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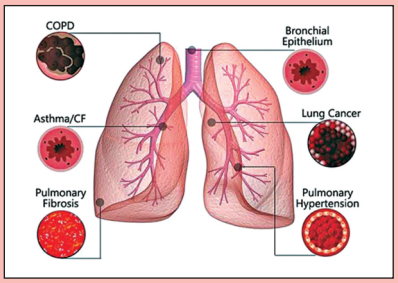

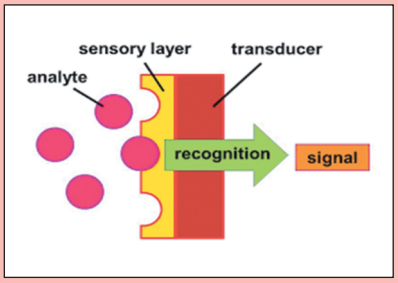

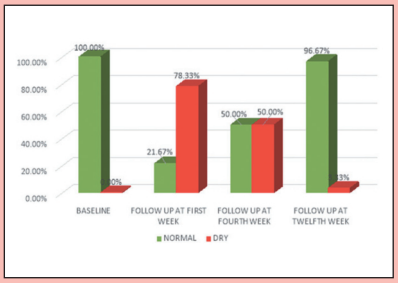

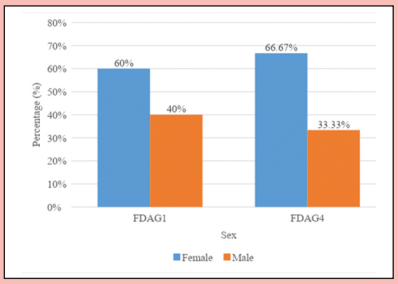

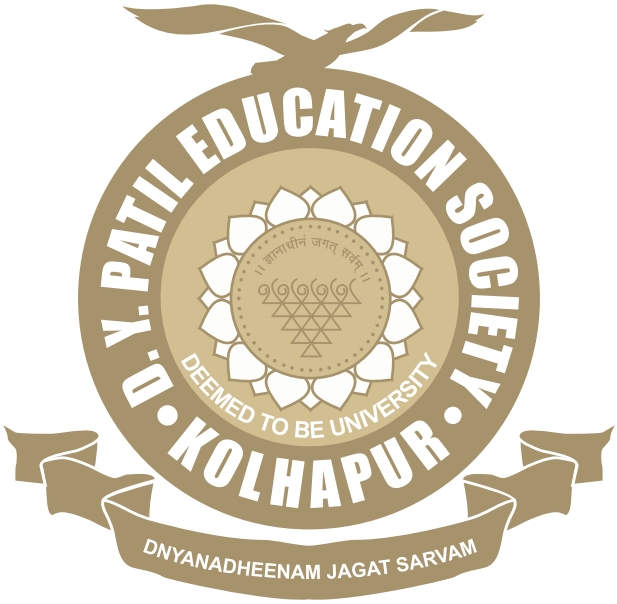

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AI-Powered Smart Platform Revolutionizes Infant Gastroesophageal Relaxation (GER) Management

Lack of non-pharmacological therapies makes treating Gastrointestinal Esophageal Relaxation (GER) in infants difficult. Pediatricians often prescribe antacids or proton pump inhibitors, which have long-term adverse effects. An alternative technique without medication-induced adverse effects is needed. Our innovative smart relaxing platform treats GER non-pharmacologically. AI-powered and working at a pioneering Technology Readiness Level (TRL) IV TO VII, this non-invasive marvel will change newborn care. The combination of AI intelligence and complex rocking and swaying motions on our platform soothes infant irritation beyond traditional limitations. The three varied positions of feeding, sleep, and transit drive our creativity. This triad offers a complex, non-pharmacological GER treatment with great promise. Each unit is comfortable and durable, made of breathable cotton and stainless-steel gauges. Innovation is typically driven by persistent problems. Gastroesophageal Relaxation (GER) has long puzzled parents and healthcare experts in baby care. Infants often regurgitate stomach contents into the esophagus, which can be stressful for both the kid and parents. Pharmacological treatments for GER have dominated. Pediatricians often prescribe antacids or proton pump inhibitors for symptoms. These treatments provide relief, but also have long-term adverse effects, which worries parents and doctors. It was evident that GER needed a non-pharmacological therapy without the risks of drugs. This urgent necessity led Dr. Abhinandan R. Patil and Dr. Vijay T. Mali (Pediatrician, D. Y. Patil Hospital Kolhapur) to create the AI-powered smart relaxing platform. This extraordinary Technology Readiness Level (TRL) IV through VII innovation redefines newborn care by treating GER non-pharmacologically. This smart relaxation platform uses AI to calm and support infants. The platform soothes newborn irritation with AI-controlled rocking and swaying. It moves between feeding, sleeping, and transporting without bounds. This trio of features guarantees the platform meets infants' and caregivers' diverse demands. This idea stands out for its non-pharmacological care. By avoiding drugs and focusing on infant comfort, it manages GER effectively and without adverse effects.

The platform has breathable cotton and stainless-steel gauges and was meticulously engineered. Each unit is comfortable and high-quality, ensuring infants' comfort while using the gadget.

As the world sees this innovative solution, the AI-powered smart relaxation platform will transform newborn care. The efforts of Dr. Patil and Dr. Mali has led to this potential alternative for GER control and a leap forward in non-pharmacological newborn healthcare. Infant care will be more peaceful, reassuring, and compassionate in the future.

Innovative Nutraceutical Formulation for GI Health and Colon Cancer

Due to the growing use of fast food containing harmful compounds like ajinomoto, present in bakery items, bowel disturbances and GI health issues have become popular concerns. This has increased gastro illnesses, requiring appropriate preventative measures. Immune booster nutraceuticals like vitamin capsules or pills are not widely accepted or palatable. In response, we identified two novel bacteria, *Lactobacillus plantarum* and *Lactobacillus rhamnosus*, that prevent gastrointestinal cancer and are used in biscuits without maida (refined wheat flour) and enhanced with prebiotics and probiotics. This unique approach overcomes toxic additive health issues and provides easy, tasty, and widely accepted preventive therapy. The contemporary period has offered quick food, but it has also brought health concerns, notably GI health. Fast food, often laden with hazardous substances like ajinomoto, has caused a rise in gastro illnesses and related problems, necessitating strong preventative measures. Dr. Abhinandan Patil began a discovery adventure to address this health issue. Their goal: to create a novel approach that addresses toxic additive health issues and provides easy, appealing, and widely accepted preventive treatment. *Lactobacillus plantarum* and *Lactobacillus rhamnosus* were their breakthrough bacteria. These microbes showed remarkable gastrointestinal cancer prevention, a breakthrough in nutraceuticals and preventative medicine. Innovative ideas continued after the discovery of these potent microbes. Dr. Patil understood healthcare delivery and palatability. He used an innovative method instead of immune booster nutraceuticals like vitamin capsules or pills. He added these helpful microorganisms to bakery products, a popular meal. Beautifully made without maida (processed wheat flour), these products are full of prebiotics and probiotics. Fusion not only ensures seamless delivery of the preventive benefits of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* in a tasty and commonly accepted diet. The innovation revolutionizes preventive care. It addresses the growing health risks of harmful chemicals in modern diets with a practical, universal palatability-compliant approach.

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STEM CELL THERAPY AND FUTURE APPROACHES ON LUNG DISEASES

Neha Kumamekar[*], ***Meghnad G Joshi***[**]

ABSTRACT

Respiratory diseases, including COVID-19 and COPD (Chronic Obstructive pulmonary disease), are the leading causes of global fatalities. There are no effective or curative treatments, only supportive care. Cell therapy is a promising strategy for refractory and unmanageable pulmonary illnesses, as preclinical studies have proven. Stem cells consist of cells with the potential to differentiate into cell types for repair, such as mesenchymal stromal cells, endothelial progenitor cells, peripheral blood stem cells, and lung progenitor cells. Numerous phase I/II clinical trials have confirmed the safety and feasibility of stem cell and extracellular vesicle administration in patients with COPD, ARDS, bronchial dysplasia, IPF, pulmonary artery hypertension, and silicosis. Different doses and routes have been tested for tolerance and advantages. This review systematically summarizes global trends of cell therapy for common airway/lung diseases registered for clinical trials, as well as future directions for new trials/preclinical trials.

Keywords : Lung, stem cells, Respiratory diseases, COPD, Regeneration.

INTRODUCTION

Respiratory diseases are a leading cause of death worldwide. Acute and chronic lung diseases, including COVID-19, ALI/ARDS, BPD, PAH, silicosis, sarcoidosis, extensively drug-resistant tuberculosis, COPD, and IPF have high morbidity and mortality. COPD is the third leading cause of global fatality. Preclinical models suggest cell-based therapy may be a promising strategy for repair, such as MSCs from umbilical cord blood, bone marrow, adipose tissue, or placenta. Stem cells may modulate the immune response, alveolar fluid clearance, cell fate, and drug delivery through paracrine and autocrine mechanisms, mainly via exosomes. Clinical trials registered to four databases from 1 are underway to study the safety and benefits of cell therapy for airway and lung diseases.

Many chronic lung diseases, such as COPD and fibrosis, are caused by changes in the cells that compose the lungs. Stem cells are a key cell population and their job is to sustain the normal lung structure. When triggered by certain cues, they divide to replace worn-out or damaged specialist lung cells. In chronic lung diseases, this process can go awry resulting in long-term breathing complications and death.

Pneumonia has a high mortality rate, particularly among the elderly. It is usually caused by a lung infection from flu or COVID-19. Severe pneumonia is

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treated with mechanical ventilation, which can damage the lungs. To repair this damage, stem cells should be activated to produce new healthy lung tissue; however, when the damage is too severe, fibrosis (when healthy cells are replaced by scar tissue) can occur instead. To discover new treatments for these ailments, it is essential to gain insight into how stem cells in the lungs facilitate repair and how stem cell behavior is altered in chronic lung diseases and severe infections, which is crucial for finding new treatments.

Lung diseases:

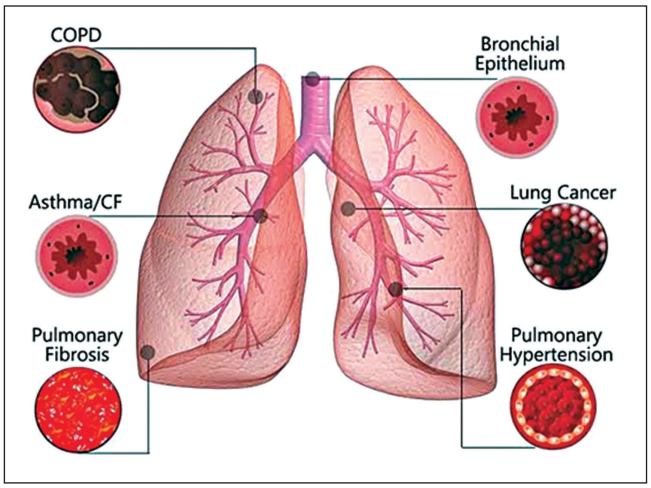

Fig. 1 : Lung Diseases

COPD is a condition that causes respiratory problems and airflow obstructions. They worsen substantially over time. The two most common types of COPD are emphysema and chronic bronchitis, but many people with COPD have both. Find below a bit of brief on both of these conditions:

Emphysema: In emphysema, the alveoli walls are damaged. As a result, damaged walls of the alveoli eventually lose their shape and elasticity, making more air trapped inside them as we breathe out. This trapped air will continue to distend the alveoli, resulting it harder and harder for us to breathe in new oxygen. The effects of hyperinflation result in the lungs losing their ability to oxygenate blood effectively which is a serious and life-threatening problem.

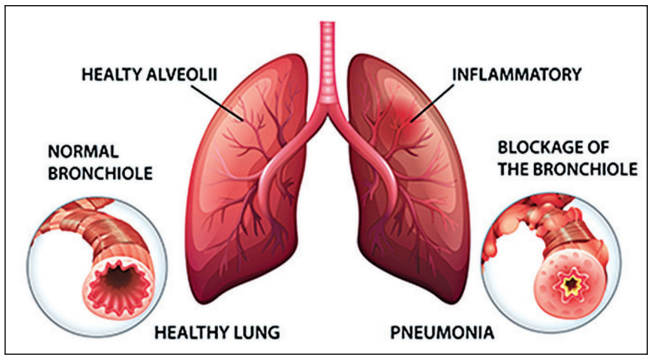

Fig. 2 : Chronic Obstructive Pulmonary Diseases

Chronic bronchitis: If you have coughing, shortness of breath, and excess mucous production lasting more than three months in a row for two years or more, this indicates chronic bronchitis. The cilia which line your bronchial tubes help to move the mucus out of your lungs by brushing against it. When this happens, you'll become more susceptible to developing chronic bronchitis because the cilia begin dying off one after the other. A loss of cilia means that there are fewer brushing motions happening along within each bronchial tube and therefore a build-up of excess mucus is created.

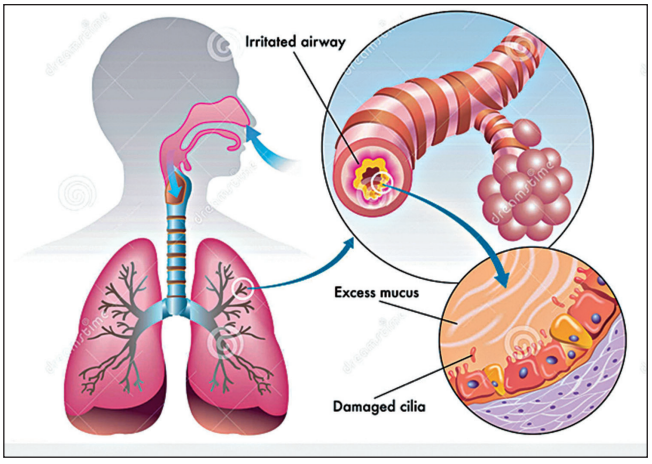

Fig. 3 : Chronic Bronchitis

Causes

Cigarette smoking is the leading cause of COPD. Most people who have COPD smoke or used to smoke.

However, up to 25 percent of people with COPD never smoked. Long-term exposure to other lung irritants—such as air pollution, chemical fumes, or dusts—also may contribute to COPD.

COPD may be due to infection by bacteria or virus. The infection causes inflammation leading to narrowing of air passages

SYMPTOMS

Understanding how the lungs function will help in understanding the disease. The air that we breathe goes down through pipes through our windpipe into the lungs called Bronchial tubes or airways. These airways branch into thousands of thinner, smaller tubes called bronchioles. These tubes end in bunches of tiny round air sacs called alveoli. When air reaches the air sacs, oxygen passes through the air sac walls into the blood in the capillaries. At the same time, CO_2 moves from the capillaries into the air sacs. Stem cell treatment for lung disease is the best cure possible.

Some of the common symptoms include:

Constant cough that won't go away, cough up mucus often, Shortness of breath, especially when you exercise, feeling of tightness in your chest, change in the color or thickness of mucus, In COPD, less air flows in and out of the airways because of one or more of the following reasons: The airways and air sacs lose their elastic quality. The walls between many of the air sacs are destroyed. The walls of the airways become thick and inflamed. The airways make more mucus than usual, which can clog them.

DIAGNOSIS

To diagnose COPD, medical history and physical exam is a must. These will give your doctor important information about your health. Following test may be performed, Lung function, Chest X-rays.

Some other tests include- Arterial blood gas test measuring the content of oxygen, carbon dioxide, and acid in your blood, an oximetry test measuring the oxygen saturation in the blood, an electrocardiogram (ECG, EKG) test to find out heart problems causing breathing problems.

Transfer factor for carbon monoxide test to find out the damage in your lungs. Stem cell therapy for lung cancer helps you with it.

Stem cells in the Lungs

Human lungs take 20-40 million breaths and experience daily airflow of 7,000-10,000 litres over an average lifetime. These lungs are composed of two regions: conducting airway tubes (trachea, bronchi, and bronchioles) and gas exchange regions (alveolar spaces). Scientists have found that each region contains unique stem cells which divide to replace old or damaged lung cells, thus maintaining lung health. These stem cells are tracheal basal cells, bronchiolar secretory cells (club cells) and alveolar type 2 cells; their division is believed to be enough to renew the lung structure in adulthood.

In lung diseases, the processes which produce functional cells are disrupted, reducing the lung's ability to supply oxygen and eventually leading to illness and death. For example, COPD patients experience hyperplasia of basal cells in the larger airways, loss of ciliated cells, increased mucous-secreting goblet

cells, and fewer alveolar type I cells in their alveoli. Further, healthy lungs can efficiently convert alveolar type II cells into type I cells; however, in COPD lungs this process is impaired. The environment surrounding stem cells also changes with disease, as activated macrophages and fibroblasts are present in COPD alveoli and can influence stem cell behaviour.

Stem cells play a role in lung development and regeneration. Issues with stem cells can lead to lung diseases. In mice, researchers have identified rare stem cells in the airway tubes that are capable of dividing and producing new cells to repair airways and gas exchange regions after severe injury, such as influenza. These stem cells have been grown in the laboratory and tested as a treatment on mouse lungs. It is still unclear if these rare stem cells are present and important in human lungs.

Spectrum of stem cell for Respiratory Diseases

Stem cells are divided into multipotent embryonic stem cells and progenitor cells, with MSCs being the most commonly used in clinical trials due to their safety profile and availability. MSCs can be obtained from bone marrow, adipose tissue, muscle, peripheral blood, umbilical cord blood and placenta. Of these sources, UCB-MSCs have the highest proliferation rate and anti-inflammatory ability, while BM-MSCs and AD-SCs are the most popular autologous stem cells. Other types of stem cells tested in clinical trials include EPCs, peripheral blood stem cells, placental mesenchymal stem cells, adult human stem cells, bronchi stem cells, menstrual blood-derived stem cells, bronchial basal cells, heart muscle progenitor cells and lung stem cells. Combining two or more types of stem cell is often used to treat lung diseases.

Lung stem cell in lung Regenaration

After adolescence, mammalian organs and tissues contain endogenous stem/progenitor cells that create a specialized environment known as a “niche”. This niche provides essential life conditions and is crucial for maintaining homeostasis and repairing tissue. Identifying proliferating cells in the lungs is straightforward, but classifying them into a hierarchy is more challenging. Evidence from mouse models and to some extent humans suggests there are adult endogenous epithelial stem/progenitor cells in the airways and alveoli. These pulmonary stem/progenitor cells, including bronchiolar stem cells, bronchioloalveolar stem cells, tracheal and bronchial stem cells, alveolar stem cells and alveolar type II cells, play an important role in tissue repair and preserving homeostasis.

Molecular markers, lineage tracing and clonal analysis are used to identify the roles of endogenous epithelial stem or progenitor cells in both human and mouse lungs However, there is still no consistent agreement on their identity, role and function. Although differentiated epithelial club cells have a low stable state proliferative index, mouse airways can self-renew and replace ciliated cells in trachea and distal airways during normal homeostasis or injury. It appears that these putative progenitor cells may not respond to less severe injuries, suggesting that they are not essential for normal airway epithelial homeostasis. Instead, it is believed that these progenitor cells act as a reserve population for maintenance and replenishment when the facultative progenitor pool is depleted.

