2</sup>, Ridwan Amiruddin<sup>3</sup>, Sukri Palutturi<sup>4</sup>, Stang<sup>5</sup>, Aminuddin Syam<sup>6</sup>, Rosmala Nur<sup>7</sup>

7535-7539



## **Development and Standardization of Capsular Pharmaceutical Dosage form from Dry Extract of Ferula Tenuisecta**

Oygul Rakhimova<sup>1</sup>, Saodat Sharipova<sup>2</sup>, Mukhammad Sodik Okhunov<sup>3</sup>, Farrukhjon Baratov<sup>4</sup>, Farrukh Shokirov<sup>5</sup>, Shokhrukhbek Abdugulomov<sup>6</sup>

7540-7547



## **Comparative Analysis of Human Kidney Venous Vessels at Various Method of Radiation Research**

Abuselim Vezirkhanov<sup>1</sup>, Edgar Kafarov<sup>1</sup>, Oleg Zenin<sup>1</sup>

7548-7552



## **Specifics of Left Ventricular Remodeling and Daily Blood Pressure Profiles in Young and Middle-Aged Servicemembers Dealing with Arterial Hypertension**

Botyrkhon Salikhov<sup>1</sup>, Bakhromkhon Alyavee<sup>1</sup>

7553-7557



## **Features of Pain Syndrome of Patients with Brucellosis if Damaged Nervous System**

Sokhiba Khakimova Ziyadullayevna<sup>1</sup>, Dildora Atokhodjaeva Alisherovna<sup>1</sup>

7558-7562



## **Adaptation Mechanisms of Shift Workers in Areas of the Far North**

Tomus I.Yu.<sup>1</sup>, Zhilyakov E.V.<sup>1,2</sup>, Prokofieva I.Yu.<sup>1</sup>

7563-7569



## **Assessment of Survivability of Vitrified Embryos Depending on the Stage of Development**

O.V. Shurigina<sup>1</sup>, S.Z. Yuldasheva<sup>2</sup>, O.V. Ivanova<sup>3</sup>, N.N. Demidova<sup>4</sup>

7570-7575



## Changes in Blood Counts of Patients with Chronic Atrophic Rhinitis

Anvar Shadiev<sup>1</sup>, Maxzuna Nasretdinova<sup>1</sup>, Hurram Karabaev<sup>2</sup>

7576-7579

 PDF

## Work-Related Musculoskeletal Symptoms among Academicians in Malaysian Universities

Syed Abudaheer K.<sup>1</sup>, Chu Rongle<sup>2</sup>, Rahul Krishnan Kutty<sup>1</sup>, Narayanan Muthukumran N.<sup>1</sup>, Mahendran Jayaraman<sup>1</sup>, Suvinlal Stalin Russel<sup>1</sup>, Arunkumar Nedunchezhiyan<sup>1</sup>

7580-7585

 PDF

## Pre-Clinical Study of General Toxicity of the Medication “Fitin-S”

Kamola Mirzaakhmedova<sup>1</sup>, Shakhida Ziyayeva<sup>2</sup>, Abdushukur Yunusov<sup>3</sup>, Gulchekhra Karimova<sup>4</sup>, Rustam Karimov<sup>5</sup>

7586-7594

 PDF

## Hemodynamic Shifts in Children During the Induction into Narcosis P

Talat Agzamkhodjaev<sup>1</sup>, Anvar Yusupov<sup>2</sup>, Makhfuza Ismailova<sup>3</sup>, Mamur Talipov<sup>4</sup>, Isfandiyor Mamatkulov

7595-7599

 PDF

## Comparative Analysis of the Results of Traditional and New Distance Learning Method Among the Master’s Degree Students and Physicians in Uzbekistan

Zokhid Abdurakhimov

7600-7602

 PDF

## The Role of Robotic Mechanotherapy in the Recovery of Mobility in Patients after an Acute Cerebrovascular Accident

Aziza A. Biykuzieva<sup>1</sup>, Durдона M. Ahmedova<sup>2</sup>, Adibakhon A. Usmankhodjaeva<sup>3</sup>, Olga N. Visogortseva<sup>4</sup>, Ekaterina F. Svyatskaya<sup>5</sup>, Dilnora H. Isabaeva<sup>6</sup>

7603-7608

 PDF

## Development and Standardization of Dental Plant-Based Preparation in The Republic of Uzbekistan

Aziza A. Djuraeva<sup>1</sup>, Viloyat N. Abdullabekova<sup>2</sup>

7609-7617

 PDF

## **A Complex of Risk Factors for Developing Dependence on the Combined Abuse of Hashish and Tramadol**

Inara Khayredinova<sup>1</sup>, Zarifjon Ashurov<sup>2</sup>, Indira Giyazitdinova<sup>3</sup>, Mark Agranovsky<sup>4</sup>  
7618-7623

 PDF

## **Obtaining and Studying Technology of Dry Substance Silver Nanoparticles Obtained by “Green Synthesis” Method Using Scutellaria Iscandaria L.**

Iroda B. Shermatova<sup>1</sup>, Mokhigul G. Ismailova<sup>2</sup>  
7624-7635

 PDF

## **Assessment of Informativity of Dopplerographic Determination of the Ankle-Brachial Index**

Aytimova G.Y.<sup>1</sup>, Rozikhodjaeva G.A.<sup>2</sup>, Matrizaeva G.Dj.<sup>3</sup>, Yakubova A.B.<sup>4</sup>, Rakhmanova N.Kh.<sup>5</sup>  
7636-7641

 PDF

## **Significance, Effectiveness and Prospects of Development of Enzyme Preparations in Modern Pharmaceutical Practice**

U.M. Tillaeva<sup>1</sup>, D.T. Gaibnazarova<sup>2</sup>, F.S. Jalilov<sup>3</sup>, B.Sh. Abdullaev<sup>4</sup>  
7642-7652

 PDF

## **Comparative Analysis of the Spectrophotometric and HPLC Method for Quantitative Determination of Fensulkal in a Substance**

Umida M. Tillaeva<sup>1</sup>, Gulnora U. Tillaeva<sup>2</sup>, Dilafruz B. Kasimova<sup>3</sup>  
7653-7659

 PDF

## **Technologies for Producing Capsules “Yuyuzin”**

Zilola Vakhobdjonovna Turdieva<sup>1</sup>, Manzura Tokhirovna Mullajonova<sup>2</sup>, Umarkhon Mukhtorovich Azizov<sup>3</sup>,  
Dilfuza Botirali qizi Mirakilova<sup>4</sup>  
7660-7663

 PDF

## **Adaptive Capabilities of Underweight Newborns and Development Features in their First Year of Life**

Kh. Akramova<sup>1</sup>, A. Djalilov<sup>2</sup>  
7664-7675

 PDF



## **The First 5 Years Characteristics of Growth and Development of Children Residing in the Regions of Uzbekistan**

Dilfuza Ashurova<sup>1</sup>, O. Tursunova<sup>2</sup>, N. Nurmatova<sup>3</sup>, G. Yusupova<sup>4</sup>, B. Sharapov<sup>5</sup>  
7676-7684

 PDF

## **Age-Related Features of Mast Cell Reactivity in Allergies**

Sattar Begmanov<sup>1</sup>, Y. Zokirov<sup>1</sup>, N. Abidova<sup>1</sup>, G. Khakimova<sup>1</sup>, Z. Shakhobutdinov<sup>1</sup>  
7685-7691

 PDF

## **Correlation of the Main Parametres of Immun Systeminchildren with Localized Scleroderma by the Stages of the Disease**

Mun Andrey<sup>1</sup>, Abdushukur Mannanov<sup>2</sup>  
7692-7699

 PDF

## **Forensic-Medical Criteria for Evaluation of Poisoning by Alcohol Surrogates**

Shokhsanam Yadgarova<sup>1</sup>, Sherzod Ruziev<sup>2</sup>, Khasankhodja Abidov<sup>3</sup>  
7700-7705

 PDF

## **Nephroprotective Effects of 2nd Type Sodium-Glucose Co-Transporter Inhibitors: A Literature Review**

Botir Daminov<sup>1</sup>, Sherzod Abdullaev<sup>2</sup>, Rano Igamberdieva<sup>3</sup>, Olimkhon Sharapov<sup>4</sup>  
7706-7716

 PDF

## **Assessment of Hearing in Children with Cerebral Palsy**

Gavkhar Khaydarova<sup>1</sup>, Aziza Madrimova<sup>2</sup>, Khalida Shaykhova<sup>3</sup>  
7717-7723

 PDF

## **Clinical Features of Early Diagnosis and a Choice of Method of Surgery for Hirschsprung Disease in Infants**

Ulugbek A. Khamroev<sup>1</sup>, Bakhtiyor B. Ergashev<sup>2</sup>, Rajabboy I. Isroilov<sup>3</sup>  
7724-7736

 PDF

## **Especially the Development of Myocarditis in Hypothyroidism in Postnatal Ontogenesis**

Mirzamukhamedov O.Kh.1, Mirsharopov U.M.2, Sodikova Z.Sh.3, Akhmedova S.M.4, Khatamov A.I.5,  
Mirzabekova O.A.6  
7737-7745

 PDF

### **Neuroimmunological Characteristics of Idiopathic and Symptomatic Epilepsy in Accordance with the Clinical Course**

Ra'no B. Azizova1, Nargiza N. Abdullaeva2, Iqrorjon I. Usmonaliev3  
7746-7752

 PDF

### **Our Experience in Treatment Acute Surgical Diseases of the Abdominal Cavity in Patients with Situs Inversus Viscerum (Transposition of Internal Organs)**

S.R. Baymakov1, S.Sh. Yunusov2, U.R. Jamilov3, Sh.Kh. Bobokulov4, Sh.Sh. Boltaev5, Sh.Sh. Jonibekov6  
7753-7757

 PDF

### **State Rubricator of Scientific and Myoma, Senior Reproductive Age and IVF/IVF+ICSI is there Any Connection?**

M.M. Maksudova  
7758-7760

 PDF

### **Inconsistency of Seams on the Uterus after Cesara Section Problems and Ways of their Solution**

Farida Mirzaevna Ayupova1, Nargiza Mirzaevna Umarova2, Gulnara Makhsudovna N  
7761-7770

 PDF

### **Stability Research of "Biomayrin" Capsules**

Nilufar E. Mamatmusaeva1, Shavkat A. Shomurotov2, Bakhodir Tahirovich Sagdullayev3, Abbaskhan S.  
Turaev4  
7771-7778

 PDF

### **Protection of Human Rights in the Context of Combating of Counterfeiting Medicines: Theoretical and Legal Issues of International Cooperations**

Filei Yurii Vladimirovich1, Davidov Pavel Grigorovich2, Dmytro Yermolenko3, Koverznieva Hanna4, Vasyl V.  
Ilkov5  
7779-7783

 PDF

### **Cytology and Genotyping of High-Risk Human Papilloma Virus from Female Sex Workers in Makassar**

Juan<sup>1</sup>, Sharvianty Arifuddin<sup>2</sup>, Abdul Rahman<sup>3</sup>, St. Nur Asni<sup>1</sup>, Syahrul Rauf<sup>2</sup>, Masita Fujiko<sup>4</sup>  
7784-7793

 PDF

### **Comparing the Effect of Combined Therapy of Extracorporeal Shock Wave Therapy and Ice Massage with Combined Therapy of Therapeutic Ultrasound with Paraffin Wax Bath in Treating Trigger Finger**

Yu Chye Wah<sup>1</sup>, Chen Zhun Yi<sup>2</sup>, Kshtrashal Singh<sup>3</sup>, Koh Kim Hua<sup>4</sup>, Susmitha Govind<sup>3</sup>, Veni Chandrakasan<sup>4</sup>  
7794-7801

 PDF

### **Correlation between Duration of the Second Stage and Pelvic Floor Muscle Strength in Primiparous Women Following Vaginal Delivery and Caesarean Section**

Witono Gunawan<sup>1</sup>, TrikaIrianta<sup>2</sup>, AjardianaIdrus<sup>3</sup>, Maisuri T. Chalid<sup>4</sup>, David Lotisna<sup>2</sup>, Rina Previana<sup>3</sup>  
7802-7811

 PDF

### **User Centered Portable Suspension Therapy Unit Design & Testing on Healthy Volunteers**

Kaviarasu Mahalingam<sup>1</sup>, Karthikeyan Jayabalan<sup>1</sup>, Sree Vamsi<sup>1</sup>, Yu Chye Wah<sup>2</sup>, Angeline Qnah Lie Mun<sup>3</sup>, Khor Yi Wei<sup>3</sup>, Winnie Tan<sup>3</sup>  
7812-7817

 PDF

### **The Comparison of Gestational Diabetes Mellitus Screening between the One Step Method and the Two Step Method**

Arini Estiastuti<sup>1</sup>, Deviana Soraya Riu<sup>1</sup>, Nur Rakhmah<sup>1</sup>, St. Maisuri T. Chalid<sup>1</sup>, Nugraha Utama Pelupessy<sup>1</sup>, Johnsen Mailoa<sup>1</sup>  
7818-7824

 PDF

### **To Compare the Effectiveness of Taping and Arch Support on the Flexible Flat Foot on a Random Population**

J. Karthikeyan<sup>1</sup>, Kshtrashal Singh<sup>1</sup>, Susmitha Govind<sup>1</sup>, Kaviarasu Mahalingam<sup>1</sup>, Sree Vamsi<sup>1</sup>, Pavithra A/P Annamalai<sup>2</sup>, Yu Chye Wah<sup>3</sup>  
7825-7832

 PDF

## **The Relationship between Prediabetes and Body Mass Index of Outpatients at Dr. Dody Sarjoto Hospital at Maros Regency, South Sulawesi, Indonesia**

Junedi Sitorus<sup>1</sup>, Nurhaedar Jafar<sup>2</sup>, Ridwan Amiruddin<sup>3</sup>, Sukri Palutturi<sup>4</sup>, Veni Hadju<sup>2</sup>  
7833-7837