Endothelial progenitor cells in lung regeneration

Endothelial progenitor cells (EPCs) are present in

adult blood and contribute to postnatal vascular repair or angiogenesis. This concept was very intriguing as it suggested that EPCs could be sourced from peripheral blood, potentially providing a new treatment for vascular diseases. Over the past 15 years, this concept has been widely discussed in over 4000 publications. Gradually, research revealed EPCs' role in the pathogenesis of a variety of lung diseases such as pulmonary hypertension, pulmonary fibrosis, asthma, acute lung injury, COPD, BPD and lung cancer in children. Injury to pulmonary microvasculature causes endothelial cells to slough off and release endothelial microparticles (EMs) and circulating endothelial cells (CECs) into circulation. Enhanced CECs have been observed in vascular diseases. This process releases a variety of angiogenic factors including vascular endothelial growth factor (VECF), which recruits EPCs to site, presumably to repair the vascular damage.

Mesenchymal stem cells (MSCs) in lung regeneration

Mesenchymal stem cells (MSCs) have been of great interest to the medical and scientific community since their discovery. They have potential applications in regenerative medicine, which could be used to treat neurological disorders, immunological diseases, cartilage damage, and irreversible lung fibrosis. MSCs are usually isolated from bone marrow, but can also be found in foetal tissues, adipose tissue and skeletal muscle and lungs. However, isolating and identifying them is complicated due to the lack of a distinguishable marker.

MSCs possess several useful functions that make them a potential therapy for regenerative medicine. These include the secretion of growth factors and anti-inflammatory cytokines, their ability to 'rescue' cells through the transfer of functional mitochondria and migration of administered cells to sites of injury. Autologous cell transplantation could be used to bypass immune rejection. This makes MSCs a potential treatment for chronic lung diseases such as obstructive bronchiolitis (OB), idiopathic pulmonary fibrosis (IPF) and COPD.

MSCs are being explored as a potential therapy for IPF, due to their anti-inflammatory properties, migration abilities and immune privilege. It is thought that MSCs can kickstart tissue regeneration through the recruitment of endogenous stem cells and by prompting local stem cell differentiation. While current treatments for COPD cannot repair or restore damage caused by emphysema, MSCs have shown promise as a therapeutic modality. This is because they can migrate to the affected area and begin repairing tissue. Stromal cell-derived factor-1 (SDF-1) is a chemokine that regulates stem cell recruitment and angiogenesis, and is secreted by MSCs. Table 1 illustrates SDF-1's role in repairing neonatal lungs. Additionally, MSCs possess anti-inflammatory and protective abilities through suppression of inflammatory cytokines and growth factor production; this could potentially be beneficial for repairing lung tissue damaged by emphysema.

Administration of MSCs is believed to inhibit the development of early airway obstruction (AO) in the heterotopic tracheal transplant model of OB. The administration of MSCs appears to be a hopeful option for the prevention of BOS in transplant patients. In many patients, transplantation or retransplantation is the only option for end-stage lung disease

Bone marrow–derived stem cells in lung regeneration

Bone marrow is the major storage site of stem cells,

which are the primary source of stem/progenitor cells outside the lungs. These potentially regenerative cells include endothelial progenitor cells (EPCs), hematopoietic stem/progenitor cells (HSPCs) and bone marrow-derived mesenchymal cells (BMSCs). During infection, acute injury or administration of a mobilizer, they leave the bone marrow pool and migrate towards the injured lung tissue under the influence of chemokines. In addition, they have a positive role in repairing differentiated cell types (8). Studies in mice with an endotoxin-induced model of ALI showed that administering BMSCs shortly after injury leads to pulmonary hemorrhage, alveolar edema and reduced vascular permeability. This is accompanied by decreased levels of pro-inflammatory cytokines (INF-γ, IL1β, IL-6 and macrophage inflammatory protein 1α) and macrophage inflammatory protein 1α) and additional up-regulation of anti-inflammatory cytokines (IL-10) are responses thought to be mediated by both soluble factors and direct cell-cell contact.

Bone marrow-derived mesenchymal stem cells (BM-MSCs) inhibit T-lymphocyte proliferation and human monocyte differentiation into dendritic cells both through direct contact and the secretion of soluble factors. BM-MSCs have been shown to increase survival in transplant recipients with graft-vs.-host disease, though the exact mechanism is still unknown. Evidence suggests that direct contact is necessary for this effect, while other studies point to a role for soluble factors. Further research is needed to determine if each system plays a role in this process. If BM-MSCs can be mobilized to sites of acute injury, reduce inflammation by regulating cytokine production, and contribute to tissue repair, they may be useful for treating conditions causing organ damage such as ALI/ARDS. In a murine model of bronchiolitis obliterans, intraperitoneal administration of BMSCs had significant effects on cytokine levels and lung tissue histopathological lesions.

Adipose-derived stem cells (ADSCs) in lung regeneration

Adipose tissue-derived stem cells (ADSCs) have shown potential for use in regenerative therapies due to their multipotency, low morbidity when collected through minimally invasive techniques, and low immunogenicity. They are able to differentiate into various cell types, such as osteocytes, adipocytes, neural cells, vascular endothelial cells, cardiomyocytes, pancreatic cells, and hepatocytes. In addition, they produce trophic factors that can support regeneration and therapy. Compared to bone marrow stem cells (BMSCs), ADSCs can be easily harvested from adipose tissue in large quantities. A pilot study showed the protective effects of ADSCs on acute lung injury and improved subacute airway remodeling in rats.

Embryonic stem cells in lung regeneration

The use of differentiating cultures of embryonic stem cells as an alternative source of committed lung progenitor or stem cells has increased recently. Reports have shown conditions that contribute to the differentiation of mouse embryonic stem cells into heterogeneous populations, with subsets expressing markers such as SP-C and CCSP. Manipulating the culture conditions for directed differentiation may improve the efficiency of producing lung progenitor cells in vitro. Human embryonic stem cell-derived progenitor cells could also be used to ameliorate sepsis-induced inflammatory lung injury, and functional airway epithelium can be obtained from human embryonic stem cells through extensive generation measures.

Many devastating lung diseases such as IPF, cystic fibrosis, and COPD have no cure and cause significant mortality and morbidity. In addition, the prevalence of lung diseases, especially COPD, is increasing. It is predicted that COPD will be the third leading cause of death worldwide by 2020. A limited number of suitable donor lungs and lung transplants are available, a fact that is further complicated by significant transplant failure and complications from immunosuppressive drugs. An alternative to classic organ replacement is urgently needed. The engineering of bioartificial organs, using either natural or synthetic scaffolds, is an inspiring option for the production of functional lung tissue for human clinical use. Natural organ scaffolds can be made using native tissues that have undergone decellularization. Acellular scaffolds maintain native organ ultrastructure and can be seeded with autologous cells to regenerate functional tissues. Several decellularization strategies have been employed for the lung; however, there is no consensus on the optimal approach. Several cell types have been investigated as potential candidates for efficient recellularization of acellular lung scaffolds

Candidate cells that may be the most suitable are those that can be easily separated, expanded in vitro, seeded onto decellularized matrices, and easily differentiated into lung lineage cells with high rates of survival to functional maturity. Whole lung cell suspensions, induced pluripotent stem cells (iPSCs), embryonic and adult stem cells, and endogenous progenitor cells were examined for their applicability to repopulate acellular lung matrices. Because of its potential to reduce immunosuppression after transplantation, lung recellularization is performed using the patient's own autologous cells. The challenges in developing complex 3D functional lung tissues ex vivo will be to recapitulate the normal 3D dynamic integrated network of cells in the appropriate environment and architecture. Other approaches such as human capillary endothelial cells and human epithelial cells attached to porous polydimethylsiloxane chips can simulate the alveolar architecture and can be used to study pathophysiological processes and also for high-throughput drug screening. However, it is still practicable to make part of the upper or lower airway or alveolar tissue. Indeed, important advances have recently been made using decellularized or synthetic scaffolds to create tracheal cartilage as well as tendon tissue in the diaphragm for clinical use. MSC-derived chondrocytes and epithelial cells are introduced into the decellularized donor trachea, adapting tracheal function.

RESPIRATORY CONDITIONS REGISTERED BY CLINICAL TRIALS

Clinical trials registered are designed to test the safety and benefits of stem cells for BPD 21 (18%), COVID-19 20 (17%), COPD/Emphysema 18 (15%), ALI/ARDS 12 (10%), pulmonary fibrosis 9 (8%), PAH 8 (7%). Few trials are for lung cancer, pneumoconiosis, silicosis, asthma, cystic fibrosis (Figure 2C). There is a significant increase since 2014 (Figure 1C), particularly after the outbreak of COVID-19.

Covid 19

In December 2019, the COVID-19 coronavirus began in Wuhan, China and has since spread rapidly to more than 1 million confirmed cases and 56,000 deaths worldwide. Twenty-four clinical trials are currently being conducted to study the therapeutic effects of mesenchymal stem cells (MSCs) on COVID-19. MSCs have anti-inflammatory, anti-apoptotic, antimicrobial, and anti-fibrotic properties which may lower the risk of developing severe complications from the virus.

ALI/ARDS

ALI is a common vital complication of systemic and pulmonary insults and developed as ARDS in the late stages. Preclinical models suggest MSCs may be beneficial for ARDS. Two clinical trials have demonstrated the short-term safety of MSCs for ARDS patients, lasting up to 6 months. This safety was further confirmed by a randomized phase Ia trial of 40 ARDS patients treated with allogeneic mesenchymal stromal cells. Both trials were registered in the United States (NCT01775774 and NCT02097641). Additionally, a new phase II trial is evaluating the safety of MSCs for ARDS. Moreover, there are 12 International Clinical Registration and 5 Chinese Clinical Trials registered to assess the benefits of MSCs from different sources. Results from completed trials indicate that while benefits remain uncertain, safety is not a concern.

COPD

Chronic Obstructive Pulmonary Disease (COPD) is an irreversible airway obstruction caused by a combination of bronchitis and emphysema. It has a high mortality rate and is the third leading cause of death globally. Common treatments include corticosteroids and bronchodilators. Preclinical studies have suggested that mesenchymal stem cells (MSCs) may be promising for COPD treatment. Of the 18 registered clinical trials to evaluate cell therapy in COPD or emphysema, three have been completed, showing cell therapy to be safe for COPD patients. The majority of these studies used adipose-derived MSCs and bone marrow-derived MSCs. To further evaluate the efficacy of MSCs for COPD, a randomized, double-blind, placebo-controlled clinical trial has been conducted to follow up on patients two years after MSC infusion.

BPD

BPD is a chronic lung disease in premature infants, causing lifelong pulmonary complications such as COPD and asthma. The current treatment strategies for BPD are unsatisfactory. To evaluate the safety and efficacy of MSCs in preclinical and clinical studies, 21 clinical trials have been registered globally. Intratracheal infusion of allogeneic UCB-MSCs in preterm infants has been found to be safe and feasible, with inflammatory markers and growth factors decreasing in tracheal aspirate samples after MSC transplantation. A Phase II clinical trial for intratracheal transplantation of UCB-MSCs to preterm infants with BPD (NCT01632475) has also been warranted. UCB-MSCs are considered the best available source for cell therapy for BPD; however, given the small sample size of these trials, caution should be exercised when interpreting the safety data, and it is too early to draw conclusions about the benefits of cell therapy.

PAH

PAH (progressive chronic disorder with high mortality and increasing prevalence) is characterized by the remodelling of the pulmonary arteries and increased pulmonary infiltrates. Interventions specifically targeting PAH have been developed, but fatality has not been reduced. Animal studies suggest that cell therapy may be the most effective approach for PAH, leading to 8 registered trials to date (Figure 2C); 2 of which have been completed. Autologous EPCs, either alone or with gene editing of endothelial NO-synthase (eNOS), are used in these trials and appear to be safe and feasible. A phase II trial evaluating eNOS gene-enhanced EPCs for PAH is ongoing (NCT03001414). Additionally, AD-MSCs are being tested for their

safety and efficacy in treating PAH. As phase I/II trials are not double blind nor placebo controlled, the efficacy of EPCs for PAH is unknown.

IPF

Idiopathic Pulmonary Fibrosis (IPF) is an irreversible, chronic lung disease caused by diffuse alveolar inflammation and extracellular matrix remodelling. Currently, there is no effective treatment for IPF. However, the administration of mesenchymal stem cells (MSCs) is being evaluated as a potential therapy. In animal models, MSCs have been shown to prevent the progression of IPF. Additionally, nine clinical trials have been conducted that demonstrate the safety and tolerability of cell therapy, along with improved quality-of-life in IPF patients. A standardized protocol is available for clinicians. Other types of MSCs are also being tested in China, Australia and Greece, including placental-derived MSCs and bronchial stem cells to compare efficacy. Thus far, no severe adverse effects have been observed during a 6-month follow up period with autologous lung spheroid stem cell infusion.

Other

In addition to pulmonary diseases, clinical trials have been registered to assess the safety and efficacy of stem cells for other refractory lung diseases, including lung cancer, silicosis, asthma, bronchiolitis obliterans, and tuberculosis (Figure 2C). Two clinical trials are recruiting bronchiolitis obliterans patients to evaluate the safety and feasibility of MSCs infusions. A phase I trial is evaluating the safety of allogeneic BM-MSCs (2-10 × 107 cells/kg, i.v.) for asthma. Additionally, a trial has initiated to assess the safety of intranasal delivery of MSC-trophic factor for asthma (NCT02192736). Autologous BM-MCs is being tested for silicosis (NCT01239862) based on a previous study. Radiation-induced lung injury is also a potential target for MSCs in the near future.

Current approaches

Researchers are primarily studying how to expand the various stem cells of the lungs indefinitely. This is typically done through organoid (mini-organ) cultures, which are well-suited for cultivating cells that resemble body cells in the laboratory. These cultures are being employed for disease modelling and drug testing in order to find potential new treatments for ailments.

Animal testing is still needed to assess the effects of new drugs on the lungs and body as a whole. Donated patient material is used to create organoid cultures, which can identify differences between healthy and diseased lungs and how they respond to stem cell behaviour cues and treatments. Laboratories worldwide are working to improve methods for growing lung cancer organoids, so similar experiments such as manipulating stem cell behaviour cues or drug screening can be conducted on them.

Research is also underway to develop methods that "gene correct" (repair damaged genes) in human cells. If successful, this could one day lead to personalized medicine that cures diseases caused by genetic defects. Scientists are exploring ways to safely alter patient DNA or introduce new lung stem cells with modified DNA.

Recent research suggests that both embryonic stem cells and stem cells sourced from adult tissues (e.g., bone marrow and umbilical cord blood) may be used to repair or regenerate damaged or diseased lungs. This is an emerging field with promising

therapeutic applications for numerous lung diseases. Initially, focus was placed on transplanting stem cells into the lungs, however, more recent studies have shown that mesenchymal stem cells (MSCs) can reduce inflammation and modify immunity in mouse models of acute lung injury and pulmonary fibrosis. Furthermore, due to initial reports of safety and efficacy after administering allogeneic MSCs to people with Crohn's disease or graft-versus-host disease, a trial is now underway to observe the effects of MSCs in patients with chronic obstructive pulmonary disease. Autologous stem cell administration has been trialled in patients with pulmonary hypertension and shown potential benefits. This review will summarise the recent advances in cell therapy concerning MSCs.

CONCLUSION

In recent years, there is rapid increase in the field of stem cell biology and its applications in various disease treatments. Lung diseases are leading cause of death in current world and new treatments need to be discovered. There is a rapid pace of clinical trials on stem cell therapy for lung diseases in the last 5 years. To date, most of these trials are at an early stage for evaluating safety, feasibility, tolerance, and potential efficacy, further studies investigating the role of transplanted stem cells will provide improved insight into the mechanisms of lung repair and development after dysfunction and may also provide novel and more efficient therapeutic strategies for medical application.

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NANOTECHNOLOGY-BASED EARLY DETECTION OF PATHOGENS BY USING TRADITIONAL AND INNOVATIVE METHODS

Radhika Jadhav[*] ***Deepak Sawant***[**]

ABSTRACT

Bacteria are unambiguously menacing to community health globally. The common reservoirs of bacteria are contaminated foods, animals, polluted water, and soil. There are several diseases causing bacteria such as *Vibrio, Listeria, Yersinia, Salmonella, Shigella*, *Clostridium, Campylobacter,* and many more. The apprehension of the transmission of bacterial infection has increased the incidence of outbreaks and several health issues across boundaries. The key challenge for the treatment of bacterial infection in the early stage is the investigation of multiplexed bacterial virulent factors. Therefore, it requisites the development of highly efficient techniques to identify, prevent and manage the growth of pathogenic bacteria for the well-being of the living system. Conventional techniques including serological tests, immunoassays, polymerase chain reaction (PCR) and isothermal microcalorimetry (IMC), and many other techniques have been used for the testing of bacterial infection. However, limitations of the aforesaid techniques have been overcome by biosensing techniques as they have offered numerous advantages including highly simple, sensitive, specific, cost-effective and quick response generating reproducible, on-site detection and ease of portability. The present review article deciphers the role of distinct types of biosensors based on gold nanoparticles for the analysis of bacteria from different reservoirs

Keywords: Diagnosis, bacterial infection, biosensor, PCR, nanoparticles

INTRODUCTION

The field of pathogenic microorganism detection is by no means a new one, and microorganisms since time immemorial have been an integral part of life on the Earth. However, some microorganisms are known to cause diseases and can have calamitous effects on humans, and can cause detrimental health issues. The ability of most microorganisms to evolve rapidly allows them to adapt and grow under various conditions like high or low temperatures, acidic or basic pH, various pressures, and a range of salinities. These abilities allow some of these microbes to cause substantial damage to life. Under this scenario, the commonly used control measures and sampling techniques are not robust enough to maintain infection control. The early detection of pathogens is very crucial and key for diagnosing and preventing diseases [1]

Currently, conventional methods are used to detect pathogens owing to their high selectivity and high sensitivity. However, these methods are prone to lengthy

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experimental turn around times for results and miss the correct sampling period. This incorrect sampling period can lead to misinterpretation of organisms and their growth responses. Some emerging methods such as biosensors are being developed, but their future is in reaching the same level of selectivity for a fraction of the cost. Furthermore, the issues of ease of use, maintenance, and in situ real-time approaches require due consideration. This review aims to give a focused overview of the field of pathogenic microorganism detection from basic conventional laboratory-based assays to the emerging state-of-the-art with an emphasis on materials sciences.

The most popular methods are, by far, those based on culture and colony counting methods and the polymerase chain reaction, PCR. This can be explained on the grounds of selectivity and reliability of both techniques. Culture and colony counting methods are much more time-consuming than PCR methods but both provide conclusive and unambiguous results. On the other hand, recent advances in PCR technology, namely real-time PCR, now enable obtaining results in a few hours. Biosensor technology comes with promises of equally reliable results in much shorter times, which is perhaps why they are currently drawing a lot of interest. However, there is still much work to do before biosensors become a real alternative. Many biosensors rely on either specific antibodies or DNA probes to provide specificity. However, the technology is splited when it comes to detection modes. It indicates that biosensors are the fastest-growing pathogen detection technology.[1]

DISCUSSION

Conventional Method : Conventional method is time-consuming and does not perfectly rely on the results Serology method. [2]

Serology is an in-vitro study of serum where we see antigen and antibody reactions. Tests may be specific and non-specific. Specific for diagnosing viral and bacterial infection, e.g., a Widal test for Enteric fever and a Brucellosis test**.** Non-specific tests give evidence for some diseases like Syphilis and SLE. These serological tests are based on antigen and antibody reactions. These are: Agglutination, Precipitation, Fluorescent antibody test, ELISA (Enzyme-linked immuno-absorbed assay), Radioimmunoassay (RIA), Electrophoresis, Immunoelectrophoresis,

2.2.1 Agglutination

The antigens are in a particulate form where antigen and antibody forms clumps. This is a direct measurement of antibody binding to antigen. It is used in the quantitative serologic assay. Antibodies are called agglutinin. The antigen may be present in the bacterium, which forms an agglutinate with the antibody. At the same time, antigen on RBC forms haemagglutination. The antigen may be bound to RBC, and antibodies may be detected; this is called passive haemagglutination.

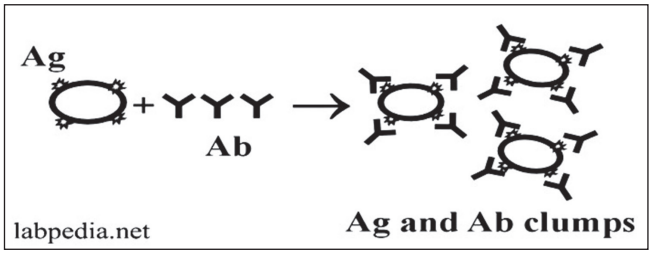

Fig. 1 : Agglutination

2.2.2 Precipitation

Ag & Ab's reaction is just like agglutination with the difference that here antigens are insoluble form. The

antibodies giving precipitation are called precipitation.

2.2.3 Fluorescent Antibody Technique

Antibodies are labelled by fluorescent material, then antigen is added. These are washed and seen with the help of a fluorescence microscope. No fluorescence will be seen if the antibody is not bound to the antigen.

2.2.4 ELISA (Enzyme-linked Immuno Absorbent Assay)

ELISA relies upon the "capture" antibody fixed to the plastic plate. Antigen or antibody-coated beads or wells are taken. Suppose there are antigen-coated wells. Now add the serum of the patient after washing and add enzyme-linked antibody to these wells; after incubation, washes the wells. Now add substrate, and colour develops. Stop the reaction and read by photometer, which will measure absorbance according to the intensity of colour, depending on the number of antibodies bound to the antigen

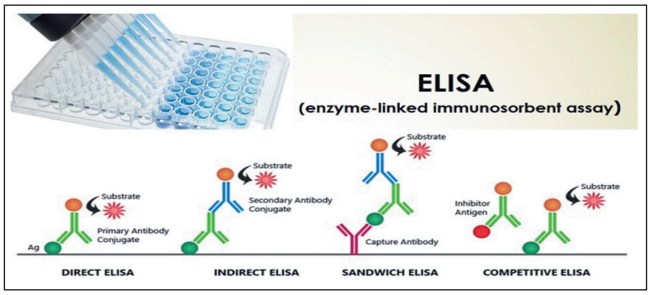

Fig. 2 : Schematic presentation of ELISA

2.2.5 Radioimmunoassay (RIA)

RIA is used to detect molecules (analytes) in circulation. It depends upon the availability of an antibody that specifically recognizes the analyte. This is basically a competitive assay. A fixed amount of antibodies is added and compete for the analyte, either in the sample or added to the sample in a radiolabeled form (e.g., bound to I^{125}). Analyte-antibody complexes form and are precipitated by physiochemical means. Gamma-Counter measures the radioactivity in the precipitate.

2.2.6 Electrophoresis

This method is used to separate proteins in serum in the electrical field. Also used to separate different types of Haemoglobins. Proteins migrate within the electrical field according to their charge. In turn, this migration is influenced by pH and the optimum separation achieved by Buffer at pH 8.6. Once separated on gel or fitter, these are stained with dye.

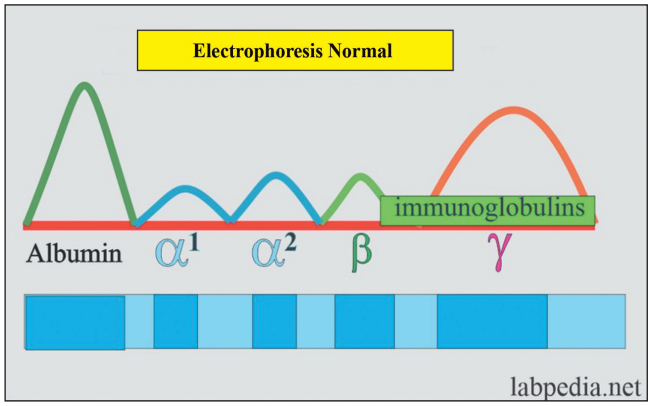

Fig. 3 : Schematic presentation of Electrophoresis

PCR

PCR is a nucleic acid amplification technique developed in the 1980s and is widely used to identify bacteria. In principle, the method is developed for the isolation, amplification and quantification of a short DNA sequence, inclusive of the target bacteria's genetic material, some examples of developed PCR methods for bacterial quantification are real-time PCR, multiplex PCR and reverse transcriptase PCR. PCR is much less time-consuming than other conventional techniques utilising culture and plating. PCR usually takes anywhere between 5 and 24 hours to produce

a result but this is dependent on the specific PCR variation and excludes enrichment stages. The PCR technique is used for amplifying a specific DNA fragment from a complex pool of DNA. The PCR assay requires template DNA, primers, nucleotides and thermostable DNA polymerase (Taq polymerase) enzyme. Nucleotides are referred to as adenine, thymine, cytosine, and guanine. [1]

Of the methods listed multiplex PCR is of most use. Multiplex PCR allows the simultaneous detection of several organisms through the introduction of different primers to amplify DNA region coding for specific genes of each bacterial strain targeted. In real time PCR faster results can be obtained without the need for manipulation of the recognition stages. Real-time PCR results are obtained through the detection of fluorescence emission by a specific dye that attaches to the targeted amplicon. Fluorescence intensity is directly proportional to the amount of amplified product and therefore it is possible to follow this response in real-time elimination of laborious post-amplification processes such as the already mentioned, gel electrophoresis. [1]

Colorimetric detection

Recently, gold nanoparticle (AuNP)-based colorimetric bio-sensing assays have attracted considerable attention in molecular recognition and diagnosis. Small AuNPs in water or glass appear deep-red in colour, this colour changes to purple or blue when AuNPs aggregate. The predictable colour change during AuNPs aggregation (or re-dispersion of an aggregate) provides an elegant platform for absorption-based colorimetric detection with AuNPs as signal reporters. This platform has been increasing application for the detection of a large variety of targets including nucleic acids, proteins, saccharides, small molecules, metal ions, and even cells. For the detection of foodborne pathogens, Deng et al. (2013) developed a novel strategy for rapid colorimetric analysis of a specific DNA sequence of *Bacillus anthracis* by combining AuNPs with an asymmetric PCR. However, this method detected the target pathogen by detecting its specific DNA, which involves the steps of DNA extraction and might generate false positives of dead cells.

Only a few literatures have been carried out on dealing with whole-cell bacteria detection by AuNP colorimetric assays. Chang et al. (2013) developed a detection method for *Staphylococcus aureus* by aptamer-conjugated AuNPs and successfully detected single *S. aureus* cells within 1.5 hr. Yuan et al. (2014) developed a detection method for *Salmonella* enteric serovar *typhimurium (S. typhimurium*) based on the recognition of aptamers coupled with nanogold labelling and silver signal amplification and the detection limit of this method was 7 cfu/ml. Dharanivasan et al. (2016) detected trace DNA by using AuNP conjugated bi-functional oligonucleotide probe. However, most reported colorimetric assays are based on mono-functional AuNP oligonucleotide probes, which are used to recognize one target binding site, and the other strand is left free. 3

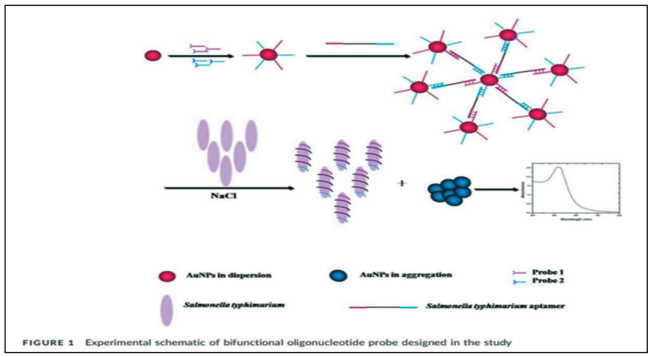

Fig. 4 : Schematic presentations of colorimetric detection

Biosensors

Biosensors have recently been defined as analytical devices incorporating a biological material (e.g., tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, natural products, etc.), a biologically derived material (e.g., recombinant antibodies, engineered proteins, aptamers, etc.) or a biomimic (e.g., synthetic catalysts, combinatorial ligands, and imprinted polymers) intimately associated with or integrated within a physicochemical transducer or transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric, magnetic or micromechanical.

Optical biosensor

Optical biosensors are a powerful alternative to conventional analytical techniques, due to their particularly high specification and sensitivity, as well as their small size and cost-effectiveness. Biosensor detection typically relies on an enzyme system, which catalytically converts analytes into products that can be oxidized or reduced at a working electrode and maintained at a specific potential. One of the best advantages of this optical transducer is the low cost and the use of biodegradable electrodes. An optical biosensor is a compact analytical device containing a biological sensing element integrated or connected to an optical transducer system. [4]

Fluorescence detection

Fluorescence occurs when a valence electron is excited from its ground state to an excited singlet state. The excitation is produced by the absorption of light of sufficient energy. When the electron returns to its original ground state it emits a photon at lower energy. Another important feature of fluorescence is the little thermal loss and rapid light emission taking place after absorption. The emitted light is at a longer wavelength than the absorbed light since some of the energy is lost due to vibrations, this energy gap is termed Stoke's shift, and it should be large enough to avoid cross talk between excitation and emission signals. Antibodies may be conjugated to fluorescent compounds, the most common of which is fluorescein isothiocyanate (FITC). There are, however, other fluorescent markers. The use of lanthanides as sources of fluorescence in luminescent assays has very recently been reviewed.

Surface plasmon resonance

SPR biosensors measure changes in refractive index caused by structural alterations in the vicinity of a thin film metal surface SPR has successfully been applied to the detection of pathogen bacteria by means of immunoreactions.

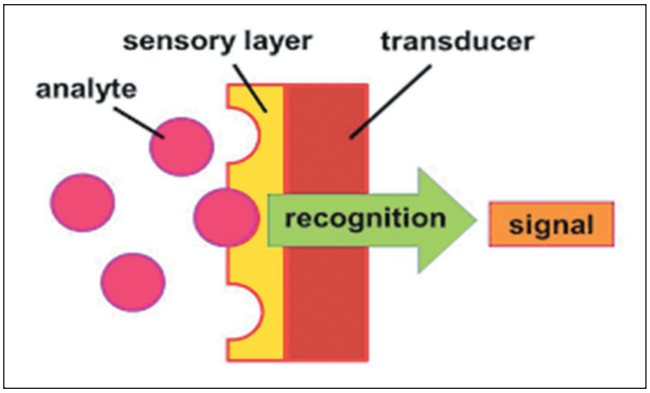

Fig. 5 : Schematic presentation of biosensor

Enzyme base biosensor

The first enzyme-based sensor was reported by Updike and Hicks in 1967. Enzyme biosensors have been devised on immobilization methods, i.e. adsorption of enzymes by van der Waals forces, ionic bonding or covalent bonding. The commonly used enzymes for this purpose are oxidoreductases, polyphenol oxidases, peroxidases, and aminooxidases. [5-7] Ghasemi-

Varnamkhasti et al. worked on the monitoring of ageing of beer using enzymatic biosensors, based on cobalt phthalocyanine. These biosensors evinced a good capability to monitor the ageing of beer during storage.

Cell based biosensor

The first microbe-based or cell-based sensor was actualized by Diviès.[8] The tissues for tissue-based sensors arise from plant and animal sources. The analyte of interest can be an inhibitor or a substrate of these processes. Rechnitz.[9] developed the first tissue based sensor for the determination of amino acid arginine. Organelle-based sensors were made using membranes, chloroplasts, mitochondria, and microsomes. However, for this type of biosensor, the stability was high, but the detection time was longer, and the specificity was reduced.

Immuno sensor

Immunosensors were established on the fact that antibodies have high affinity towards their respective antigens, i.e. the antibodies specifically bind to pathogens or toxins, or interact with components of the host's immune system.

DNA biosensor

The DNA biosensors were devised on the property that single-strand nucleic acid molecule is able to recognize and bind to its complementary strand in a sample. The interaction is due to the formation of stable hydrogen bonds between the two nucleic acid strands.[10] Mahmoud Amouzadeh Tabrizi et al. [11] developed an electrochemical DNA biosensor based on nanoporous glassy carbon electrodes to detect *Salmonella* DNA sequences. E. Sheikhzadeh et al. [11] established a label-free impedimetric biosensor to detect *S. Typhimurium* in apple juice. Modifying the amino group at the 5' end of aptamer before immobilizing on electrode surface. LOD of devThe eloped biosensor was 3 CFU/mL, which achieved a satisfying detection result

Magnetic biosensor

Magnetic biosensors miniaturized biosensors detecting magnetic micro- and nanoparticles in microfluidic channels using the magnetoresistance effect have great potential in terms of sensitivity and size. [12] magnetic IONP-based nano biosensors possess biosensing as well as pathogen detection applications and lead to multifunctional approaches in the area of disease diagnosis. [13] IONP-based nano biosensors have gained increasing heed due to their specific detection with depleted concentration regimes. It has been reported that melittin-conjugated IONPs are responsible for electric diagnosis of food pathogen with sensitive detection. One of the principal advantages of IONPs is they can be separated and combined with IONPs according to the functionalization. A low-cost separation methodology has been described. IONPs are reported as effective candidates against major multi-drug resistant bacteria like *E. coli, S. aureus,* etc

Thermal biosensor

Thermal biosensors or calorimetric biosensors are developed by assimilating biosensor materials as mentioned before into a physical transducer. Recently, microelectromechanical (MEMS) thermal sensors are being used to monitor metabolic applications on the basis of temperature detection. Low-cost integration of miniaturized devices and low-cost batch fabrication are the advantages of MEMS technology. MEMS thermal sensors exhibited improved thermal isolation, low thermal mass, and sample volume of the MEMS thermal biosensor providing linear range and high

sensitivity, low measurement time, and low power consumption. It is possible to measure multiple samples in parallel. [14]

Piezoelectric biosensor

Piezoelectric biosensors are of two types: the quartz crystal microbalance and the surface acoustic wave device. They are based on the measurement of changes in the resonance frequency of a piezoelectric crystal due to mass changes on the crystal structure.

Genetically encoded biosensor

Green fluorescent protein and the subsequent autofluorescent protein (AFP) variants and genetic fusion reporters have aided the development of genetically-encoded biosensors. This type of biosensor is user-friendly, easy to engineer, manipulate and transfer into cells. Single-chain FRET biosensor is another example. They consist of a pair of AFPs, which are able to transfer fluorescence resonance energy between them when brought close together. Different methods may be used to regulate changes in Förster resonance energy transfer (FRET) signals based on the intensity, ratio, or lifetime of AFPs. Peptide and protein biosensors are easily manufactured through synthetic chemistry followed by enzymatic labeling with synthetic fluorophores. Due to their independence of genetically-encoded AFPs, they are readily utilized to control target activity and constitute attractive alternatives, and have the added advantage of being able to enhance signal-to-noise ratio and sensitivity of response through the introduction of chemical quenchers and photoactivatable groups. [15-25]

CONCLUSION

Biosensors have become ideal alternative of traditional methods and molecular detection methods for food borne pathogens detection. Most nanobiosensor devices used in biomedical applications require a large sample for detection, which may lead to false-positive or false-negative results. Very few biosensors have attained commercial success at the global level, apart from electrochemical glucose sensors and lateral flow pregnancy tests. There is also a need for making nanostructure-based biosensors at an affordable cost that give rapid results with accuracy and are user-friendly. For example, nanomaterials should be integrated with a tiny biochip (lab-on-chip) for sample handling and analysis for multiplexed clinical diagnosis. More research should be done in this area and we expect the ongoing academic research to be realized into commercially viable prototypes by industries in near future.

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ASSESSMENT OF CARDIOVASCULAR MORBIDITY IN POLYCYSTIC OVARIAN SYNDROME IN ASSOCIATION WITH NON-ALCOHOLIC LIVER DISEASE

Jasmine Nath, **Shimpa Sharma*****

ABSTRACT

Introduction : Polycystic Ovarian Syndrome remains as the most common endocrine disorder among females and are at increased risk for developing early onset atherosclerosis. Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common chronic liver disease associated with significant mortality and morbidity and occur in higher prevalence in patients with Polycystic Ovarian Syndrome. (PCOS). **Methodology :** 60 PCOS patients diagnosed by Rotterdam's Criteria were included in the study. Abdominal ultrasound screening was used to identify patients with NAFLD and patients without NAFLD. Demographic and anthropometric data was collected. Cardiovascular morbidity was assessed for all patients using lipid profile, fasting blood sugar levels, Echocardiography and Carotid Intima Media Thickness. Data analysis was done based on the compiled information. **Results :** Out of 60 PCOS patients, 30 patients presented with NAFLD with mean age of 27.7 + 8.03 and 30 patients without NAFLD with a mean age of 24.7 + 7.4. (p value<0.05).Presence of NAFLD shower higher FBS (Fasting Blood Sugar) and TGL (Triglycerides) compared to patients without NAFLD (p <0.01).There was a significant corelation of mean CIMT with FBS and TGL levels. (p value <0.05) and also with BMI. (p value <0.05). Study suggested that presence of NAFLD was not associated with either diastolic dysfunction or increased CIMT with a p value >0.05. Regression analysis was done with age, BMI, LVEF, FBS and Total cholesterol levels, and multicollinearity was found to be absent. (p value>0.05) Regression equation : Average CIMT = 0.66 + 0.01 * Age **Conclusion :** This study showed that PCOS patients with presence of NAFLD had higher BMI and dyslipidaemia. It also showed a positive corelation of BMI with Mean CIMT which suggested that weight reduction could reduce cardiovascular mortality. Presence of NAFLD was not associated with increased cardiovascular morbidity in PCOS patients.

INTRODUCTION

NAFLD : Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease and is associated with significant morbidity and mortality.[1] The prevalence of NAFLD has been increasing dramatically worldwide, particularly in Western countries.[1] Although NAFLD can progress from accumulation of fat alone (steatosis) without inflammation, to necroinflammation (steatohepatitis), to cirrhosis, and the sequelae of portal hypertension, majority of patients with NAFLD are asymptomatic.

PCOS : Polycystic ovarian syndrome (PCOS), also known as hyperandrogenic anovulation (HA), is one

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of the most common endocrine disorders in women of reproductive age. The three essential components of PCOS are hyperandrogenism, ovulatory dysfunction, and/or ultrasound findings of polycystic ovarian morphology.[3]-

Besides these clinical features, patients are often insulin resistant, obese, and present with dyslipidaemia, impaired glucose tolerance or Type 2 diabetes mellitus, and arterial hypertension. Impaired glucose tolerance is seen in these patients due to post binding defect in receptor signalling likely due to increased receptors or insulin receptor substrate 1 serine phosphorylation that affects metabolic pathways in classic targets of insulin.