## **Pandemic Corona (Covid-19) and Health Law Protection**

Birkah Latif  
7838-7846



## **The Analysis of Antenatal Care During Covid-19 Pandemics in The Working Area of Public Health Center of Tangerang City**

Triana Indrayani<sup>1</sup>, Vivi Silawati<sup>1</sup>, Retno Widowati<sup>2</sup>, Sukri Palutturi<sup>3</sup>, Ridwan Amiruddin<sup>4</sup>  
7847-7851



## **The Effectiveness and Safety of Convalescent Plasma as a Therapy for COVID-19 Patients: A Literature Review**

Tri Setyawati<sup>1</sup>, Virgiana<sup>2</sup>, Rosa Dwi Wahyuni<sup>3</sup>, Sumarni<sup>3</sup>, Rosmala Nur<sup>3</sup>  
7852-7855



## **Clinical Characteristic of Covid-19 Infection in Pediatric: A Systematic Review**

Annisa Istiqamah Ahmad<sup>1</sup>, Tri Setiawaty<sup>2</sup>, Rosmala Nur<sup>3</sup>, Rahman Kadir<sup>4</sup>  
7856-7860



## **Tobacco Smoke and Pregnancy Outcome: Literature Review**

Haerani Harun<sup>1</sup>, Anwar Daud<sup>2</sup>, Veni Hadju<sup>3</sup>, Muh Syafar<sup>4</sup>, Ridwan Amiruddin<sup>5</sup>, Rahma<sup>6</sup>, Abdul Faris<sup>1</sup>  
7861-7867



## **Correlation Between Oxygen Saturation, Uric Acid and Creatinine Level to Maternal Outcome of a Woman with Severe Preeclampsia**

Fadlia Pratiwi Suyuthi<sup>1</sup>, Maisuri T. Chalid<sup>1</sup>, Samrichard Rambulangi<sup>1</sup>, Deviana S. Riu<sup>1</sup>, Eddy Tiro<sup>1</sup>, Firdaus Kasim<sup>2</sup>  
7868-7872



## **Marriage Tradition of Indigenous Community of Baduy and The Covid-19 Prevention**

Farida Patittingi<sup>1</sup>, Desti Novita<sup>2</sup>, Kahar Lahae<sup>1</sup>

7873-7877

 PDF

## **Overcoming of COVID-19 Transmission: The Fulfillment of Right to Health and Education**

Donna Okthalia Setiabudhi<sup>1</sup>, Irwansyah<sup>2</sup>, Toar Neman Palilingan<sup>1</sup>, Ahsan Yunus<sup>2</sup>

7878-7883

 PDF

## **Medical Tourism Awareness of Health Workers**

Hüseyin ER??<sup>1</sup>, Ebru KEMER<sup>2</sup>

7884-7889

 PDF

## **Evaluation of the Results to Digestive Endoscopy Patients in Azadi Teaching Hospital in Kirkukcity**

Rebaz Ismael Ali<sup>1</sup>, Marghoob Hussein Yaas<sup>2</sup>

7890-7896

 PDF

## **Non Genetic Study for Autism Child in Related to Age And Sex of Autism Child, Parental and Maternal age And Degree of Relative and Family History with the Disease or Any Other Psychological Disorder in Anbar Governorate**

Raida Noori Hamid<sup>1</sup>, Shakir Mahmood Ali<sup>2</sup>, Shaimaa Hamid Mudher<sup>1</sup>

7897-7902

 PDF

## **Parent's Knowledge upon Nutritional Status of Primary School Children toward Low-Income Households in Al-Hilla City, Iraq**

Abdulmahdi A. Hasan

7903-7907

 PDF

## **Right To Health Protection in the Former-Soviet Constitutions: Constitutional and Legal Analysis**

Maksakova Ruslana<sup>1</sup>, Yurii Viktorovych Kyrychenko<sup>2</sup>, Maryna Leonydovna Smoliarova<sup>3</sup>

7908-7913

 PDF

## **The Influence of Labor Environment Settings on Labor Progression**

Dewi Sartika Siagian<sup>1</sup>, Arum Meirani<sup>2</sup>, Nanik Cahyati<sup>3</sup>, Lusi Lestari<sup>4</sup>, Fania<sup>5</sup>

7914-7921



## **Development of Spectrophotometric Method for Analysis of Framicetine Sulphate in a Medicinal Product and its Validation**

Gapparov Dilshodbek Davronbekovich<sup>1</sup>, Smanova Zulaykho Asanalievna<sup>2</sup>

7922-7928



## **Comprehensive Integrated Assessment of Health, Lifestyle and Medical Services for Children at the Age of their First Year**

Ilshod Isayev

7929-7935



## **Walkability Index Based on Pedestrian Needs in the Losari Beach area of Makassar City**

Susy Asteria Irafany<sup>1</sup>, Shirly Wunas<sup>2</sup>, Slamet Trisutomo<sup>3</sup>, Arifuddin Akil<sup>4</sup>, Mimi Arifin<sup>5</sup>, Abd. Rachman Rasyid<sup>6</sup>

7936-7947



## **Features of Neuroimaging Diagnostics of Transient Ischemic Attacks**

Muborak B. Abdullaeva<sup>1</sup>, Yoqutkhon N Majidova<sup>2</sup>, Malika M. Raimova<sup>3</sup>, Nozima R. Babadjanova<sup>4</sup>, Umida G. Yodgorova<sup>5</sup>, Asliddin B. Kalanov<sup>5</sup>

7948-7952



## **Results of Transurethral Correction of Stenotic Developmental Abnormalities of Ureterovesical Junction in Children**

Makhmudjon Aliev<sup>1</sup>, Akmal Rakhmatullaev<sup>1</sup>, Bilim Terebaev<sup>1</sup>

7953-7959



## **Epidemiological Characteristic of Aphthous Stomatitis and Real Clinical Practice of Management of Patients in Uzbekistan**

D.M. Alimova<sup>1</sup>, O.E. Bekjanova<sup>2</sup>

7960-7967



## **The Approach to Managing the Patients with Idiopathic Thrombocytopenic Purpura: A Case-Control Study**

Gaybulla Mustafakulov<sup>1</sup>, Ulug'bek Ergashev<sup>1</sup>, Aminjon Kuryazov<sup>1</sup>, Davron Yakubov<sup>1</sup>, Fotima Atakhadjaeva<sup>1</sup>  
7968-7972

 PDF

## **Study of Hematological Changes in Local Goats (Capra Aegagrus) at Al-Najaf Province, Iraq**

Mujeeb Towfik Shamsa  
7973-7978

 PDF

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7979-7983

 PDF

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Mirsagatova M.R.<sup>3</sup>  
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 PDF

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 PDF

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Gaziev Zoir Tokhirovich<sup>1</sup>, Kurbonov Erkin Kurbonovich<sup>2</sup>, Rakhmankulov Eldor Zhurakulovich<sup>3</sup>, Chakonov Ulmasbek Aslonovich<sup>4</sup>, Kurbonov Yorkin Tulkinovich<sup>5</sup>  
8004-8007