PCOS women are at increased risk of developing early-onset atherosclerosis.[4] Studies have also demonstrated increased Carotid Intima-Media Thickness (CIMT), which is a predictor of coronary and cerebrovascular events among relatively younger women with PCOS. PCOS remains an independent predictor of higher IMT even after adjustment for age and BMI.[5]

LVH is one of the several metabolic and cardiovascular risk factor associated with insulin resistance and visceral obesity.[6] Hyperinsulinemia and insulin resistance have been major linking factor to the development of coronary heart disease in association with hypertension, lipid abnormalities and glucose intolerance. Studies have suggested that PCOS women report LVM, Diastolic dysfunction along with no difference in e'/a' ratio in echocardiography.[7]

Pathogenesis of NAFLD is multifactorial. The major two factors contributing to its development are obesity and insulin resistance which is often seen in PCOD patients.

Insulin resistance is recognized as the main determinant of NAFLD pathogenesis but as to how it is responsible for accelerated atherosclerosis, independent of metabolic abnormalities, remains unclear. Probably, the hepatic necro-inflammation with the consequent systemic diffusion of cytokines and chemokines leads to vascular damage and coagulation system abnormalities.

NAFLD patients have been shown to develop diastolic LV dysfunction as well as LV hypertrophy. Diastolic dysfunction later leads to heart failure. The diagnosis in these patients is delayed mostly as the patients remain asymptomatic with preserved systolic function in the early stages.

Echocardiography may provide evidence of subtle cardiac dysfunction using e'/a' ratio. When associated with the presence of obesity and hypertension, LV hypertrophy is reported in patients as a form of end-organ damage.[8] Studies have suggested that LV diastolic dysfunction may precede LV hypertrophy.

Insulin resistance (IR) plays a central role in the pathogenesis of NAFLD and PCOS. Also, hyperandrogenism enhances IR in these patients. IR present in the NAFLD-PCOS association could decrease the hepatic production of sex hormone-binding globulin through a possible regulation mediated by hepatocyte nuclear factor 4 alpha. On the other hand, apoptotic processes initiated by androgens actively contribute to the progression of NAFLD. Considering the association between the two conditions, the screening of women with PCOS for the presence of NAFLD appears reasonable.

It was noted in studies that there is a proportionate increase in the prevalence of PCOS and NAFLD with the degree of insulin resistance and adipose tissue.[9] PCOS patients are found to have a higher

prevalence of NAFLD and thus tend to have a higher cardiovascular morbidity.[10]

AIM

To Estimate the cardiovascular morbidity in Polycystic Ovarian Syndrome with or without NAFLD.

OBJECTIVES

To Estimate the cardiovascular morbidity in PCOS patients with or without NAFLD, a cardiovascular morbidity assessment will be done based on demographic, anthropometric, biochemical, and radiological findings for all the patients, to Compare cardiovascular morbidity with the presence and absence of NAFLD and to analyze the results by demographic and biochemical data.

MATERIALS AND METHODOLOGY

Type of Study: Comparative Observational Prospective Study.

Study setting: OPD & IPD of the tertiary care center.

Sample Size: 30 NAFLD and 30 NON-NAFLD using simple consecutive sampling.

An observational Prospective study was performed with 60 participants of PCOS that satisfied Rotterdam's criteria.

PCOS participants diagnosed by Rotterdam's criteria and satisfying inclusion and exclusion criteria were included in the study.

Rotterdam's criteria: Polycystic ovarian syndrome (PCOS) is defined by the presence of two of three of the following criteria: oligo-anovulation, signs of hyperandrogenism and polycystic ovaries (≥ 12 follicles measuring 2-9 mm in diameter and/or an ovarian volume > 10 mL in at least one ovary).

Informed consent was taken from all the participants.

Detailed history of all the patients was taken along with demographic details and then were followed up with a detailed clinical examination.

Asymptomatic individuals included observational findings of hirsutism (facial hair) that represented Rotterdam's criteria.

Anthropometric measurements including height, weight, waist-hip circumference ratio, and BMI was calculated.

Screening of all the patients was done with ultrasonography to identify the presence of fatty liver. Based on that fact they were further divided into two groups, One with NAFLD and the other without NAFLD. Ultrasound was performed using the LOGIQF8 machine.

Routine investigations were done that included complete blood count, fasting blood sugar, lipid profile, SGOT, and SGPT. A 12-h fasting blood sample was obtained at baseline for lipid and hormone assays. Lipid Profile was done using ABX Pentra XL 80 machine that included total cholesterol, HDL, LDL, and total serum triglycerides. SGOT and SGPT were done using MISPA Nano Plus and CBC using ABX Pentra XL 80 machine.

Apart from routine investigations special investigations were performed to assess cardiovascular morbidity that included echocardiography, and carotid intima body thickness.

CIMT was assessed using a diabetic risk profiler and the echo was done with a Vivid e machine.

Echocardiography was done for all the patients to determine e/a values, with ejection fraction and presence of diastolic dysfunction was assessed through the same.

Carotid intima media thickness was also identified separately left and right and mean CIMT was calculated for all the patients.

All the compiled information was presented in the form of a master chart and statistical analysis was done in MS Excel.

INCLUSION CRITERIA : PCOS patients diagnosed with Rotterdam's Criteria, Females above 18 yrs of age, with presence and absence of NAFLD.

EXCLUSION CRITERIA : Known CV diseases, chronic kidney disease, malignancy, pregnant or lactating mothers, patients with other endocrine disorders apart from PCOS, patients already on statins, metformin, patients of Acute or chronic known liver disease, conditions with accelerated atherosclerosis: Autoimmune disorders, patients on anticancer drugs, immunosuppressant, steroids, Cox-2 NSAIDS.

RESULTS

Table 1 : Age distribution of total patients:

AGE	FREQUENCY	PERCENT
18-30	41	68.33
30-51	19	31.66
TOTAL	60	100

The above **Table 1** shows that among 60 participant's majority of them were in the age group of 18-30 yrs (68.33%) followed by 30 – 51 yrs (31.66%)

Table 2 : BMI AND WAIST HIP RATIO

	NAFLD	NNAFLD	P value
BMI	**26.78 + 2.3**	**24.34+ 2.002**	**< 0.001**
WAIST HIP RATIO	**0.75 +.05**	**0.75 + 2.002**	**0.32**

Patients with NAFLD had significantly higher BMI p value <0.001. No significant difference was noted in waist hip ratio between patients with and without NAFLD.

Table 3 : Association of Lab parameters between NAFLD and Non NAFLD

	NAFLD	NNAFLD	P value
FBS	115.04 + 19.42	103.26 + 16.45	**0.014***
LDL	110.5 + 42.12	109.06 + 26.97	0.85
TCHL	166.56 + 52.83	143.44 + 29.23	0.04
TGL	164.4 + 57.69	126.6+ 30.8	**0.002****

(*- p value is significant <0.05) (- p value is significant <0.001)**

Patients with NAFLD showed higher FBS and TGL values compared with non-NAFLD patients with a significant p-value of <0.001.

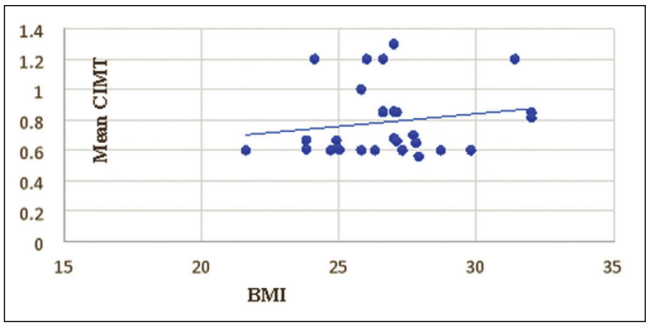

Fig. 1 : Correlation between mean CIMT and BMI

TABLE 4 : Correlation between Mean CIMT with FBS and TGL

	FBS	TGL	MEAN CIMT
FBS	NA	0.00	0.06
TGL	0.00**	NA	0.00
MEAN CIMT	0.06	0.00**	NA

The analysis of variance showed a significance of <0.05 and step-wise analysis was performed. The parameter estimates a revealed significance of less than 0.05 for age. All other parameters in the model showed p>0.05.

Regression equation : Average CMIT = 0.66 + 0.01 * AGE

DISCUSSION

According to Jokoben MV et al, NAFLD patients were high with high BMI and high waist circumference[11].

Angulo et al found obese individuals with high frequency of getting NAFLD and will have severe form of the disease. Comparative findings were reported in Sau M and Chakraborty S where cross sectional study was done among 120 NAFLD patients with 69.2% as obese and 30.8% patients being lean[12].

Young bin won et al also presented a study of 586 patients where 51 were found to have NAFLD and 548 without NAFLD. According to the study, obese PCOS were significantly higher in NAFLD compared to Non-NAFLD with p-value <0.001[13].

Lab Parameters:

Significantly higher values of FBS, TOTAL CHL, TGL 0F 115.04 +19.42, 166.56 +52.83, AND 164.4 + 57.69 were seen in the NAFLD group as described in Table no 7. Similar findings were reported in a study done by Fariborz Mansour et al among 333 patients of which 199 were NAFLD. TCHL were significantly higher in the NAFLD group with a p-value <0.05[14]. The frequency of FBS, TCHL, and LDL were found higher with NAFLD (p-value <0.05).

According to Chatrale H, NAFLD individuals presented with altered lipid metabolism along with metabolic syndrome, presented with increased TGL, LDL and HD[15]. Altered lipid metabolism is due to the overproduction of VLDL. According to study of David e cohen, NAFLD patients presented usually with an atherogenic lipid profile[16].

According to Young bin won et al, 586 PCOS patients were included with 51 NAFLD patients. MET S diagnosis was done among 62.7% in NAFLD patients with a p-value <0.01. Univariate analysis was done for risk factors that included obesity, increased TGL and decreased HDL and were found statistically significant[17]. Sookan S Pirola did study with 3497 patients and found a significant association between NAFLD and PCOS[18].

A study by Alison. J. Dawson, Jaqueline et al did a study with 25 oligo anovulatory women with PCOS, and 13 had evidence of NAFLD. PCOS with NAFLD were found to have higher BMI compared to PCOS alone but found no difference considering cardiovascular risk facto Corelation between FBS, TGL and Mean CIMT was found to be significant with p-value <0.001. BMI and waist hip ratio showed a positive correlation with mean CIMT in the present study[19].

Similarly, Vanjiappan.S et al presented an observation prospective study with 300 patients with Diabetes, 124 patients had NAFLD. CIMT was found to be elevated in the NAFLD group with p value <0.001[20]. According to a study by Anastasia Garoufi et al, 32 patients with PCOS were considered. CIMT values were compared among both groups and were found to have no correlation of right, left, and mean CIMT. P value was 0.745 for the corelation[21].

According to a systematic review done by Pacific et al

15 studies provided data on subclinical atherosclerosis, 10 were evaluated with CMIT and found no difference in CMIT and arterial stiffness[22].

CONCLUSION

The present study was done among 60 Polycystic Ovarian Syndrome participants and was equally distributed among 30 participants with the presence and absence of Non -Alcoholic Liver Disease using ultrasound. The majority of the PCOS patients were in the age group of 18 - 30 years (68%) and they were diagnosed using Rotterdam's criteria. The mean age of NAFLD was found significant compared to the Non-NAFLD group with a p-value of <0.01.WHR and BMI were also found to have a significant correlation among the total population with a p-value of <0.01.

Asymptomatic patients were found to be more in number as most of the patients were diagnosed incidentally with evidence of facial hair. On general examination, only 3 patients presented with hepatomegaly among the total population. NAFLD group showed higher BMI indicating a predominance of obesity. Patients of NAFLD also showed evidence of dyslipidemia and insulin resistance with higher Fasting blood sugar levels as well as triglyceride levels.

Mean Carotid intima-media thickness was found to be higher in patients with increased fasting sugar and triglyceride levels. CIMT and BMI were also found to have a significant correlation indicating that patients with higher BMI showed an increased in Mean CIMT levels. Thus, weight reduction can reduce the risk of CV morbidity. Cardiovascular morbidity was assessed with diastolic function as well as carotid intima-media thickness. The present study suggested that the presence of NAFLD was not associated with either diastolic dysfunction or increased CIMT with a p-value of >0.05. This could be due to a smaller sample size and also due to the mean age group of less than 30 yrs. Multicollinearity was also not found among NAFLD patients in comparison with BMI, LVEF, CIMT, Total Cholesterol and Fasting sugar levels.

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ORIGINAL ARTICLE

THE STUDY OF GLYCEMIC VARIABILITY IN PATIENTS WITH TYPE II DIABETES MELLITUS AND ITS CORRELATION WITH NERVE CONDUCTION STUDY AND HbA1c

*Amrish Ranjan**, ***Sushma Jotkar*****

ABSTRACT

Introduction-Glycemic variability is defined as the degree of variation in blood glucose levels over a given time period. Glycemic variability can be measured intraday and inter-day. It indicates the occurrence of excess glycemic excursions. GV is elevated in patients with DM and insubjects with altered blood glucose management. Continuous glucose monitoring is a reliable method for determining the short- and long-term glycemic variability in diabetic patients. Thecornerstone of blood glucose monitoring is HbA1c, which is utilized as a marker of average blood sugar readings over several months. HbA1c, however, only offers a rough indication ofglucose control; it doesn't consider either glycemic variability or hypo glycemic episodes. **Methodology-** The study was conducted at Dr.D.Y. Patil Hospital & Research Institute, Kolhapur. Total 93 patients fulfilling the inclusion & exclusion criteria were included for the study. GV was evaluated in the subjects for atleast72 hours by using continuous glucose monitoring device. Nerve conduction study using NCV machine was done for both motor conduction and sensory conduction to check diabetic peripheral neuropathy in T2DM patients. HbA1c level were checked. Glycemic variability was correlated with nerve conduction study and HbA1c.Result- In the majority of subjects, NCS was abnormal (80.65%, n=75) whereas, it was normal in (19.35% n=18) of patients. Among the patients who had abnormal NCS, (n=59) patients had high GV and (n= 65) patients had high HbA1c.. The most common type of neuropathy was sensory-motor(mild n=15, moderate n=9, severe n=20). Time above range, Time below range, MAGE, and CV were significantly found to be high in patients with HbA1c levels >6.5% than in patients with HbA1c level <6.5%. Patients(n=62) with HbA1c >6.5% were found to have increased GV whereas (n=13) patients with HbA1c <6.5% were also found to have increased GV. **Conclusion-** Majority of patients with abnormal NCS had high Glycemic Variability. GV was significantly associated with HbA1c >6.5, abnormal NCS, history of hypoglycemia. Few patients who had normal HbA1c levels were also found to have high glycemic variability and diabetic peripheral neuropathy.

INTRODUCTION

The cornerstone of blood glucose monitoring is HbA1c, which is utilized as a marker ofaverage blood sugar readings over several months. HbA1c, however, only offers a rough indication of glucose control; it doesn't consider either short-term glycemic variability or hypoglycemic episodes.[1] It has the following drawbacks:[2,3] Glycaemic excursions, or intra-day and inter-day glycemic fluctuations, which have been connected to both microvascular and macrovascular problems, are notconsidered. In patients with anaemia,

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certain hemoglobinopathies, liver disease, and iron insufficiency, it is an unreliable measurement. It doesn't give specific instructions on how to modify the treatment plan. HbA1c does not include information on hypo- and hyperglycemic excursions; rather, it represents the average glycemia for the two to three months prior. The size and frequency of intra- and inter-day glucose change cannot be determined by HbA1c. Numerous non-glycemic variables, including changed RBC lifespan, hemoglobinopathies, renal insufficiency, and the use of (non-diabetic) medications, can have an impact on HbA1c. Thus, HbA1c dependence could cause error in treatment decision as it does not provide clear clinical picture. The main difficulties in improving glycemic control are thought to be hypoglycemia and glucose fluctuation. It's possible that individualised glycaemicobjectives, like HbA1c, don't always result in better clinical outcomes.[4] Therefore, glycemia measurements other than HbA1c may be utilised to forecast the likelihood of problems caused by diabetes. In this situation, diabetes patients' micro- and macrovascular problems can be linked to CGM measures. For instance, GV is linked to a higher risk of negative cardiovascular outcomes, mostly due to hypoglycemia. Additionally, patients with more severe diabetic retinopathy have been found to spendless time inside the target range and had higher GV measurements.[5]Although HbA1c was once thought to be the gold standard for measuring glycaemic control, GV is unquestionably now being accepted as a more useful indicator of glycaemic control in clinical practice than HbA1c due to its limitations.[6] GV is elevated in patients with DM and in subjects with altered blood glucose management. Continuous glucose monitoring (CGM) sensor automatically measure glucose in the interstitial fluid at real time every 1-5 min. Sensor is subcutaneously implanted (often on the upper arm) and a reader periodically scans the sensor to gather data. The operate for a total of 14 days. The sensor is barely noticeable, and insertion is practically painless. Software is used to compress all CGM data from numerous days or weeks into a single 24-hour period. The software also computes other factors like glycaemic variability, time in-, above-, and below range. Elevated free radical generation and the amount of glucose variations in diabetes were linearly correlated, as measured by the mean amplitude of glycaemic excursion (MAGE)[7-8] Free radical production did not significantly correlate with the HbA1c level. Studies have shown that hyperglycaemia and dysglycaemia play a role in the development of different microvascular and macrovascular problems in people with diabetes (peaks and nadirs).[9] Micro angiopathy lesion in T2DM individuals has become a common and a matter ofconcern to physicians. Increased glycation end products, oxidative stress, acute inflammation,and neovascularization of the vasa vasorum are linked to the micro- and macro vascular problems.[10] Thus, patients are at high risk of long-lasting impairment and failure to several organs of the systems resulting in retinopathy, neuropathy, nephropathy, coronary artery disease, and stroke.[11]Diabetic neuropathy (DN) is a long-term complication of diabetes that isassociated with diabetic foot ulcers, exclusion, gait instabilities, and injuries inferior to falls. Therefore, early assessment of signs and symptoms of DN is crucial as it provides an option to identify the neuropathy at its earlier asymptomatic periods with the help of nerve conductionstudy (NCS).[12]

METHODOLOGY

The present prospective observational study was conducted at Tertiary care center, Kolhapur for 2 years

after getting the Institutional ethical approval.

A total of 93 patients were included in the study.

Inclusion criteria - Patients of either gender, age ≥35 years. diagnosed with T2DM for a duration of more than 5 years were included in the study.

Exclusion criteria- Peripheral neuropathy due to causes other than T2DM such as chronic alcoholics, severe renal impairment (creatinine >1.5mg/dl), VIT-B12 deficiency, HIV, post-herpetic neuralgia, Guillian Barre syndrome were excluded from the study.

A detailed history with general and neurological examination were obtained according to proforma. HbA1c level was checked for assessment of glycemic control of last 2-3 months. GV was evaluated in the subjects for at-least 72 hours by using continuous glucose monitoring device (Freestyle librepro, Abbott). Instructions were given to patients regarding care of glucose monitoring device sensor. GV parameters such as mean standard deviation (SD), coefficient of variance (CV), mean amplitude of glucose excursion (MAGE), time in range, below range, and above range were assessed. CV was calculated using the formula SD/average glucose x 100. CV ≥36% was considered as high GV. Nerve conduction study using NCV machine was done for both motor conduction and sensory conduction to check diabetic peripheral neuropathy in T2DM patients. Data were evaluated using SPSS V 1.2.5001 software. Continuous variables were expressed in terms of mean±SD whereas, categorical variables were presented as percentage and frequency. Pearson's correlation test was used find the correlation between the HbA1c, NCS and GV. $P<0.05$ was considered as statistically significant.

RESULTS

Age distribution

The mean age of the patients was 55.38±11.71 years. Most of the subjects were belong to the 46-55 year's age group (n=29, 31.19%) followed by 56-65 years (n=24, 25.80%), 35-45 years (n=23, 24.73%), 66-75 years (n=13, 13.98%), and 76-85 years (n=4, 4.30%).

Sex distribution

In this study, 51.61% and 48.38% of the participants were male and female respectively.

Duration of DM

The mean duration of DM was 10.64±5.16 years. The duration of DM in the majority of subjects (n=77, 82.80%) was 5-14 years. Whereas, 15-24 years, 25-34 years, and ≥35 years ofduration of DM were seen in 15.06% (n=14), 1.07% (n=1), and 1.07% (n=1) of patients respectively. The distribution of subjects according to the duration of DM is depicted in Table1.

Table 1 : Distribution of subjects according to the duration of Diabetes.

Duration of DM	Frequency (n)	Percentage (%)
5-14	77	82.80
15-24	14	15.06
25-34	1	1.07
≥35	1	1.07
Total	93	100

Glycated hemoglobin

The mean HbA1c in study subjects was 8.32±1.88%. In

33.33% (n=31) patients, HbA1c was found to be 5-6% whereas, in 30.10% (n=28), 26.89% (n=25), 8.61% (n=8),and 1.07% (n=1) of patients had 7-8%, 9-10%, 11-12%, and ≥13% of HbA1c levels respectively. The detailed distribution of patients according to HbA1c categories is depicted in Table 2.

Table 2 : Distribution of subjects according to HbA1c categories

HbA1c (%)	Frequency (n)	Percentage (%)
5-6	31	33.33
7-8	28	30.10
9-10	25	26.89
11-12	8	8.61
≥13	1	1.07

Nerve conduction study

In the majority of subjects, NCS was abnormal (80.65%, n=75) whereas, it was normal in 19.35% (n=18) of patients. The distribution of subjects according to NCS findings is illustrated in Table 3.

Table 3 : Distribution of subject according to NCS findings

NCS	Frequency (n)	Percentage (%)
Normal	18	19.35
Abnormal	75	80.65

Glycemic variability

High GV (CV ≥35%) was observed in 86.64% (n=75) of patients whereas, only 19.36% (n=18) of patients showed low GV. The distribution of subjects according to Glycemic variability is depicted in Table 4.

Table 4 : Distribution of subjects according to glycemic variability

Glycemic variability	Frequency (n)	Percentage (%)
High	75	80.64
Low	18	19.36

Use of insulin

Among n=93 T2DM subjects, n=30 (31.57%) were treated with insulin whereas, n=63 (66.31%) were treated without insulin. The detailed distribution of subjects according to use of insulin is depicted in table 5.

Table 5 : Distribution of subjects according to use of insulin

Treatment	Frequency (n)	Percentage (%)
With insulin	30	32.25
Without insulin	63	67.75
Total	93	100

Distribution of subjects according to GV and insulin use

Among n=30 patients treated with insulin, GV was high and low in n=20 (26.66%) and n=10 (55.56%) patients respectively. In patients without insulin use (n=63), n=55 (73.34%) patients had high GV whereas in n=8 (44.44%) patients GV was low. The detailed distribution of subjects according to GV and insulin use is depicted in Table 6.

Table 6 : Distribution of subjects according to GV and insulin use

Treatment	High GV (>36% CV)		Low GV (<36%CV)	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
With insulin	20	26.66	10	55.56
Withoutinsulin	55	73.34	8	44.44
Total	75	100	18	100

Comparison of demographical variables according to use of insulin and GV

There was no significant difference in demographical variables when compared according to insulin use and GV (P>0.05). The duration of DM was more in patients with insulin and high GV compared to patients with insulin and low GV however the difference was statistically insignificant (P=0.0656) (Table 7).

Table 7 : **Demographical variables according to use of insulin and GV**

Variables	With insulin		P value	Without insulin		P value
	Low GV	High GV		Low GV	High GV	
Age (years)	48.50±15.93	54.55±13.03	0.3147	51.12±10.12	56.56±12.30	0.1972
Sex (f/m)	6/4	9/11	0.6985	3/5	27/28	0.8146
Duration of DM (years)	8±2.45	10.20±3.76	0.0656	13.38±11.02	10.89±4.66	0.548

Comparison of clinical and glycemic variables according to use of insulin and GV.

A significant proportion of high GV patients with and without insulin were overweight and obese than low GV patients with and without insulin (P=0.02352 and P=0.0022). A significant proportion difference was found regarding the previous history of hypoglycemia between high GV patients with and without insulin compared to low GV patients with and without insulin (P=0.00274 and P=0.00005) (Table 8).

Table 8 : Comparison of clinical and glycemic variables according to use of insulin and GV

Variables	Subcategories	With insulin		P value	Without insulin		P value
		Low GV	High GV		Low GV	High GV	
BMI (%)	Normal	100	50	0.02352	100	34.55	0.0022
	Overweight	0	40		0	40	
	Obese	0	10		25	25.45	
NCS (%)	Normal	0	20	0.3424	25	21.82	1
	Abnormal	100	80		75	78.18	
History of hypoglycemia (%)	Yes	0	65	0.00274	0	78.18	0.00005
	No	100	35		100	21.82	
HbA1c (%)	<6.5	20	10	0.8494	10	12.50	0.9817
	>6.5	80	90		90	87.50	
Oral antidiabetics	Yes	60	30	0.8494	87.50	78.18	0.9817
	No	40	70		12.50	21.82	

Association between GV and clinical variables

GV was found to be significantly associated with HbA1c >6.5 (P=0.0036), abnormal NCS (P=0.00217), history of hypoglycemia (P=0.00172), and oral antidiabetics (P=0.00074) (Table9)

Table 9 : Association between GV and clinical variables

Variable		Odds Ratio	CI 95%	P-Value
Age in Years		1.0405	(0.99, 1.08)	0.06294
Gender	Female	Reference	-	-
	Male	1.083	(0.38, 3.07)	0.8788
Duration of DM in Years		1.0126	(0.92, 1.14)	0.0342
BMI	Normal	Reference	-	-
	Overweight	0.78	(0.34, 1.28)	0.1533
	Obese	0.62	(0.22, 1.03)	0.1821
HbA1C	< 6.5	Reference	-	-
	> 6.5	1.83	(0.82, 2.23)	0.0036
Complications	Normal	Reference	-	-
	Abnormal	2.17	(0.53, 14.62)	0.00217

Variable		Odds Ratio	CI 95%	P-Value
History of hypoglycemia	No	Reference	-	-
	Yes	2.54	(1.03, 4.72)	0.00172
Oral Antidiabetics	No	Reference	-	-
	Yes	2.343	(1.25, 4.36)	0.0074

Comparison of GV parameter with HbA1C

At a 5 % level of significance, here all the P-values are less than 0.05. Therefore, there was a significant average difference in GV parameter between the patients with HbA1C < 6.5 & HbA1C > 6.5 (Table 10).

Table 10 : Comparison of GV parameter with HbA1C

Glycemic Variability Parameter	HbA1C < 6.5		HbA1C > 6.5		P-value
	Mean	SD	Mean	SD	
Time in Range	58.73	3.07	53.82	3.92	0.0489
Time Above Range	14.71	6.53	20.14	7.77	0.0423
Time Below Range	27.50	9.12	32.65	8.79	0.0471
MAGE	164.84	59.34	158.42	58.42	0.0389
COV	84.34	37.23	89.40	36.73	0.0488

Comparison of nerve conduction study with HbA1C

Most of the patients with abnormal NCS had HbA1C >6.5 (69.89%, n=65) compared to patients with normal NCS (12.90% m=12). The difference between proportions was statistically significant (P=0.0435) (Table 11)

Table 11 : Comparison of nerve conduction study with HbA1C

NCS / HbA1C	Abnormal		Normal		P value
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	
<6.5	10	10.75	6	6.45	0.0435
>6.5	65	69.89	12	12.90	0.0435

Comparison of time in range with NCS, hypoglycemia, and HbA1C

The mean time in range was found to be significantly decreased in patients with history of hypoglycemia and HbA1c when compared according to hypoglycemia and HbA1c(P=0.000153 and P=0.0489). No significant difference was seen in the meantime in the range compared to NCS findings (P=0.5912) (Table 12).

Table 12 : Comparison of time in range with NCS, hypoglycemia, and HbA1C

Variables		Time in Range		P-Value
		Mean	SD	
Nerve Conduction Study	Normal	54.08	16.14	0.5912
	Abnormal	56.51	20.44	
Hypoglycemia	Yes	61.38	22.86	0.000153
	No	47.95	8.61	
HBA1c	< 6.5	58.73	3.07	0.0489
	> 6.5	53.82	3.92	

DISCUSSION

In this study, the average age of the patients was 55.38±11.71 years and most of the participants were male (51.61%). The mean duration of DM was found to be 10.64±5.16 years. These findings are similar to the previous study[11] Hypoglycemia and GV-associated oxidative stress cause dysfunction of endothelial consequent arteriosclerosis.[12-13] Marchand L. et al. in their study suggested that CV 36% is an appropriate cut-off value for the determination of GV.[14] Similarly, in this study, we used CV >36% as the cut-off value for GV in patients. We found high GV in 86.64% of the patients whereas, in the study of Gómez AM et al. and Marchand L et al it was 20.4%.[11, 14] Variations

in HbA1c and fasting plasma glucose levels are reported to be more associated with diabetic vascular complications than with HbA1c alone.[10,15] In this study the average HbA1c was 8.32±1.88%. Among the study patients, 17.20% (n=16) and 82.80% (n=77) of patients had HbA1c <6.5% and >6.5% respectively. In the patients with HbA1c >6.5%, n=62 had high GV whereas, in patients with HbA1c<6.5%, n=13 had high GV. A significant association was found between HbA1c>6.5% and GV.

Moreover, the other GV parameters such as TAR, TBR, MAGE, and CV were significantly increased in patients with HbA1C >6.5% than in patients with HbA1C<6.5%. Similarly, various studies have shown a significant association between GV and HbA1c is reported.[11,16-17] These findings suggested that even in good glycemic control, the GV can be abnormal which might the possible explanation be associated with long-duration diabetes complications. In this study, the majority of participants (80.65%) had abnormal NCS. In these patients, n=59 had high GV andn=65 had HbA1c>6.5%. Furthermore, a total of n=16 patients had sensory neuropathy including with mild (n=8), moderate (n=2), and severe (n=6) severity, among these patients n=7 had polyneuropathy. Motor neuropathy was found in n=15 patients among which n=9, n=3, and n=3 subjects had mild, moderate, and severe motor neuropathy respectively. In these patients n=4 patients had polyneuropathy. Whereas, in n=44 patient sensory-motor neuropathy was seen this including mild n=15, moderate n=9, and severe n=20, while in n=12 patients out of n=44 patients polyneuropathy was observed. A significant association was observed between NCS and GV (P=0.00217). Similarly, Akaza M et al., Yapanis M et al., Raj R. et al., Shen Z. et al., Mayeda L. et al.[5,18-21] have shown a significant association between increased GV parameters such as SD, MAGE, TIR, CV with DPN.[16] In this study, the time in range in the patient with and without abnormal NCV findings (54.08±16.14 vs 56.5±154.08, P=0.5912). The time in range in the patients with episodes of hypoglycemia was significantly less compared to patients without hypoglycaemia incidences. Moreover, time in range was found to be significantly less in patient with HbA1c >6.5 than patient with HbA1c <6.4. These findings suggest that the patients with less time in range had increased glycemic variability. These findings are comparable with the Mayeda L. et al.[21] The study suggested that assessment of HbA1c >6.5%, NCS, and history of hypoglycemia, were associated with a high GV. The strength of the study was the adequate sample size and uniform application of the protocol. Moreover, our study is one of few studies to use the consensus definition of high GV (CV>36%).

CONCLUSIONS

This study aimed to study glycemic variability in patients with type II diabetes mellitus in correlation with nerve conduction study and HbA1C. The conclusion of the study is that majority of study subjects had high GV. Among the study patients, 17.20% (n=16) and 82.80% (n=77) of patients had HbA1c <6.5% and >6.5% respectively. In the patients with HbA1c >6.5%, n=62 had high GV whereas, among n=13 patients with HbA1c <6.5% 6 patients had high GV. The majority of subjects had abnormal NCS with high GV and HbA1c. GV was significantly associated with HbA1c >6.5, abnormal NCS, history of hypoglycemia.

CONFLICT OF INTEREST- Nil

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A STUDY OF HACOR SCORE IN PREDICTING CLINICAL OUTCOME IN HYPOXAEMIC PATIENTS ON NIV

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ABSTRACT

Introduction- Mechanical ventilation (MV) is used to assist or replace spontaneous breathing. It is implemented with special devices that can support ventilatory function and improve oxygenation. Exclusively in conditions of acute respiratory failure (ARF), mechanical ventilation can be lifesaving. **Methodology-** The study was performed at tertiary care hospital. The sample size taken for the study was 140. Subjects who fulfilled the inclusion and exclusion criteria were recruited into the study. Routine clinical and laboratory investigations were performed on all the patients. In allincluded patients heart rate (HR), acidosis, consciousness (Glasgow coma score), O2 saturation, and respiratory rate (RR) were assessed at the 0th hr, 12th hr, 24th hr, and 48thhr and the HACOR score was calculated. Result- The study suggested that a 1hr HACOR score >5 has better predictability compared to other cut off values at different time points. **Conclusions-** The mean HACOR score was significantly more in patients who had failed NIVcompared to successful NIV.1hr HACOR score >5 has better predictability compared to other cut-off valuesat different time points. Early prediction of NIV failure can reduce ICU mortality HACOR at 1hr with >5 cut off score for the prediction of NIV failure in hypoxemic patients can play a crucial role in early intervention and prevent death.

Keywords : Hypoxemia, ventilatory function, HACOR, respiratory, NIV

INTRODUCTION

Mechanical ventilation (MV) is used to assist or replace spontaneous breathing. It is implemented with special devices that can support ventilatory function and improve oxygenation. Exclusively in conditions of acute respiratory failure (ARF), mechanical ventilation can be lifesaving. Among emergency admissions, ARF is the main factor. The yearly cost is US$54 billion and the death rate is approximately 20%.[1] Although there are no official statistics on the effects of ARF, a recent study that examined 45 public and private ICUs for a period of two months found that patients with ARF had a death rate of 34% and hospitals had a mortality rate of 42%. There are two basic methods of MV: Non-invasive ventilation and Invasive ventilation. Invasive mechanical ventilation was employed by 80% of the patients, and non-invasive mechanical ventilation by 20%.[2] Non-invasive ventilation (NIV) is a technique that does not require endotracheal intubation. Regardless of the evidence for the latter, the use of NIV during ARF has increased since the late 1990s for all diagnoses, including patients with and without chronic obstructive pulmonary disease (COPD).[3] NIV denotes to the administration of ventilatory provision without using an aggressive artificial airway (endotracheal tube

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or tracheostomy tube). Over the past two decades, the use of non-invasive ventilation has significantly risen, and it is now a crucial tool in the management of both acute and chronic respiratory failure, in both the home setting and the critical care unit. NIV is especially recommended in COPD with a respiratory acidosis pH 7.25–7.35 (H^+ 45–56 nmol/l), Hypercapnic respiratory failure secondary to chest wall deformity (scoliosis, thoracoplasty) or neuromuscular diseases, Cardiogenic pulmonary oedema unresponsive to CPAP, weaning from tracheal intubation, cystic fibrosis - e.g. bridge to transplant patients awaiting lung transplantation. NIV enhances gas conversation, counteracts inherent positive end-expiratory pressure (PEEP), and reduces the labour of breathing, according to physiologic study.[4-5] NIV reduces the need for intubation for invasive mechanical ventilation in patients with hypoxemic or hypercapnia respiratory failure.[6-8] This study mainly focused on the calculation of HACOR score of all hypoxemic patients on NIV and its comparison with clinical outcome in patients on NIV admitted in MICU.

MATERIAL AND METHODS

The present prospective interventional study was performed at tertiary care hospital. Institutional ethical committee approval was obtained before the initiation of the study. The sample size taken for the study was 140 patients and it was calculated using Solvin's formula. Patients >18 years of age, either gender, and patients on NIV were included in the study. While patients with hypercapnic respiratory failure, hypoxemic patient other than respiratory failure, already intubated patients were excluded from the study. Valid written informed consent was taken from the patients before the beginning of the study. Routine clinical and laboratory investigations were performed on all the patients. In all included patients heart rate (HR), acidosis, consciousness (Glasgow coma score), O2 saturation, and respiratory rate (RR) were assessed at the 0^{th} hr, 12^{th} hr, 24^{th} hr, and 48^{th}hr and the HACOR score was calculated (Table 1). The data was collected in predesigned proforma. The failure of NIV was defined based on need of endotracheal intubation or death. Success was defined as enhancement in gas exchange, health related quality of life and Heart Rate, improvement in PH, Consciousness level, Respiratory Rate, PaO2/FiO2 ratio without adverse effects. Five objectives must be met before home non-invasive ventilation (NIV) can beused: daily use of >4 hours, an improvement in gas exchange, health-related quality of life (HRQL), and side-effect-free sleep. Our objective was to determine the parameters associated with success and the frequency with which these five goals were attained.

Table 1 : HACOR Score

Variables	Values	Score
HR/min	<120	0
PH	>7.35	0
	7.30-7.34	2
	7.25-7.29	3
	<7.25	4
Consciousness (Glasgow)	15	0
	13-14	2
	11-12	5
	<10	10
PaO2/FiO2	>201	0
	176-200	2
	151-175	3
	126-150	4
	101-125	5
	<100	6
RR	<30	0
	31-35	1
	36-40	2
	41-45	3
	>46	4

STATISTICAL ANALYSIS

Data were evaluated using SPSS V 1.2.5001 software. Continuous variables were shown in mean±SD whereas, categorical variables were presented in percentage and frequency. Paired T-Test and Wilcoxon signed-rank test were used to compare the variables. P<0.05 was considered statistically significant.

RESULTS

In this study, a total of n=140 patients with hypoxemia, who were receiving NIV were recruited. Among these patients, n=91(65%) patients had successful NIV while 49 (35%) subjects had failed NIV. Detailed distribution of subjects according to NIV success and failure is depicted in Table 2

Table 2 : Distribution of subjects according to NIV success and failure

NIV	Frequency (n)	Percentage (%)
Success	91	65
Failure	49	35
Total	140	100

Age

The average age of the patients with successful and failed NIV was 56.33±16.80 years and 56.91±15.89 years respectively. There was no significant difference in mean age when compared between the groups (P=0.8484). The detailed comparison of mean age according to NIV success and failure is depicted in Table 3

Table 3 : Comparison of mean age according to NIV success and failure

NIV	Age (years)		P value
	Mean	SD	
Success	56.33	16.80	0.8484
Failure	56.91	15.89	

Sex

In the total of n=140 patients, n=92 (65.71%) were male followed by females (n=48, 34.29%). In NIV success and failure subjects males were predominantly presentcompared to females.