 PDF

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Vaisov ?.Sh.<sup>1</sup>, Fuzailov D.N.<sup>2</sup>, Munir Akhmad<sup>3</sup>, Umarov Zh.?.<sup>4</sup>  
8008-8012

## **Circulating and Renal Endothelin-1 Expression Lead to Glomerular Impairment in High Fat-Induced Diet Wistar Rats**

Haslindah Dahlan<sup>1</sup>, Muhammad Husni Cangara<sup>2</sup>, Rina Masadah<sup>2</sup>, Irfan Idris<sup>3</sup>, Upik Anderiani Miskad<sup>2</sup>, Andi Alfian Zainuddin<sup>4</sup>, Miswani Mukani<sup>1</sup>

8013-8018



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8019-8022

## **The Study of Antiallergic Activity of the Phytocollection of “Phytoallergoderm”**

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8023-8029



## **The Effect of Social Support on Pregnant Women Health in Minangkabau Matrilineal Communities**

Devi Syarief<sup>1</sup>, Andi Zulkifli Abdullah<sup>2</sup>, Suriah<sup>3</sup>, Healthy Hidayanti<sup>4</sup>, Dewi Susilawati<sup>5</sup>, Ety Aprianti<sup>6</sup>, Guslinda<sup>7</sup>

8030-8034



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Snigdha Pattanaik

8035-8040



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8041-8044



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Susant Mohanty<sup>1</sup>, Kalimireddy L. Priyanka<sup>2</sup>

8045-8048



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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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8124-8127

 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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8145-8149

 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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8172-8174

 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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8211-8215

 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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8241-8244

 PDF

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8245-8249

 PDF

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 PDF

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8258-8263



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8264-8266



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8267-8271



### **Commercially Available Toothpaste in India**

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8272-8276



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8277-8281



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8282-8285



### **Desquamative Gingivitis in Cicatricial Pemphigoid: A Diagnostic Paradox!**

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8286-8290



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Satya Ranjan Misra

8291-8294



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8295-8298



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Gayatri Potukuchi

8299-8302



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8303-8308



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Debarchita Sarangi

8309-8313



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 PDF

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8338-8342

 PDF

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8343-8347

 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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8365-8371



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8372-8375



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8376-8380



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8381-8388



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8389-8393



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Dharmashree Satyarup<sup>1</sup>, Manish Kumar<sup>2</sup>, Radha Prasanna Dalai<sup>3</sup>, Karishma R. Rathor<sup>4</sup>  
8394-8400



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8401-8404



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 PDF

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8410-8414

 PDF

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8415-8419

 PDF

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 PDF

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 PDF

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8429-8432

 PDF

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8433-8437

 PDF

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8438-8442

 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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 PDF

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8471-8475

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8961-8965



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8966-8971



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8972-8975



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8976-8979



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Swati Patnaik<sup>1</sup> , Shakti Swaroop<sup>2</sup>  
9006-9010?

 PDF

### **Periodontal Microsurgery: An Evolution in the Progression**

Aseema Samal<sup>1</sup> , Manoj Kumar<sup>2</sup> , Rashmita Nayak<sup>3</sup> , Anurag Satpathy<sup>3</sup> , Abhaya Chandra Das<sup>2</sup> , Gatha Mohanty<sup>4</sup>  
9011-9015

 PDF

### **Dental Management of Children with Special Health Careneeds: A Review**

Fungbili Mahilary  
9016-9021

 PDF

### **Chondroblastic Osteosarcoma: A Review**

K. Kiran Kumar Patro  
9022-9026

 PDF

## **Deleterious Oral Habits and Management in Pediatric Patients: A Review**

Fatma Shaghaf

9027-9031



## **Recent Advances in Non-pharmacological Behavior Management Technique in Children**

Tirtharaj Brahma

9032-9034



## **Pit and Fissure Sealant: A Review**

Pratyusha Mohanty

9035-9039



## **Swellings of the Palate: A Review**

Srishti Chatterjee

9040-9045



## **Glucocorticoids: A Boon or Bane in Dentistry**

Subhashree Sahoo

9046-9049



## **Microbes and Immunity: The Periodontal Battle Zone**

Priyanka Jena<sup>1</sup>, Gatha Mohanty<sup>2</sup>

9050-9054



## **Treatment of Chronic Periodontal Disorders: A General Practitioners Perspective**

Ayesh Das<sup>1</sup>, Gatha Mohanty<sup>2</sup>

9055-9058



## **Effect of Fluoride Varnish in Dental Caries: A Review**

Rupsita Sahu<sup>1</sup>, Ramesh Nagarajappa<sup>2</sup>, Debashruti Naik<sup>3</sup>

9059-9063

 PDF

## **Mineral Trioxide Aggregate v/s Calcium Hydroxide: A Review Article**

Gayatri Padhee<sup>1</sup> Susant Mohanty

9064-9067

 PDF

## **Microbiological Aids in Periodontics**

Soumya Ranjan Panda

9068-9070

 PDF

## **Ectodermal Dysplasia: An Overview**

Sruti Prava Panda

9071-9074

 PDF

## **Plaque Control with Newer Dimension in Children**

Harshita Singh

9075-9078

 PDF

## **Complications of Local Anesthesia**

Stutishree Tripathy

9079-9082

 PDF

## **Role of Interleukin (IL-1?) In Periodontal Disease Progression: A Literature Review**

Lipsa Behera<sup>1</sup> , Abhaya Chandra Das<sup>2</sup> , Sourav Panda<sup>2</sup>, Rashmita Nayak<sup>3</sup> , Rinkee Mohanty<sup>3</sup> , Gatha Mohanty<sup>4</sup>

9083-9087

 PDF

## **Recent Advances in Surface Characteristics of Dental Implants: A Mini Review**

Pooja Mohanty<sup>1</sup> , Manoj Kumar<sup>2</sup> , Saurav Panda<sup>2</sup> , Rinkee Mohanty<sup>3</sup> , Rashmita Nayak<sup>4</sup> , Anurag Satpathy<sup>4</sup>

9088-9090

 PDF

## **Isolation of Teeth in Children: A Review**

Prajna Nibedita Behuria

9091-9094

 PDF

### **Immunity and Periodontal Disease**

Prangya Elina Singh<sup>1</sup>, Manoj Kumar<sup>2</sup>, Rinkee Mohanty<sup>3</sup>, Rashmita Nayak<sup>4</sup>, Anurag Satpathy<sup>4</sup>, Gatha Mohanty<sup>5</sup>