Heart rate

A significant difference was observed in mean HR when compared between patients with NIV success and failure (P<0.05). Success was defined as reduction in heart rate less than 120.Failure was defined as HR more than 121.(Table 4)

Table 4 : Comparison of mean HR between subjects with NIV success and failure

Time intervals (Hr)	HR (mean±SD)		P value
	NIV success	NIV failure	
1	121.08±20.87	131±12.23	0.00057
12	117.18±14.84	131±12.23	$3.91e^{-08}$
24	111.22±13.73	120.95±13.41	0.00010
48	105.56±19.10	113.02±15.66	0.0149

pH

There was no significant difference observed in the pH when compared betweenpatients with NIV success and failure. Success was defined as increase in ph. Failure was defined as decrease in ph. (Table 5)

Table 5 : Comparison of mean pH between patients with NIV success and failure

Time intervals (Hr)	PH (mean±SD)		P value
	NIV success	NIV failure	
1	7.36±0.20	7.38±0.18	0.6144
12	7.38±0.23	7.38±0.18	0.9375
24	7.38±0.24	7.40±0.23	0.6051
48	7.34±0.44	7.43±0.26	0.1613

Glasgow Coma Score

A significantly decreased Glasgow coma score was observed in NIV failure patients than in NIV success patients at all-time intervals (P<0.05). Success was defined as improvement in GCS or gcs more than 14 failure was defined as GCS less then equal to 14. (Table 6)

Table 6 : Comparison of mean Glasgow coma score between patients with NIV success and failure

Time intervals (Hr)	GLASGOW (mean±SD)		P value
	NIV success	NIV failure	
1	13.61±1.12	11.95±1.60	$1.32e^{-8}$
12	13.42±0.83	11.95±1.60	$1.34e^{-07}$
24	13.56±1.77	12.59±1.68	0.000508
48	13.85±1.80	13.20±1.77	0.04377

Oxygen saturation

There was no significant difference in O2 saturation when compared between patients with NIV success and failure (P>0.05). Success was definedas pao2/fio2 ratio more than equal 201. Failure was defined as pao2/fio2 ratio less than equal to 200. (Table 7)

Table 7 : Comparison of mean O2 saturation between patients with NIV success and failure

Time intervals (Hr)	Pao2 / Fio2 (mean±SD)		P value
	NIV success	NIV failure	
1	166.99±87.84	161.44±26.61	0.1757
12	167.34±30.70	160.44±26.61	0.1702
24	170.93±32.03	171.59±32.08	0.9082
48	174.52±43.12	191.61±20.83	0.00213

Respiratory rate

In patients with NIV failure, the RR was significantly increased at all- time intervals except at 48 hours than NIV success patients (P<0.05). Successwas defined as respiratory rate less than 30. Failure was defined as more then equal to 30. (Table 8)

Table 8 : Comparison of mean RR between subjects with NIV success and failure

Time intervals (Hr)	RR (mean±SD)		P value
	NIV success	NIV failure	
1	30.28±5.51	33.12±2.95	$1.99e^{-05}$
12	29.47±3.67	33.12±2.95	$3.92\ e^{-09}$
24	28.32±4.50	29.95±3.54	0.0205
48	26.84±6.29	26.93±4.66	0.9189

HACOR score

The mean HACOR score at 1hr, 12hr, and 24hr was significantly high in subjects with NIV failure as compared to subjects with NIV success (P<0.05) (Table 9)

Table 9 : Comparison of mean HACOR score between subjects with NIV success and failure

Time intervals (Hr)	HACOR (mean±SD)		P value
	NIV success	NIV failure	
1	6.01±1.78	10.44±3.48	$1.07e^{-11}$
12	6.24±2.01	10.44±3.48	$7.5\ e^{-11}$
24	6.31±3.80	7.77±4.01	0.0398
48	5.52±6.75	4.87±3.93	0.4762

HACOR score at 1hr

In 80% (n=112) of patients, the HACOR score was >5. Whereas, it was <5 in 20% (n=28) of patients

respectively. The detailed distribution of subjects according toHACOR score is illustrated in Table 10.

Table 10 : Distribution of subjects according to HACOR score

HACOR score	Frequency (n)	Percentage (%)
<5	28	20
>5	112	80
Total	140	100

Comparison of NIV outcome according to HACOR score

Among the n=28 subjects with HACOR score <5, n=25 had successful NIV andn=3 had NIV failure. Out of n=112 patients with HACOR >5, NIV was failed in n=46 subjects whereas it was successful in n=66 patients (Table 11).

Table 11 : Comparison of NIV outcome according to HACOR score

NIV HACOR	Success	Failure	Total
<5	**25**	**3**	**28**
>5	**66**	**46**	**112**
Total	**91**	**49**	**140**

Predictive power HACOR score assessed at 1 hr, 12 hr, 24 hr, and 48 hr in the prediction of failed NIV

After 1hr NIV assessment, the HACOR score at cut-off point >5 showed 80.8%sensitivity, 89.84% specificity, 91.45% of PPV, 70.2% of NPV, and 83% of diagnostic accuracy. Whereas after 12 hr, 24hr, and 48hr, HACOR score at cut off point >6 diagnostic measures less compared to >5 cut off at 1hr interval. The detailed distribution of the predictive power of the HACOR score at different time intervals is depicted in Table 12.

Table 12 : Predictive power HACOR score assessed at 1 hr, 12 hr, 24 hr, and 48 hrin the prediction of failed NIV

Diagnostic measures	1hr (HACOR >5)	12hr (HACOR >6)	24hr (HACOR >6)	48hr (HACOR >6)
Sensitivity (%)	80.8	77.94	75.4	75.2
Specificity (%)	89.84	87	85	85
Positive predictive value (%)	91.45	89.2	88.36	88.2
Negative predictive value (%)	70.2	71.2	70.4	70.1
Diagnostic accuracy (%)	83	82	83	85

DISCUSSION

The study was undertaken to identify NIV failure in hypoxemic subjects using the HACOR score. In this study, the incidence of NIV failure was 35%. The mean age of the patients with failed and successful NIV was 56.33±16.80 years and 56.91±15.89 years respectively. Moreover, males were predominantly present compared to females (65.71% vs 34.29%). There was no significant difference seen in patients with NIV failure and with NIV success (P>0.05). These findings are comparable with previous reports. [9, 11]

Duan J. and their associates were the first who studied the HACOR score in thedetection of INV failure among hypoxemic subjects. The score is calculated based on the HR, pH, responsiveness, O2 saturation, and RR which are easily available bedside. They asserted that despite the scale's assessment of diverse subgroups based ondiagnosis, age, illness severity, or time points, this scoring system with a cutoff value of >5 exhibits a remarkable capacity for predicting NIV failure.[11] Similarly, in this study, we adopted a HACOR score >5 cutoffs as the risk of NIV failure at 1hr, 12hr, 24hr, and 48hr time intervals.

In this study patients with and without successful NIV when assessed according to HACOR score found that the mean score in patients with failed NIV was significantly more compared to successful NIV at 1hr (6.01±1.78 vs 10.44±3.48, P=1.07e^{-11}), 12hr (6.24±2.01 vs 10.44±3.48 P=7.5 e^{-11}), 24hr (6.31±3.80 vs 7.77±4.0 P=10.0398), and 48hr (5.52±6.75 vs 4.87±3.93 P=0.4762) time points. Moreover, among study subjects, 80% of the patients had a HACOR score >5 whereas, it was <5 in 20% of the subjects. Similar findings are reported in the studies of Duan J. et al, Magdy DM. et al. and other studies. [9-12] These findings suggest that the HACOR score increases in NIV-failed subjects.

In this study, among the n=28 subjects with HACOR score <5, n=25 had successful NIV and n=3 had NIV failure. Out of n=112 patients with HACOR >5, NIV was failed in n=46 subjects whereas it was successful in n=66 patients. We found that the HACOR score at 1st hr of NIV and cutoff score >5 has a sensitivity of 80.8%, specificity of 89.84%, PPV of 91.45%, NPV of 70.2%, and DA of 83%. Whereas, at 12hr with a cut-off score >6 has sensitivity, specificity, PPV, NPV, and DA of 77.94%, 87%, 89.2%, 71.2%, and 82% respectively. At 24hr it was 74.4%, 85%, 88.36%, 70.4% and 83% respectively. While, at 48 hr at cut-off score >6, HACOR has 75.2%, 85%, 88.2%, 70.1%, and 85% of sensitivity, specificity, PPV, NPV, and DA respectively. These findings suggested that the HACORE cut-off score >5 at 1hr has better predictability compared cutoff score >6 at the 12th hr, 24th hr, and 48th hr. Similarly, Magdy DM. et al suggested that HACOR cutoff score 6 at 1hr has 81%, 91%, and 85% of sensitivity, specificity, and diagnostic accuracy. They suggested HACOR cutoff score >5 at 1hr has better predictability compared cutoff score >6 at the 12th hr, 24th hr, and 48th hr.[9] Whereas, Ishikawa O et al. validated the HACOR scale and reported thatpatients with a score >5 are more prone to intubation and thus can be important in the determination of NIV failure to reduce mortality.[13] Bai L. et al. conducted a study to evaluate the efficacy of NIV using HACOR in ARDS patients and suggested that the reduction of HACOR score after 1–2 h of NIV can identify the patients who respond well to NIV.[14]

The study suggested that a 1-hr HACOR score >5 has better predictability compared to other cutoff values at different time points. The strength of the study wasthe adequate sample size and uniform application of the protocol. Determination of HACOR score at various time points with diagnostic measures makes this study uniqueas there is a lack of data in the literature concerning after 1-2hr of intervention.

LIMITATIONS OF THE STUDY

Investigator was not blind and the study was single centered all together couldhave led to some bias. The performance of NIV was assessed by the attending physician. Patients other than hypoxemia were not included.

CONCLUSION

The mean HACOR score was significantly more in patients who had failed NIV compared to successful NIV.1hr HACOR score >5 has better predictability compared to other cut-off values at different time points. Early prediction of NIV failure can reduce ICU mortality HACOR at 1hr with >5 cut-off score for the prediction of NIV failure in hypoxemic patients can play a crucial role in early intervention and prevent death. Further studies are warranted to confirm the present study findings.

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ORIGINAL ARTICLE

TO STUDY INCIDENCE AND PATTERNS OF DRY EYE CHANGES FOLLOWING MANUAL SICS AND PHACOEMULSIFICATION

Sanket Patil, **Shadakshari S. Math*****

ABSTRACT

Introduction : The tear-film-cornea interface forms the strongest refracting surface of the eye. Any abnormality in the tear film causes sub-optimal visual performance. A dry eye leads to significant morbidity due to its symptoms, commonly surface discomfort like foreign body sensation, pricking and gritty feeling, and visual disturbance. On studying the incidence and pattern of dry eye after cataract surgery, we can improve the quality of life of the patients, and counsel them about the symptoms of dry eye. **Materials and Method :** The study was conducted on 120 patients divided into two groups of 60 each undergoing Manual SICS and Phacoemulsification surgery. Incidence and pattern of dry eye were analyzed at days 0, 1 week, 4 weeks, and 12 weeks post-operatively using the Tear break-up test, Schirmer's I, Tear meniscus height, and Lissamine green B staining. **Results :** Seven days after cataract surgery the incidence and severity of dry eye was observed to be higher in the Manual SICS group in comparison with the Phacoemulsification group. The peak of dry eye incidence was found to be at seven days' post-operative period in both groups. The symptoms improved gradually over the 12-week period in both groups. **Conclusion :** Following cataract surgery, the incidence of dry eye increases by the first week and most of the symptoms are resolved by 12 weeks after surgery. The incidence and severity is found to be higher after Manual SICS. We should evaluate patients pre and post-operatively to be able to manage the patient's discomfort promptly and efficiently.

INTRODUCTION

The definition of Dry eye is a "multifactorial disease of the tears and surface of the eye that causes symptoms of loss of comfort, disturbed vision, and loss of stability of tear film with likely damage to the surface of the eye. It is accompanied by increased osmolarity of the tear film and subacute inflammation of the surface of the eye". [1] The main lacrimal gland, meibomian glands, the nerve supply between them, and the surface of the eye (cornea, conjunctiva, accessory lacrimal glands), specialised sebaceous glands present at the eyelid edge, that generate the external lipidiform tier of the tear film, all work together as a functional unit. Dry eye illness may impact some or all of these structures.[2] The tear film which coats the surface of the eye is vital for shielding the eye from the environment, providing lubrication to the surface of the eye, keeping a smooth surface for refracting light, and maintaining conjunctival health and the corneal avascularity. The tear film is produced at a rate of about 2 µL/min, it is about 3 to 10 µL in volume

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and 3 μm in thickness. [3-4] The pH of tears is about 7.45 and varies between 7.14 to 7.82, based on diurnal and seasonal changes.[4] Closure of lids for long time, like while sleeping, results in an increase of carbon dioxide, thus decreasing the pH. It has three layers– mucin, aqueous, and lipid layer. Primary lacrimal glands secrete almost all the the aqueous layer, while some is secreted by the goblet cells of conjunctiva and supplementary lacrimal glands.[4] The tears either vaporise or get emptied via the punctum. Tears are of three varieties. Basal tears provide nutrients, ocular comfort and help in shedding of debris, and are present on the surface of the eye. Tears that are shed as a reaction to a source of irritation like chemicals and alien objects are known as reflex tears. Reflex tears help clear the surface of the eye of irritants and are released in greater amount than basal tears. Tears that are secreted behind closed eyes while you sleep help in lubrication. Some elements of the tear film, including lactoferrin, lipocalin-1, and lysozyme, largely hold true throughout many varieties of tears.[3] Protein and lipid content are higher in basal tears, but overall amounts of protein, lipid, and secretory IgA differ among types. The osmolarities in various tear types are largely stable despite compositional variations.[5] Cataract at present is the root cause of avoidable blindness mainly in the developing world responsible for almost 3/4th of blindness. Phacoemulsification is the primary technique used to do cataract surgery in the developed world. However, due to the density of cataracts involved and the highly expensive equipment, phacoemulsification is not an option in many poor nations, which accounts for the bulk of cataract blindness in the globe today.[6] According to a theory, alterations in the surface of the eye brought on by surgical trauma and post-operative inflammation are what causes dry eye following cataract surgery. Following cataract surgery, many people report experiencing a pricking sensation, discomfort, red eye, and blurred vision, all of which are thought to be unfavourable side effects.[7] It's critical to understand the prevalence of dry eye following cataract surgery and risk factors for this particular type of dry eye because it can influence the functional recovery.[8] As per the long-duration research based on a population, the incidence of dry eye in people between ages 43 and 86 are 13.3 percent and 21.6 percent, respectively, at 5 and 10 years of follow-up.[9] To evaluate dry eye, there is no one gold standard study. The sensitivity and specificity of various diagnostic techniques utilised as a result vary. In research investigations involving dry eye tests in several situations, surface-of-the-eye surveys are frequently used.[1-6] According to a study by Sitomptu R. et al., the OSDI score decreased two weeks after SICS surgery, while total OSDI dramatically increased following phacoemulsification surgery.[10] Previous research that looked at how cataract surgery affected tear film parameters noted a temporary disturbance in tear production. Schirmer's score and TBUT decreased in 23 post-cataract surgery patients, according to Ram et al.[6,11] Phacoemulsification is a good alternative to manual small incision cataract surgery in situations where extremely large volume surgery with low-cost instrumentation is necessary. The most frequent procedure carried out in ophthalmic units is cataract extraction, and several patients have reported postoperative dry eye and irritation problems. Symptoms like pain, irritation, and decreased vision can occur due to dry eye. The surface of the eye disease usually gets worse following cataract surgery. A number of things can cause this, such as the cleaving of corneal nerves that causes corneal sensation loss

and slowed healing, damage to the corneal epithelium from intense microscopic light exposure, prolonged irrigation of the surface of the eye with irrigating solutions, irritation of surface of the eye that causes an increase in inflammatory mediators, and the use of preservatives in topical anaesthetic drops.[6] The studies done prior evaluated the incidence of dry eye following cataract surgery. Therefore, current study was performed to assess and compare the incidence of dry eye following Manual SICS and Phacoemulsification.

AIMS AND OBJECTIVES

Aim : To study incidence and pattern of dry eye changes following Manual SICS and Phacoemulsification.

Objectives : To study incidence and pattern of dry eye changes following Phacoemulsification, to study the incidence and pattern of dry eye changes following Manual SICS, and to compare incidence and pattern of dry eye changes following Manual SICS and Phacoemulsification.

MATERIALS AND METHOD

Current randomized prospective, observational study was conducted under the Ophthalmology department, a tertiary care center. Approval was taken from Institution Ethics Committee before beginning of study. An informed written consent has been taken from the patients before including them in the study.

Study Design : Randomized, prospective, observational study.

Duration of Study : March 2021 - July 2022

Study Population : Patients having senile cataracts undergoing cataract surgery and meeting the inclusion and exclusion criteria.

Inclusion Criteria: Patients of either gender and aged 55 to 80 years, patients having senile cataracts on slit-lamp biomicroscopy, and patients signing consent for taking part in the study.

Exclusion Criteria: Patients under 55 years and above 80 years age, with other types of cataract except senile cataract, with pre-existing dry eye disease. on medications likely to cause dry eye, having lid abnormalities, who have undergone previous ocular surgeries. wearing contact lenses for prolonged period having thyroid disease, DM, collagen vascular disease, vitamin A deficiency and autoimmune diseases, like SLE, Sjogren's syndrome, with history of ocular trauma, not consenting to be a part of the study.

Sample Size : Considering Type I error to be 5% and based on a prevalence of Phacoemulsification of 4% at our hospital, a sample size of 59.006≈60 was calculated.

Therefore, the total sample size for the study was 60 for each group: Manual SICS and Phacoemulsification.

SAMPLING TECHNIQUE :

All the patients confirmed with senile cataract and attending OPD in the Department of Ophthalmology during the study period and who after inclusion and exclusion criteria were met, were included in the study. All consecutive patients were included until desired sample size was met. Then patients were allocated to one of the two Groups on random basis.

ETHICAL CONSIDERATIONS : Approval was taken from the Institutional Ethics Committee prior to performing the study.

INSTRUMENTS USED

Snellens Chart, Slit Lamp, Indirect Ophthalmoscope (+20D lens), Slit Lamp Biomicroscope. (+90D lens), Slit Lamp Biomicroscope with attached graticule, Schirmer's Strip (Whatman no.41 filter paper), Fluorescence Strip, Lissamine Green B staining of conjunctiva and cornea. All instruments mentioned above were available in the OPD and were used for ophthalmic evaluation regularly for no extra cost to the patient.

METHOD OF DATA COLLECTION : Demographic details were recorded. Detailed history of present and past illness along with personal history were taken from all the patients and noted. A detailed ophthalmic examination was done to rule out ocular pathology after a general and systemic workup.

All the included cases underwent a distant visual acuity test using Snellen's visual acuity chart at a distance of 6 meters in a well-illuminated room. The patient was made to sit 6 meters distance from the chart and put on a well-fitted trial frame. Left eye of the patient was occluded using an occluder while testing right eye and vice versa. The patient was then asked to start reading from the topmost line of the chart and continue doing so until he/she reached a line where more than half the letters (eg. 3 of 5) were read incorrectly or he/she read all letters on the chart including the bottom (6/6) line. If the patient had previously prescribed spectacles, the visual acuity was tested with spectacles as well. Patients were allowed to read the chart only once and visual acuity was noted. Monocular testing was done.

The details of the affected side were noted. The patients were divided to one of the two groups as follows on random: Group A: Treated with Manual SICS. Group B: Treated with Phacoemulsification. The baseline tear film was analysed by the following tests:

Tear Meniscus Height (TMH) test: It was recorder using slit lamp biomicroscopy. The eyepiece had an attached measuring graticule. The lower lid TMH was recorded under low magnification(8x) and at high magnification(32x). The findings were recorded and graded as follows: 0.5 mm or more: Normal, Less than 0.5 mm: Dry eye

Schirmer-I test: This test was done to measures the aqueous tear secretion without topical anesthetics. It was used for the diagnosis and grading of dry eye. Whatmann no.41 (5 mm x 35 mm) filter paper was placed in inferior cul-de-sac at the junction of outer one-third and inner two-third and amount of wetting of filter paper after 5 minutes was measured. The findings were recorded and graded as follows:

More than 15 mm: Normal, 10 to 15 mm: Mild dry eye, 5 to 10 mm: Moderate dry eye, Less than 5 mm: Severe dry eye, Special care was taken to prevent alteration of test results due to evaporation of tear from the film. Lissamine Green B test: This test is done to evaluate the dead and devitalised cells on the surface of the eye.

The topical 1% Lissamine Green stain was applied with a pipette in lower conjunctival cul-de-sac. It stained the devitalized epithelium not protected with a mucus layer. It showed the surface of the eye damage. The interpretation of Lissamine Green staining in dry eye was based on factors that are intensity and location. Van Bijsterveld[9] reported a grading scale that evaluates intensity of staining based on scale of

0 to 3 in three areas : Nasal conjunctiva, Temporal conjunctiva, and Cornea. The highest possible grading score with this system is 9. The scores were recorded and graded as follows : Upto 3 : Normal, More than 3 : Dry eye.[13]

Tear Break-Up Time (TBUT) test: This test was performed to evaluate the lipid components of the tear film. An A dry fluorescence strip was touched to the inferior fornix with patient asked to look up. This was done to stain the tear film. The patient was then instructed to blink rapidly for a couple of seconds and then stop. The cornea was seen on a slit lamp with low magnification using cobalt blue light. At the time of appearance, the first small black spot the cornea from the last complete blink was noted. The findings were recorded and graded as shown below: 10 to 30 seconds: Normal, 5 to 10 seconds: Mild-to-Moderate dry eye, Less than 5 seconds: Severe dry eye

The patients then underwent Manual SICS or Phacoemulsification according to the assigned Group. Pre-operative preparation was done according to the standard Institution protocols. The Manual SICS and Phacoemulsification operative procedures were done as per standard guidelines. Patients were discharged the following day on being hemodynamically stable. They were followed up at the first week, fourth week, and twelfth week post-operatively. The findings of the TMH test, Schirmer-I test, Lissamine Green B test, and TBUT test were noted at each follow-up visit. **Statistical Analysis**: The data was analyzed using statistical0software (IBM SPSS, IBM Corporation, Armonk, NY, USA).

OBSERVATIONS AND RESULTS

Table 1: Comparison of the findings of the TMH test during the study period in the two groups

Time	Findings	MANUAL SICS		PHACO-EMULSI-FICATION		P VALUE
		N	%	N	%	
Baseline	Normal	60	100%	60	100%	--
	Dry	0	0%	0	0%	
First Week Fup	Normal	13	21.67%	11	18.33%	0.648
	Dry	47	78.33%	49	81.67%	
Fourth Week Fup	Normal	30	50%	41	68.33%	0.041*
	Dry	30	50%	19	31.67%	
Twelfth Week Fup	Normal	58	96.67%	60	100%	0.248
	Dry	2	3.33%	0	0%	

Table 1 and Figures 1 and 2 show the distribution of the findings of the TMH test in the two Groups. All the cases had normal eyes at baseline. There was an increased incidence of dry eye in both the Groups at the first follow-up week: 78.33% in the Manual SICS Group vs 81.67% in the Phacoemulsification Group; P value: 0.648. There was a recovery in both the Groups by the fourth follow-up week (Phacoemulsification>Manual SICS); P value: 0.041. By the twelfth follow-up week, near-complete recovery was noted in almost all the cases; P value: 0.248.

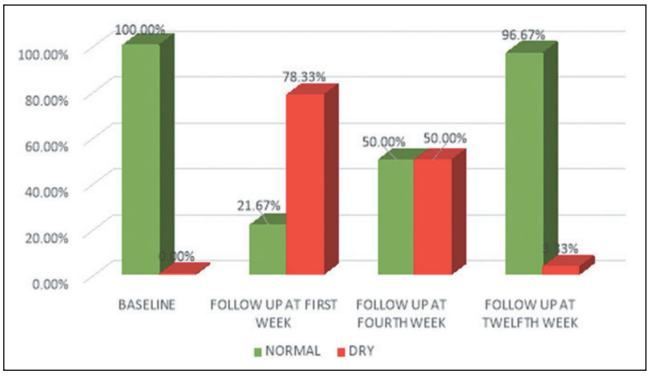

Fig. 1 : Intra-Group comparison of findings of TMH test in the Manual SICS Group

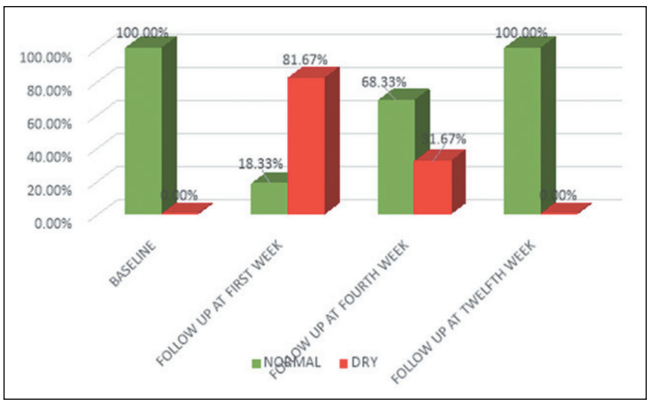

Fig. 2 : Intra-Group comparison of findings of TMH test in the Phacoemulsification Group

Table 2: Comparison of the findings of the Schirmer-I test during the study period in the two groups

Time	Findings	MANUAL SICS		PHACO-EMULSI-FICATION		P VALUE
		N	%	N	%	
Baseline	Normal	60	100%	60	100%	--
First Week Fup	Normal	2	3.33%	6	10%	<0.001*
	Mild	10	16.67%	50	83.33%	
	Moderate	40	66.67%	4	6.67%	
	Severe	8	13.33%	0	0%	
Fourth Week Fup	Normal	11	18.33%	34	56.67%	<0.001*
	Mild	21	35%	26	43.33%	
	Moderate	28	46.67%	0	0%	
	Severe	0	0%	0	0%	
Twelfth Week Fup	Normal	45	75%	60	100%	<0.001*
	Mild	15	25%	0	0%	
	Moderate	0	0%	0	0%	
	Severe	0	0%	0	0%	

Table 2 and Figures 3 and 4 show the distribution of the findings of the Schirmer-I test in the two Groups. All the cases had normal eyes at baseline. There was an increased incidence of dry eye in both the Groups at the first follow-up week: 66.67% moderate dry eye in the Manual SICS Group vs 83.33% mild dry eye in the Phacoemulsification Group; P value: less than 0.001. There was a recovery in both the Groups by the fourth follow-up week; 46.67% moderate dry eye in the Manual SICS Group vs 43.33% mild dry eye in the Phacoemulsification Group; P value: less than 0.001. By the twelfth follow-up week, 75% cases in the Manual SICS Group had normal eyes as compared to 100% in the Phacoemulsification Group; P value: less than 0.001.

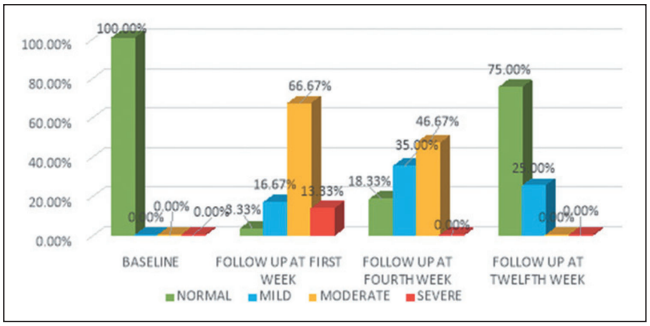

Fig. 3 : Intra-Group comparison of findings of the Schirmer-I test in the Manual SICS Group

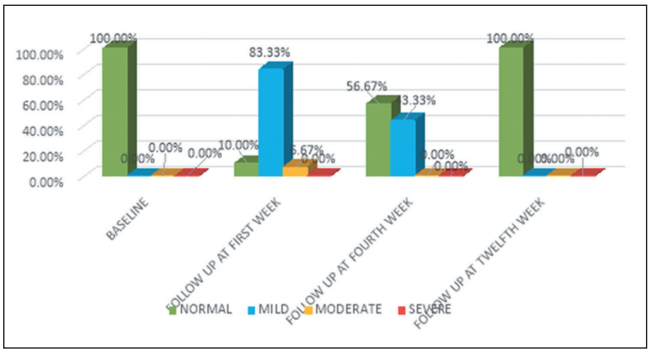

Fig. 4 : Intra-Group comparison of findings of Schirmer-I test in the Phacoemulsification Group

Table 3: Comparison of the findings of Lissamine Green B test during the study period in the two groups

Time	Findings	MANUAL SICS		PHACO-EMULSI-FICATION		P VALUE
		N	%	N	%	
Baseline	Normal	60	100%	60	100%	--
	Dry	0	0%	0	0%	
First Week Fup	Normal	12	20%	37	61.67%	<0.001*
	Dry	48	80%	23	38.33%	
Fourth Week Fup	Normal	35	58.33%	57	95%	<0.001*
	Dry	25	41.67%	3	5%	
Twelfth Week Fup	Normal	58	96.67%	60	100%	0.248
	Dry	2	3.33%	0	0%	

Table 3 and Figures 5 and 6 show the distribution

of the findings of the Lissamine Green B test in the two Groups. All the cases had normal eyes at baseline. There was an increased incidence of dry eye in both the Groups at the first follow-up week: 80% in the Manual SICS Group vs 38.33% in the Phacoemulsification Group; P value: less than 0.001. There was recovery in both Groups by the fourth follow-up week with the prevalence of normal eyes being 58.33% in the Manual SICS Group vs 95% in the Phacoemulsification Group; P value: less than 0.001. By the twelfth follow-up week, 96.67% of cases in the Manual SICS Group had normal eyes as compared to 100% in the Phacoemulsification Group; P value: 0.248.

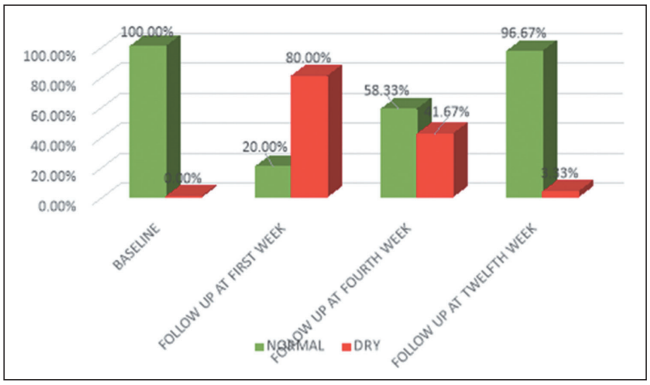

Fig. 5 : Intra-Group comparison of findings of the Lissamine Green B test in the Manual SICS Group

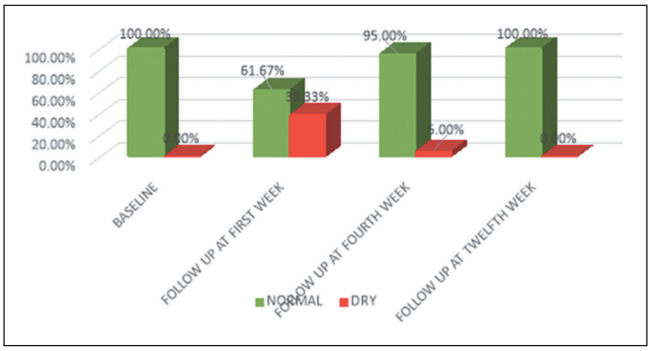

Fig. 6 : Intra-Group comparison of findings of Lissamine Green B test in the Phacoemulsification Group

Table 4: Comparison of the findings of TBUT test during the study period in the two groups

Time	Findings	MANUAL SICS		PHACO-EMULSI-FICATION		P VALUE
		N	%	N	%	
Baseline	**Normal**	60	100%	60	100%	--
First Week Fup	**Normal**	5	8.33%	28	46.67%	**<0.001***
	Mild	0	0%	0	0%	
	Moderate	54	90%	32	53.33%	
	Severe	1	1.67%	0	0%	
Fourth Week Fup	**Normal**	35	58.33%	59	98.33%	**<0.001***
	Mild	0	0%	0	0%	
	Moderate	25	41.67%	1	1.67%	
	Severe	0	0%	0	0%	
Twelfth Week Fup	**Normal**	60	100%	60	100%	--
	Mild	0	0%	0	0%	
	Moderate	0	0%	0	0%	
	Severe	0	0%	0	0%	

Table 4 and Figures 7 and 8 show the distribution of the findings of TBUT test in the two Groups. All the cases had normal eyes at baseline. There was an increased incidence of dry eye in both the Groups at the first follow up week: 90% moderate dry eye in the Manual SICS Group vs 53.33% moderate dry eye in the Phacoemulsification Group; P value: less than 0.001. There was recovery in both the Groups by the fourth follow-up week; 58.33% normal eye in the Manual SICS Group vs 98.33% normal eye in the Phacoemulsification Group; P value: less than 0.001. By the twelfth follow up week, all the cases had normal eyes in both the Groups.

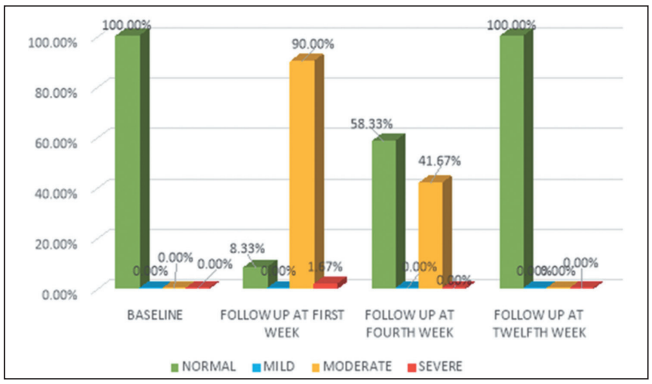

Fig. 7 : Intra-Group comparison of findings of TBUT test in the Manual SICS Group

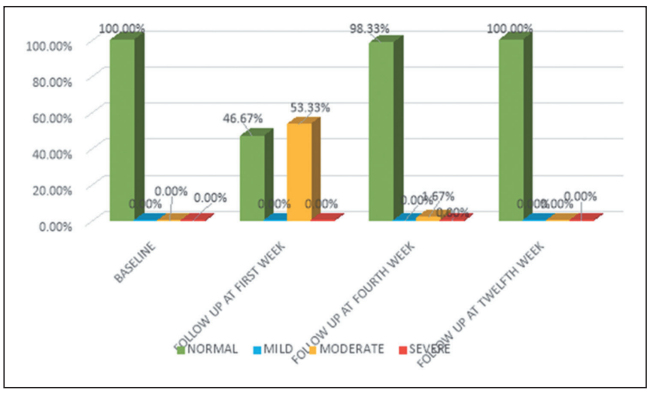

Fig. 8 : Intra-Group comparison of findings of TBUT test in the Phacoemulsification Group

DISCUSSION

Dry eye disease is a spectrum of disorders ranging from tear instability, to neurosensory abnormalities. These can cause chronic dysfunction.[12] In addition pre-existing dry eye is challenging for cataract surgery, as it causes chronic inflammation, cicatrizing changes, lid abnormalities, and compromised media clarity,[14] It may also worsen following cataract surgery causing complications and compromised visual outcomes. [15-17] This is probably due to increased inflammation, toxicity from eye drops containing preservatives like benzalkonium chloride (BAK) and corneal nerve damage from limbal incisions. Besides, phacoemulsification, by itself, has been reported to cause a reduction in tear film breakup time, tear meniscus height, and corneal sensitivity post-operatively. Though several studies have documented the increased incidence of dry eye following cataract surgery, the studies comparing incidence after different techniques of cataract surgery are scarce, particularly in the Indian scenario. Hence, the current study was conducted to evaluate the incidence of dry eye following Manual SICS and Phacoemulsification and to compare incidence and resolution. The present study was conducted after obtaining approval from the Institutional Ethics Committee. A total of 120 patients having senile cataract and undergoing surgery were included in the study after obtaining written informed consent. Demographics and detailed histories were recorded. Ocular pathologies were ruled out. Detailed ophthalmologic examination was done. Baseline tear film was assessed by the TMH test, Schirmer-I test, Lissamine Green B test and TBUT test. Patients were assigned randomly into one of two Groups: Manual SICS and Phacoemulsification. The surgery and post-operative management was done as per the standard guidelines. The patients were followed up post-operatively at first, fourth and twelth weeks. The tear film was assessed by the afore mentioned tests at every visit. Excel was used to enter and evaluate the data. The following sections provide an overview of the findings.

Demographics : In the present study, it was observed that the mean age of the population in the Manual SICS Group was 66.65 ± 7.84 years and in the Phacoemulsification Group was 63.93 ± 7.36 years. The age range was 51 to 80 years. The age distribution was similar in the two groups; P value: 0.53. There was almost equal involvement of both the genders. The gender distribution was also similar in the two Groups; P value: 1.000.

In the study by Ishrat S. et al[18], they included a total of 96 cases. They observed that the mean age of the study population was 63.1 ± 8.3 years. This was similar to the present study. Similar age distribution was observed in the study by Singh H. et al.[19]

Kumari P. and Lakra M.[20], they included 187 patients with cataract in their study. They observed similar gender distribution in their study 55.08% males and 44.91% females. This was similar to the present study.

Findings of the TMH test: In the present study, it was observed that all the cases in both the Groups had normal eyes. At the first follow up week, 78.33% cases in the Manual SICS Group had dry eyes as compared to 81.67% in the Phacoemulsification Group. The incidence was similar in the two Groups; P value: 0.648. However, by the second follow up in the fourth week, most of the cases had recovered in the Phacoemulsification Group with the prevalence of dry eyes being 31.67% as compared to 50% in the Manual SICS Group; P value: 0.041. By the end of the twelfth week, most of the cases had recovered in both the Groups (3.33% in the Manual SICS Group vs 0% in the Phacoemulsification Group); P value: 0.248.