9095-9098

 PDF

### **Hypochondriac Disorder in OSMF Patient**

Baba Sankar Sahu<sup>1</sup>, Abhishek Ranjan Pati<sup>2</sup>

9099-9101

 PDF

### **Management of Epileptic Patient in Pediatric and Preventive Dentistry**

Prangya Elina Singh

9102-9105

 PDF

### **Intracanal Medicaments in Paediatric Endodontics**

Praspita Parlin Patro

9106-9110

 PDF

### **TMJ Ankylosis: A Reveiw**

Ethena Priyadarsani

9111-9114

 PDF

### **Management of Cleft Lip and Palate in Pediatric Dentistry: A Review**

Subhangi Chandan

9115-9119

 PDF

### **Topical Fluoride for Prevention of Dental Caries: A Review**

Padmaja Nayak

9120-9123

 PDF

### **A Traumatic Restorative Technique: A Review Article**

Anwasha Subuddhi

9124-9128

 PDF

### **Stainless Steel Crown: A Review Article**

Harsha Agrawal

9129-9132

 PDF

### **Assessment of Restorative Skills for Amalgam Restoration**

Jagruti Nanda<sup>1</sup>, Lora Mishra<sup>2</sup>

9133-9137

 PDF

### **Benefits of Early Orthodontics Intervention in Children: A Review**

Debashreeta Nanda

9138-9141

 PDF

### **Bell's Palsy: A Review**

Sumanta Sourav

9142-9145

 PDF

### **Ossifying Fibroma of the Anterior Maxilla: A Case Report**

Satya Ranjan Misra<sup>1</sup>, Shivani Sahu<sup>2</sup>

9146-9151

 PDF

### **Inhalational Inducing Agents**

Manisha Mohapatra

9152-9156

 PDF

### **Artificial Intelligence and How it Renovates the World of Dentistry**

Rabina Mohanty

9157-9160



### **Infections of the Surgical Site: An Overview**

Mohammed Wasif

9161-9164



### **Pulpotomy**

Rojalin Jena

9165-9168



### **Evolving Trends in Forensic Odontology**

J. Shirisha

9169-9173



### **Complication & Management of Local Anesthesia: A Systemic Review**

Alisha Pradhan

9174-9178



### **Oral Hygiene Practice and Oral Health Status among Tribal Children of Odisha, India**

Biswabandita Mohanty<sup>1</sup>, Susant Mohanty<sup>2</sup>, Antarmayee Panigrahi<sup>3</sup>

9179-9182



### **Dental Implants in Pediatric Dentistry: A Review Article**

Sristi Das

9183-9186



### **SARS-CoV-2 and Oral Health: Implications and Recommendations for Dental Practice Reformation Post Pandemic**

Swagatika Panda<sup>1</sup>, Rohit Kunnath Menon<sup>2</sup>, Neeta Mohanty<sup>3</sup>, Lora Mishra<sup>4</sup>, Gunjan Srivastava<sup>5</sup>, Divya Gopinath<sup>6</sup>

9187-9193



 PDF

### **Effect of Modern (Junk) Food in Dental Caries**

Manisha Nayak

9194-9197

 PDF

### **Adenocystic Carcinoma of Salivary Gland: A Literature Review**

Santosh Kumar Subudhi<sup>1</sup>, Sthitaprajna Lenka<sup>1</sup>, Kalyan Sundar Pal<sup>2</sup>, Satchidananda Meher<sup>2</sup>, Aditi Avasthi<sup>2</sup>

9198-9201

 PDF

### **Glandular Odontogenic Cyst**

Arpita Kar

9202-9205

 PDF

### **Caries Risk Assessment-Protocols and Paraphernalia**

Swati Patnaik

9206-9210

 PDF

### **Osteomyelitis of Jaw: A Review**

Ronisa Swain

9211-9216

 PDF

### **Accidental Periodontitis Induced by Orthodontic Treatment: A Review**

Sadaf Nayab

9217-9221

 PDF

### **Space Maintainer: A Review**

Subhashree Samal

9222-9225

 PDF

## **A Comparative Study on Effect of Motivation and Hope on the Well-being of Geriatric Population**

Apoorva Sharma<sup>1</sup> , Shikha Srivastava<sup>2</sup>

9226-9230



## **Challenges of Allied Healthcare Professionals in India-Establishing the Council is Need of the Hour**

Alka Rawekar<sup>1</sup> , Shashank Gotarkar<sup>2</sup> , Swapnil Patond<sup>3</sup> , Sarika Dakhode<sup>2</sup>

9231-9236



## **The Study of Location of Mandibular Foramen in Dry Adult Human Mandible**

Chimurkar Vilas<sup>1</sup> , Patond Swapnil<sup>2</sup> , Anjankar Vaibhav<sup>3</sup> , Pande Varsha<sup>4</sup>

9237-9242?



## **A Study of Rhodamine-6G Stain Expression for Detection and Association of Copper Content in Potentially Malignant Disorders and Squamous Cell Carcinoma**

Himanshu Singh<sup>1</sup> , Sushruth Nayak<sup>2</sup> , Prachi Nayak<sup>3</sup>

9243-9248



## **Effectiveness of Deep Brain Stimulation in Iraqi Patients with Parkinson Disease**

Bassam Mahmood Flamerz Arkawazi<sup>1</sup> , Anwer Noori Hafdh<sup>2</sup> , Zaki Noah Hasan<sup>3</sup> , Awfa Aktham

Abdullateef<sup>4</sup>

9249-9254



## **Study of Some Immunological Parameters with Level of Iron in Women Infected with Trichomonas Vaginalis**

Lima Tariq Youash Lazar<sup>1</sup> , Kasim Sakran Abass<sup>2</sup>

9255-9260



## **Heuristic "Bottom-up": COVID-19**

Taranjeet Duggal<sup>1</sup> , Ayush Sundriyal<sup>2</sup> , Abhishek Kakkar<sup>2</sup> , Neeti Mittal<sup>2</sup>

9261-9268



## **Effect of Vestibular Stimulation on Postural Stability and Mobility in Elderly**

Jain Ankit<sup>1</sup> , Sarkar Aparna<sup>2</sup> , Gupta Meena<sup>3</sup>

9269-9278

 PDF

## **Factors Affecting Recurrent Lateral Ankle Sprain in Athletes**

Pooja Sharma<sup>1</sup> , Shaurya Dayal<sup>2</sup>

9279-9283

 PDF

## **Impact of COVID-19 Pandemic on the Mental Balance among Health Care Workers**

Savita Pohekar<sup>1</sup> , Ranjana Sharma<sup>1</sup> , RuchiraAnkar<sup>1</sup>

9284-9288

 PDF

## **L-ascorbic Acid Supplementation Ameliorates Sodium Fluoride Induced Oxidative and Nitrosative Hepatic Damage in Hypoxic Rats**

Jaya Simha Reddy<sup>1</sup> , R. Chandramouli Reddy<sup>2</sup> , Raju H. Taklikar<sup>3</sup> , Kusal K. Das<sup>2</sup>

9289-9296

 PDF

## **To Assess Knowledge, Attitude and Practice on the Use of Automated External Defibrillation (AED) by Emergency Medical Services (EMS) Providers in Pune, India**

Parag Rishipathak<sup>1</sup> , Shrimathy Vijayaraghavan<sup>2</sup> , Anand Hinduja<sup>3</sup>

9297-9301

 PDF

## **Whither to Public Interest-The Curious Case of Compulsory Drug Licensing in Indian Pharmaceutical Industry**

Rakesh Kumar Sehgal<sup>1</sup> , R.L. Koul<sup>2</sup>

9302-9306

 PDF

## **A Study on Sociodemographic Profile and Comparison of Pattern of Facial Injuries in Victims of Two Wheeler Road Traffic Accident with and without usage of Helmet in South Chennai**

P. Shruthi<sup>1</sup> , R.Venkat<sup>2</sup>

9307-9312

 PDF

## **Quality of Life among Post CABG Patients**

Ambina K.1 , Shalimol U.S.2 , Anjana A.P.2  
9313-9320



### **Pulpectomy: A Comprehensive Review**

Akankhya Jena  
9321-9324



### **Ethical Marketing in Orthodontics: A Review**

Sumita Mishra<sup>1</sup>, Ananya Panda<sup>2</sup>, Smruti B. Nanda<sup>3</sup>  
9325-9328



### **Effect of Nursing Intervention on Prevention of Exclusive Breastfeeding Discontinuation**

Sahar S. Kamal<sup>1</sup>, Wafaa E. Ouda<sup>2</sup>, Safy S. Al-Rafay<sup>2</sup>, Fatma M. Mohamed<sup>3</sup>  
9329-9334

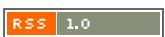
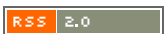


### **Relationship between the Safe Benzene Concentration with Blood Profile of Printing X Surabaya Workers**

Aisyah Noor Shofi Hanifah, Abdul Rohim Tualeka, Dani Nasirul Haqi, Syamsiar S Russeng, Pudji Rahmawati, Ahsan Ahsan, Indri H Susilowati  
6211-6218



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# Comparative Test of Midazolam Hydrochloride Stability in Different Storage and Temperature Container

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## Abstract

**Background:** Midazolam hydrochloride is an injection form of benzodiazepines and included in the high alert category in the Dr. Soetomo Teaching Hospital Intensive Care Unit (ICU), Surabaya, Indonesia. Nowadays, the technical preparation of midazolam drugs in the ICU room by doctors and nurses was performed by reconstituting drugs in the injection syringes to be stored for a while in the room. **Objectives:** To evaluate the preparation process for midazolam hydrochloride injection which has been analyzed in Dr. Soetomo Teaching Hospital. **Methods:** Physical, chemical, and microbiological stability of 1 mg/mL midazolam hydrochloride in aqua pro injection solvents have been evaluated at points 0, 8, 12, and 24 hours after preparation, as well as a comparison of stability in storage conditions at room temperature compared to refrigerator temperatures. **Results:** The results showed all the preparations were no foreign particles in the sample container. The midazolam did not experience precipitation in both room and refrigerator temperatures. The pH of midazolam solution during storage proved that the preparation was relatively stable (pH 4.3–4.5). There were no significant differences of midazolam levels in both storages which was in range of 95%. The Microbiology Stability Test showed negative germ growth after 24 hours incubation in both storage. **Conclusion:** This study showed no changes in physical, chemical, or microbiological stability in the midazolam injection samples up to 24 hours after the manufacturing process.

**Keywords:** Midazolam Hydrochloride, Injection Preparation, Aseptic Technique, Stability Test

## Introduction

The stability of pharmaceutical dosage forms is strongly influenced by chemical, physical, and microbiological reactions. These reactions can lead the changes of medicinal properties during the process of shipping, storage, and use. Chemical degradation is known as the cause of a potential decrease or increase in drug toxicity. The storage condition also cause dissolution and pollination process of other medicinal ingredients, so that affect to the stability of the drug<sup>1</sup>.

Midazolam hydrochloride is an injection form of benzodiazepines with a short reaction period. Intramuscular route is often used for midazolam administration, then continuous to iv infusion and iv slow bolus<sup>2</sup>. Intramuscular midazolam administration can terminate acute seizure in children besides intranasal midazolam<sup>3,4</sup>. Previous research showed the stability of midazolam is around 24 hours in normal saline 0.9% or Dextrose 5%<sup>5,6</sup>. Technical midazolam preparation is usually carried out by the drug reconstitution of injection syringes preparation and drugs storage in the room. It aims to facilitate the administration in emergency condition, which needs immediate administration<sup>7,8</sup>.

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Patient safety becomes priority in the management of drug use (MDU). The policy of MDU, namely standard operational procedure for mixing injectable

medicines, explains that the single component does not recommend storing reconstitution drugs in injection syringes, but in sterile vial containers covered by film paper<sup>9</sup>. The reconstitution drugs do not stored for more than 8 hours<sup>9</sup>. However, the MDU policy on injecting drug reconstitution is not supported by accurate data from preliminary research results both qualitative and quantitative aspects.

Baseline reference for the MDU policy for the reconstruction of single preparations is existed in Dr. Soetomo Teaching Hospital. This preliminary study determined the qualitative and quantitative stability of 1 mg/mL midazolam injection in aqua pro injection solvents, whether the stability of the drug was more than 8 hours or 24 hours. The stability test compared to the parameters of the storage container will be observed from the room temperature. The room temperature was 20-25 °C, compared to the refrigerator temperature (2-8 °C). The qualitative stability measured by physical, chemical, and microbiological stability. However, quantitative stability is conducted by determining levels using High-Performance Liquid Chromatography (HPLC) tools. The results of this study can be a reference data for the guidelines evaluation for injecting drug mixing policy in Dr. Soetomo Teaching Hospital. The purpose of this study determined by using qualitative and quantitative stability of midazolam reconstitution HCl 1 mg/mL with storage container parameters.

## Methods

### *Material*

Raw midazolam hydrochloride was a gift from Kalbe Farma Factory. Acetonitrile and potassium hydrogen phosphate were Wako products (Osaka, Japan). Sterile glass vials and injection syringes (Terumo®, Tokyo, Japan) were purchased from the Medical Pharmacy Service Unit, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. Other research materials were analytical grade.

### *Test Solution Preparation*

Preparation of the test solution sample was examined by the same possible conditions that was conducted by the nurse or doctor when reconstituting midazolam hydrochloride in the ICU inpatient ward of Dr. Soetomo

Teaching Hospital. Two midazolam hydrochloride test solutions of 1 mg/mL were prepared for replication three times per test procedure. The first solution was prepared in a Terumo® injection syringe container (containing polypropylene material) and a second solution was prepared in a sterile vial storage container covered with parafilm paper. The first mixing container (5 mL injection syringe, Terumo®) was filled by midazolam hydrochloride 1 mg/mL. The concentration was obtained by mixing technically as follows: A total of 1 ampule of midazolam hydrochloride 5 mg/mL was diluted and a number of 20 mL aqua pro injection to obtain a 1 mg/mL level test solution. The solution was taken 1 mL to be stored in a 5 mL injection syringe. The second solution was prepared in a sterile vial storage container with a dilution procedure: 1 ampoule midazolam hydrochloride 5 mg/mL diluted and 20 mL aqua pro injection to obtain a 1 mg/mL level test solution. Then, 1 mL solution was stored in a sterile vial container.

This study also revealed the effect of storage temperature on the stability of midazolam hydrochloride reconstitution preparations. Three solutions test were stored at room temperature (20-25 °C) and at refrigerator temperature (3-4 °C). At the time of testing, the sample was taken by 1 mL from the storage container using aseptic techniques. The sampling point in this study was carried out at 0 o'clock (shortly after preparation), 8, 24, and 48 hours after preparation.

### **Preparation Stability Test**

The samples were taken at 0, 8, 12, and 24 hours after preparation, then evaluated for physical, chemical, and microbiological stability. Physical stability tests was performed, including observe color's solution and it's clarity. The solution clarity was tested by placing a sample above lighting and observed by rotating the sample and flipping the container against a black or white background (Erweka Apparatus Gmens Hensen Stamm, Germany). Thus, it could clarity and the presence of foreign particles in the test sample were observed.

The sample was taken by 5 mL for physical stability checks. Microbiological stability tests were examined by the sterility requirements of intravenous preparations. Sterility tests were conducted on bacterial growth media in microbiology laboratories. Chemical stability tests was examined including pH testing and levels determination

using HPLC. PH measurement was performed because the pH can affect the preparation stability. the pH measurements of midazolam preparations after storage was conducted by using a pH meter (Eutech Instruments pH 700, Germany) at 25 °C.

*Midazolam Hydrochloride Analysis Method Using HPLC*

Midazolam hydrochloride levels were quantitatively measured using HPLC. The analysis conditions were as follows: the stationary phase uses an HPLC C18 column, the detector used was a photodiode array (PDA), and the analysis was analyzed at a wavelength of 220 nm, the mobile phase was acetonitrile: 0.3 M potassium dihydrogen phosphate pH 3.3 (3:7 v/v) and a flow rate of 1 mL/minute, and the injection sample volume was 10 µL

Standard solute was prepared by dissolving midazolam hydrochloride at a level of 1 mg/mL in methanol to be injected in the HPLC. Furthermore, it analyzed the peak area of the chromatogram. The results will be used to measure midazolam levels quantitatively by using a comparison of the chromatogram peak area of the sample with the standard 1 mg/mL level midazolam solution.

**Statistic Analytic**

For qualitative analysis, the results were compared to the initial solution visually. If there were differences, there was a qualitative change in stability. For quantitative analysis, the test solution resulted stable if midazolam hydrochloride levels in the test solution >90% initial levels. Statistical analysis used one-way analysis of variance followed by Tukey’s post hoc test with a confidence level of 0.05 to determine the significance differences between all the results The test used SPSS version 23.0 (SPSS, Inc., Chicago, IL).

**Results**

**Solution Clarity test**

This study found that all preparations were clear and there were no deposits or foreign particles in the sample container (Table 1). During storage phase, the midazolam did not experience precipitation in both storage. However, there were substances in most vial samples such as fibers (unpublished data) observed in >50% of the test samples (±50 vials) which were not observed in the test samples with polypropylene syringes.

**Table 1. The results of the clarity observation of the midazolam solution in Intensive Care Unit’s inpatient ward Dr. Soetomo Teaching Hospital (n=3).**

No	Sampling-Time	pH Midazolam injection			
		Sput		Vial	
		Room temperature	Refrigerator temperature	Room temperature	Refrigerator temperature
1	0th hour	*	-	*	-
2	1st hour	*	*	*	*
3	8th hour	*	*	*	*
4	12th hour	*	*	*	*
5	24th hour	*	*	*	*

Note: \* = there is no sedimentation



*pH Test*

All of samples did not experience significant pH changes, where the mean pH of the samples was around 4.3 to 4.5, at the 1<sup>st</sup> and 24<sup>th</sup> hours (Table 2). The test

sample showed pH in the range of 4.4 as for the 0th hour. Thus, there was no change in the pH of the midazolam solution during storage, both room temperature and refrigerator temperature, proving that the relatively stable preparation was kept until the 24<sup>th</sup> hour.

**Table 2. The results of the pH test in midazolam in the Intensive Care Unit inpatient ward of Dr. Soetomo Teaching Hospital (n=3).**

No	Sampling-Time	pH Midazolam injection			
		Sput		Vial	
		Room temperature	Refrigerator temperature	Room temperature	Refrigerator temperature
1	0th hour	4.41±0.05	-	4.38±0.05	-
2	1st hour	4.36±0.05	4.47±0.03	4.39±0.01	4.47±0.13
3	8th hour	4.41±0.06	4.42±0.05	4.40±0.05	4.50±0.02
4	12th hour	12th hour	4.49±0.08	4.45±0.06	4.58±0.01
5	24th hour	4.32±0.05	4.50±0.02	4.36±0.07	4.49±0.05

**Microbiology Stability Test**

The sterility test resulted that 6 test containers representing about 3% of the total sample and showed no microbial contamination in the vial container. In general, after incubation, the test results showed negative results, it can be declared as sterile. However, there was a sample from 3 replications showed a *Staphylococcus pseudintermedius* culture at 8 o'clock (Table 3).

**Table 3. The results of the microbiological stability test of midazolam in Intensive Care Unit's inpatient ward Dr. Soetomo Teaching Hospital (n=3).**

No	Sample	Storage temperature	Microbiological Test Results at the hour			
			0	1	12	24
1	Sput	Room temperature	Negative	Negative	Negative	Negative
		Refrigerator temperature	-	Negative	Negative	Negative
2	Vial	Room temperature	Negative	Negative	Negative	Negative
		Refrigerator temperature	-	Negative	Negative	Negative

*Stability Test for Midazolam Levels*

By using a comparative analysis of the chromatogram peaks of the raw midazolam material. A quantitative level of the drug sample was obtained (Table 4).

**Table 4. HPLC test results on drug levels of midazolam injection samples after 24 hours in the Intensive Care Unit's inpatient ward of Dr. Soetomo Teaching Hospital (n=3).**

Sampling-Time	Midazolam Level (Persect)			
	Sput		Vial	
	Room temperature	Refrigerator temperature	Room temperature	Refrigerator temperature
24th hour	90.31±7.59	91.97±19.93	95.68±3.14	96.85±8.92

The level of midazolam at the 0 minute was lower than the data at the 24th hour, both stored at room temperature and refrigerator. After 24 hours, the levels of medicinal ingredients were still 90.31%-96.85%, both samples stored in vials and polypropylene syringes. There were no significant differences between the storage temperature in the room and the refrigerator. Moreover, midazolam concentration were in the range of 95% after 48 hours in refrigerators, either by using vial containers or injection syringes. At this point, samples at room temperature were not tested due to the technical problems. The coefficient between measurement replications was still too large (in range 3.28%-21.67%).

### Discussion

This study proved that midazolam hydrochloride injection, which was prepared using aseptic technique, has good stability until the 24<sup>th</sup> hour after the manufacturing process. Both glass vial and injection syringes didn't show significant effect on drug content as well as 24-hours storage temperatures. Previous research explained that midazolam stability was influenced by pH, storage temperature, light, and storage containers<sup>6</sup>. This study found that the midazolam injection was still stable, both physically, chemically, and microbiologically until the 24<sup>th</sup> hour after its mixture. Recent study showed stable diluted concentration of midazolam was 0.03 mg/mL to 0.5 for 24 hours with 5% dextrose or 0.9% sodium chloride injection<sup>10</sup>. On the other hand, midazolam 5 mg/mL was stable at room temperature for 100 days in polypropylene syringes<sup>11</sup>.

Treatment of samples was divided into 2 categories which used different containers, sterile glass vials and injection syringes containing polypropylene. The results showed that there was no significant difference between midazolam injection which prepared in sterile glass vial containers and injection syringes in any storage conditions. PH measurement showed unchanged results between observation times, which around 4.3-4.6 with stable physical conditions and observed the deposition of drug ingredients during storage. Moreover, more than 90% of the initial concentration of midazolam can be observed in injection samples at 24 hours after preparation, even at 48 hours in samples stored in the refrigerator. This data was in line with the previous studies which have shown that midazolam solution was stable even up to 5-weeks after manufactured and there was no midazolam adsorption process in injection syringe containers<sup>5</sup>. In the 0-hour sample, the results of the determination of the levels were smaller than the 24<sup>th</sup> hour which was about 81-86%. This was possible because of over filling after the dilution process of 5 mg/mL midazolam levels with aqua pro injection to a level of 1 mg/mL which results in a diluted final concentration.

In addition, sterility tests for injection samples have also been revealed. This is an important parameter for preparations of parenteral routes. The results showed that most of the samples showed sterility to germs/microbes. However, there were 1 in 3 sample replications which showed contamination by *Staphylococcus pseudintermedius* contained in the free air. This was possible because there was of working technique on a sample that less accurate due to drugs preparation.

Thus, it was necessary to limit the number of midazolam injection samples to avoid incorrect technique, so that will obtain perfect sterility. Besides, there were several contaminations, such as fine fibers in most of the glass vial container samples which were not found in the injection syringe container. This was the main evaluation in the sterile vial sterilization process.

These data were expected to be a guide or reference for the preparation process of midazolam injection in hospitals, especially Dr. Soetomo Teaching Hospital, and generally other hospitals in Indonesia with proper aseptic techniques. Further studies needs to concern about dose uniformity after the dilution process as important information for proper preparation of midazolam hydrochloride in the ward. This was an attempt to ensure dose uniformity between injection sample, which this study found that the coefficient of variation between replications was still too large, around 3-22%.

### Conclusion

This study showed midazolam hydrochloride injection had good physical, chemical, and microbiological stability until the 24<sup>th</sup> hour after the manufacturing process. Vial and injection syringes did not show a significant effect on drug content in 24-hours storage temperature. However, midazolam injection was possible to be contaminated by microorganism because of less accurate technique during preparation.

### Acknowledgement

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**Ethical Clearance:** This study has been approved ethically by the Medical Research Ethics Committee of Faculty of Medicine, Universitas Airlangga Surabaya.

**Conflict of Interest:** There is no conflict of interest

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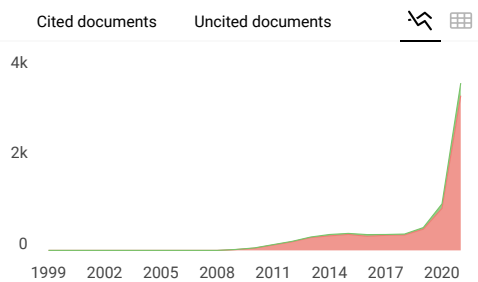
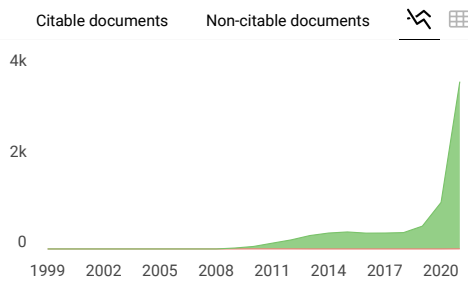
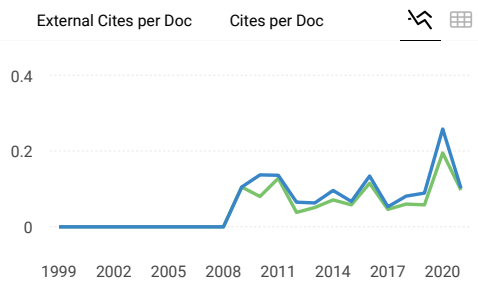
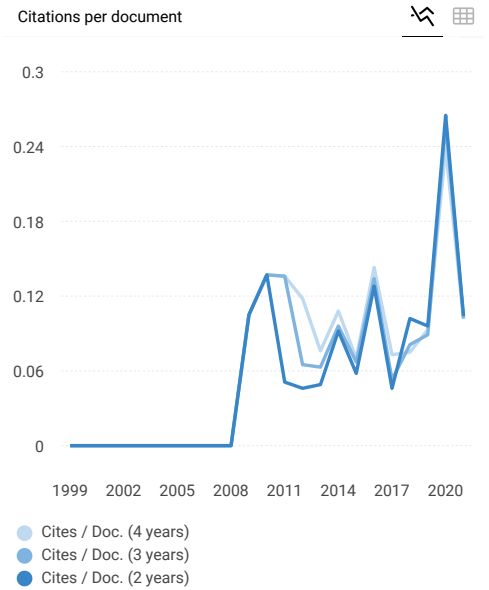
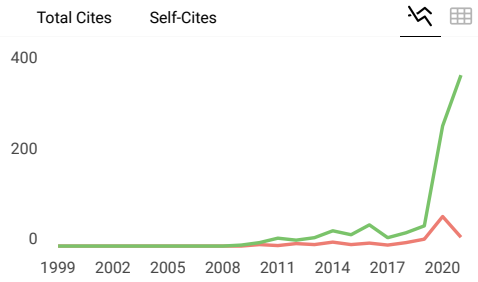
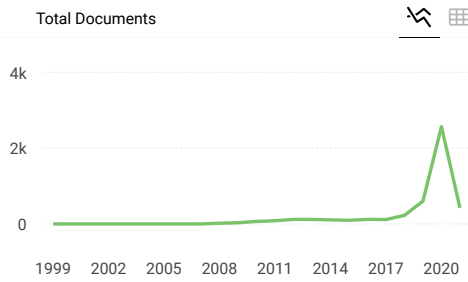
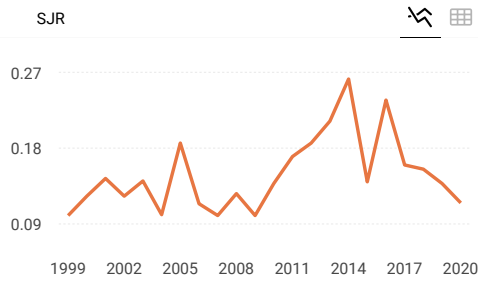
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