Findings of the Lissamine Green B test: In the present study, it was observed that all the cases in both the Groups had normal eyes. At the first follow up week, the incidence of dry eyes was significantly more in the Manual SICS Group as compared to the Phacoemulsification Group (80% vs 38.33%, respectively); P value: less than 0.001. By the second follow up in the fourth week, significant recovery was noted in both the prevalence of dry eyes being 5% in the Phacoemulsification Group as compared to 41.67% in the Manual SICS Group; P value: less than 0.001. By the end of the twelfth week, most of the cases had recovered in both the Groups (3.33% in the Manual SICS Group vs 0% in the Phacoemulsification Group); P value: 0.248.

Findings of the Schirmer-I test: In the current investigation, it was shown that both groups of individuals had normal eyes in all cases. At the first follow up week, most of the cases in both the Groups had dry eyes with the prevalence of normal eyes being 3.33% in the Manual SICS Group and 10% in the Phacoemulsification Group. Amongst the cases having dry eyes, most of the cases had moderate dry eyes in the Manual SICS Groups (66.67%) while most of the cases in the Phacoemulsification Group had mild dry eyes (83.33%). Severe dry eyes were noted only in the Manual SICS Group (13.33%). The distribution was significantly different in the two Groups; P value: less than 0.001. By the second follow up in the fourth week, significant recovery was noted in the Phacoemulsification Group as compared to the Manual SICS Group with the prevalence of normal eyes being 56.67% in the Phacoemulsification Group as compared to 18.33% in the Manual SICS Group. Amongst the cases having dry eyes, most of the cases still had moderate dry eyes in the Manual SICS Group (46.67%) as compared to mild dry eyes in the Phacoemulsification Group (43.33%). The distribution was significantly different in the two Groups; P value: less than 0.001. By the end of the twelfth week, all the cases had recovered in Phacoemulsification Group as compared to only 75% cases in the Manual SICS Group; P value: less than 0.001.

Findings of the TBUT test: In the current investigation, it was shown that both groups of individuals had normal eyes in all cases. At the first follow up week, the incidence of dry eyes was significantly more in the Manual SICS Group as compared to the Phacoemulsification Group with the prevalence of normal eyes being 8.33% and 46.67% respectively. Amongst the cases having dry eyes, most of the patients had moderate dry eyes in both the Groups while severe dry eyes were observed in Manual SICS Group only (1.67%). The distribution was significantly different in the two Groups; P value: less than 0.001. By the second follow up in the fourth week, significant recovery was noted in the Phacoemulsification Group

as compared to the Manual SICS Group with the prevalence of normal eyes being 98.33% in the Phacoemulsification Group as compared to 58.33% in the Manual SICS Group. Amongst the cases having dry eyes, moderate dry eyes were present in 41.67% cases in the Manual SICS Group as compared to 1.67% in the Phacoemulsification Group. The distribution was significantly different in the two Groups; P value: less than 0.001. By the end of the twelfth week, all the cases had recovered in both the Groups.

In the study by Bista B. et al [21], they included 100 cases, 50 underwent Manual SICS and 50 underwent Phacoemulsification. They used Schirmer's I test, TBUT, Lissamine Green Surface Staining and OSDI. They observed that per-operative values of the tests were similar in the two Groups; P value: more than 0.05. They observed that the values of Schirmer's I test and TBUT were significantly lower in the SICS Group while Lissamine Green Stain values were higher as compared to the Phacoemulsification Group; P value: less than 0.001. Thus, they concluded that the incidence of dry eyes was significantly more after SICS than after Phacoemulsification. They also observed the return to baseline values after 12 weeks post-operatively. These results were comparable to those of the current study. Similar findings of higher incidence of dry eye after SICS than after Phacoemulsification was observed in other studies. [18-19] Kumari P. and Lakra M.[20], they included a total of 187 cases and assessed the dry eyes using TF-BUT test, Schirmer's II test and corneal fluorescein staining. They observed that overall there was an increase in incidence of dry eyes in the study population by the first week post-operatively with return to near-baseline by the third month post-operatively. Similar recovery periods were also found in other studies.[22-23] The incidence of dry eye is much higher in the Manual SICS Group than in the Phacoemulsification Group, it can be successfully deduced from the current study. The severity of dry eyes is also more in the Manual SICS Group. The recovery rate seems to be faster with earlier recovery in the Phacoemulsification Group. In certain studies, postoperative dry eye after cataract surgery has been linked to an anomaly in the lipid layer of the tear film. Dry eye following cataract surgery is reportedly brought on by aberrant meibomian gland activity, according to Park Y. et al. In a different study, researchers discovered that cataract surgery thins the lipid layer. [24] As a result of these modifications, the precorneal tear film becomes less stable, which promotes the onset of dry eye. **[25]** According to research by Sutu C. et al., one of the iatrogenic mechanisms for dry eyes following cataract surgery is corneal sensory nerve damage caused by the incision, impaired epithelial wound healing, increased permeability, decreased epithelial metabolic activity, and loss of cytoskeletal structures, which results in decreased corneal sensitivity and a subsequent decrease in tear production.[26] Another important element in the emergence of dry eye is the rise of the inflammatory response, which results in the recruitment of neutrophils and macrophages as well as the formation of free radicals, proteolytic enzymes, and cyclooxygenase. Pre- and postoperative eye drops with preservatives also contribute to the inflammatory response, as can topical anaesthetics.

In SICS, with a big corneoscleral tunnel incision, there occurs denervation of a bigger part of cornea, which is associated with persistent foreign body sensation and pooling of mucus and debris within the groove. On the contrary, in phacoemulsification cataract surgery, incision is much smaller. Therefore, there is minimal corneal denervation. Thus, the incidence

of dry eye is more after Manual SICS than after Phacoemulsification. Size of the incision has been correlated with the severity of dry eyes in the study by Fine I. H. et al [27] and by Oh T. et al.[28] Khanal S. et al[29], in their study, speculated that Phacoemulsification incisions on 3 and 9 o'clock positions lead to less tear secretion and resultant neurotropic keratopathy. Reduced corneal sensitivity and tear production after cataract surgery has been reported by other studies as well. [22,29-30]

Limitations: The present study was limited by the OPD attendance of the patients. Therefore, the results may not be generalized.

SUMMARY

All the cases that satisfied the inclusion and exclusion criteria's in the study period from March 2021 to July 2022 in tertiary care centers were included in this study. A total of 120 patients were included in our study and examined for the incidence and pattern of dry eye following Manual SICS and Phacoemulsification. The 120 patients were randomly assigned to group A or group B undergoing Manual SICS and Phacoemulsification respectively. Out of the 60 patients that underwent Manual SICS 47 patients had a dry eye on Tear Meniscus Height Test, 58 patients had dry eye of various grading (40 patients had moderate dry eye) on Schirmers I test, 48 patients had dry eye on the Lissamine Green B test and 55 patients had dry eye on Tear Breakup time test on first-week follow-up. Out of the 60 patients that underwent Phacoemulsification, 49 patients had a dry eye on Tear Meniscus Height Test, 54 patients had dry eye (mild) on Schirmers I test, 23 patients had a dry eye on the Lissamine Green B test and 32 patients had dry eye on Tear Breakup time test on first week follow-up. From the four tests performed for diagnosing dry eye it is observed that the incidence of dry eye is more in patients that underwent Manual SICS in comparison with patients who underwent Phacoemulsification. It is observed that the severity of dry eye is greater in patients undergoing Manual SICS in comparison with the patients undergoing Phacoemulsification.

CONCLUSION

Dry eye syndrome is a multifactorial disease that results in ocular discomfort, visual disturbance, and tear film instability, with potential damage to the surface of the eye. Dry eye occurs due to disruption of the tear film. Amongst the many extrinsic and intrinsic cause, cataract surgery is an important contributing factor, particularly because of the greater incidence of cataracts. Therefore, our present study was conducted to assess and compare the incidence of dry eye following two such surgical procedures: Manual SICS and Phacoemulsification. It can be effectively concluded from the present study that the mean age of presentation of cases with senile cataracts is above 60 with an almost equal gender distribution. Both eyes are equally involved. Following cataract surgery, most of the cases undergoing Phacoemulsification developed mild dry eye and only a few cases of moderate dry eye as compared to the Manual SICS group which showed moderate to severe dry eye cases at the first week of follow-up. The majority of cases resolved back to normal by the twelfth week of follow-up in both groups. Therefore, we can effectively conclude that Phacoemulsification surgery has the advantage of better comfort and early recovery in comparison to Manual SICS post-operatively.

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A STUDY OF THE CHANGES OCCURRING IN THE CORNEAL CELL MORPHOLOGY, CELL COUNT & CENTRAL CORNEAL THICKNESS IN TYPE II DIABETES MELLITUS USING SPECULAR MICROSCOPY ALONG WITH ITS CORRELATION IN VARIOUS STAGES OF DIABETIC RETINOPATHY & GLYCOSYLATED HAEMOGLOBIN (HbA1C) LEVELS

Aishwarya Ambre*, *Shadakshari S. Math*****

ABSTRACT

INTRODUCTION : Diabetes mellitus (DM) causes chronic hyperglycemia as a result of relative insulin insufficiency, resistance, or both. Diabetes affects cornea, lens as well as retina over a period of time. It also causes physiological instability in the cornea. Studies showed increased polymegathism, pleomorphism (percentage of hexagonal cells) with increased central corneal thickness (CCT) and decreased endothelial cell density (ECD) combined with further morphological modifications that ultimately result in diminished endothelium functionality, increased corneal moisture, and central corneal thickenings This study was conducted to observe the changes occurring in the corneal cell morphology, cell count and central corneal thickness in type II diabetes mellitus using specular microscopy and to correlate with various stages of diabetic retinopathy and glycosylated hemoglobin (HbA1c) levels. **MATERIAL AND METHODS :** This is hospital-based observational cross-sectional study conducted by simple consecutive sampling among 144 patients (38 controlled diabetes and 106 uncontrolled diabetes) from March 2021 to August 2022. The corneal endothelial morphological features were evaluated using a non-contact model SP 1P specular microscope. The morphological characteristics of endothelial cells like endothelial cell density (ECD), hexagonal cells (HEX), coefficient of variance (CV), and central corneal thickness(CCT) were compared between controlled and uncontrolled diabetes. The changes were compared with the duration of diabetes as well. **RESULTS :** The mean HbA1c level of the study population was 8.32 ± 1.84. The majority of the study subjects (73.6%) had HbA1c levels >=7. There is a statistically significant association between HbA1c and central corneal thickness, cell density, coefficient of variance, and hexagonality. The majority of the study subjects had mild NPDR, followed by moderate and severe NPDR in both eyes. A statistically significant association was there between HbA1c levels and diabetic retinopathy among the study subjects (P value = 0.001). The mean duration of diabetes mellitus among the study subjects was 9.70 ± 6.02 years. There is a statistically significant association between the duration of diabetes mellitus and central corneal thickness, cell density, and coefficient of variance. **CONCLUSION :** Our research found that type II diabetes mellitus has an effect on corneal morphology. Uncontrolled type II DM patients had slightly thicker central corneas and also showed an increase in the coefficient of variance compared to those with controlled type II DM. Corneal endothelial cell density as well as hexagonality shows an inverse relation with HbA1c levels which implies that

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both the cell density and hexagonality decrease with an increase in HbA1c levels. Duration of diabetes also plays a significant role in the changes of endothelial cell characteristics with increased duration of DM,

Keywords : Diabetes mellitus, corneal cell morphology, retinopathy, corneal thickness, HbA1c

INTRODUCTION

Diabetes mellitus (DM) causes chronic hyperglycemia as a result of relative insulin insufficiency, resistance, or both. DM is a deep-rooted non-communicable disease affecting the entire globe and has extensive hazardous effects on almost all systems of the body including the eyes. Diabetes affects the cornea, lens as well as retina over a period. It affects each layer of cornea i.e., epithelium, endothelium, and stroma [1]. It also causes physiological instability in the cornea. The diabetic cornea is susceptible to a wide range of abnormalities, including corneal endothelial damage, repeated corneal erosions, punctate epithelial keratopathy, persistent epithelial defects, reduced corneal sensitivity, ulcers, sluggish wound healing, and superficial keratitis. Diabetes affects the neural cells and the vasculature of the retina leading to diabetic retinopathy. Diabetic retinopathy is one of the primary causes of visual loss in middle-aged adults in developed countries.[2] The two most common consequences are diabetic macular oedema and proliferative diabetic retinopathy which affects our eyes and can impair our sight. It can also affect visual acuity resulting from retinal microvascular degeneration. Advanced diabetic eye disease is a serious vision-threatening consequence of diabetic retinopathy that arises in patients who have had insufficient or inefficient treatment. Studies showed increased polymegathism, pleomorphism (percentage of hexagonal cells) with increased central corneal thickness (CCT) and decreased endothelial cell density (ECD) combined with further morphological modifications that ultimately result in diminished endothelium functionality, increased corneal moisture, and central corneal thickenings.[3] Corneal function abnormalities can be detected even before the appearance of clinically evident lesions and symptoms. One of the earliest clinically discernible changes in the diabetic eye is thought to be an increase in corneal thickness. CCT or central corneal thickness, is a crucial measurement in evaluating patients for refractive surgery as diabetes makes cornea more fragile and riskier for intraocular surgeries.[4] Diabetic individuals have a much higher coefficient of variation (standard deviation of cell areas/mean cell area) of corneal endothelial cells. Since the endothelial cells enlarge to cover the spaces between adjacent cells, this increase suggested the presence of polymegathism.

In the quantitative examination of corneal endothelium, errors can occur. When there are a significant number of border cells, the fixed frame approach can produce substantial inaccuracies (endothelial cells cut by one border of the frame). The variable frame cell counting approach eliminates border mistakes and is hence recommended over the fixed-frame method. Subjective decisions in calculating cellular borders in the center-to-center approach and cell border intersections in the corner method are other causes of mistake. Regardless of the technique's strategy, the accuracy of the assessment is determined by the quality of the endothelium image obtained using

specular microscopy.[5] As endothelial imaging is based on specular reflex, any optical impediments in front of the endothelium monolayer will impact the image's quality in delineating the endothelial cells. A poor ocular surface tear film, epithelial haze, stromal scarring, and changes in the Descemet's membrane (excrescence/guttae) are all factors that can interfere with the quality of endothelium imaging. It is possible to assess the endothelial image quality obtained by specular microscopy as good, fair, poor, or difficult to study.[5] The endothelial cell analysis provided by specular microscopy is based on the detection of cellular borders. When individual cell borders are properly outlined, the automatic method of analysis is fairly accurate. However, in eyes with fair endothelial image quality (where legitimate outlines of cell borders are not recorded very clearly due to optical hindrances during endothelial image capture), it is recommended to execute a manual counting approach by a technician well-skilled in specular microscopy. Inter-observer variability in cell density analysis is estimated to be between 0 and 6% for excellent to good quality endothelium pictures, and between 6% and 11% for bad quality photos.[5-6] Endothelial anomalies have been found in diabetes and chronic renal disease patients, particularly those on haemodialysis.[7-8] When contemplating cataract surgery in such individuals, endothelial imaging should be included in the pre-operative evaluation. This research was conducted to study the changes occurring in the corneal cell morphology, cell count, and central corneal thickness in type II diabetes mellitus using specular microscopy and to correlate with various stages of diabetic retinopathy and glycosylated haemoglobin (HbA1c) levels.

MATERIALS AND METHODS

This observational cross-sectional study was conducted at the Ophthalmology OPD of the tertiary care center, Kolhapur. The study was started after getting the necessary ethical permissions from the Institutional Research Committee. The duration of the study was from March 2021 to August 2022. The sample size taken for the study was 144 patients. Written informed consent was taken from the patients. All patients diagnosed with type 2 diabetes mellitus by the physician, patients belonging to the age group of 40-65 years, as well as both genders were included for the study. Whereas, patients with Type 1 Diabetes Mellitus, Type 2 Diabetes mellitus with hypertension, glaucoma, and corneal pathology due to any cause will be excluded. Also, those with past history of ocular surgeries, ocular trauma, and uveitis. pregnant and lactating women, drugs that alter the corneal morphology and thickness will be excluded from the study. Detailed history and clinical examination will be done. History of time since diagnosis of type II diabetes mellitus will be recorded. HbA1c levels will be evaluated. Detailed Ophthalmic examination will be done comprising of best corrected visual acuity, and anterior segment evaluation. Dilated fundus examination using indirect ophthalmoscope +20D lens and slit lamp biomicroscopy with +90D lens and diabetic retinopathy will be grouped into NPDR and PDR based on ETDRS classification. Specular microscopy will be used to assess the endothelial cell density, cell morphology, central corneal thickness and mean central corneal thickness will be used for comparison. Based on the duration of type II diabetes mellitus three groups will be formed as follows-

Group A – 0 - 5 years
Group B – 6 - 10 years

Group C – > 10 years

Diabetic patients will be divided into controlled (glycosylated Hb <7%) and uncontrolled (glycosylated Hb >7%) type II DM based on HbA1c levels (ICMR guidelines 2018). The master chart was prepared using MS Excel 365 and data analysis was done on SPSS software version 20. Data was represented by Mean+/- SD (Standard deviation).

RESULTS

GENDER DISTRIBUTION

In our study the following Figure.1 and Figure.2 show the demographic distribution of gender and age in diabetic patients respectively.

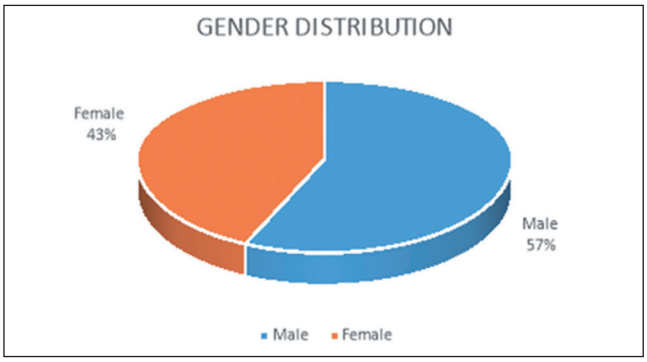

Fig. 1: Gender wise distribution of patients

In our study, out of total 144 patients, 82 patients (56.9%) were males and 62 patients (43.1%) were females.

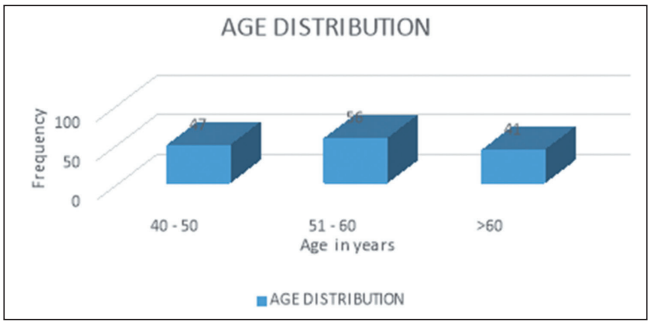

Fig. 2: Graph showing the age distribution

The mean age of the study population was **54.85 ± 7.56 years**.

HbA1c

In our study, 38 patients (26.4%) were under controlled diabetes mellitus and 106 patients (73.6%) were under uncontrolled diabetes mellitus. The mean HbA1c level of the study population was **8.32 ± 1.84.**

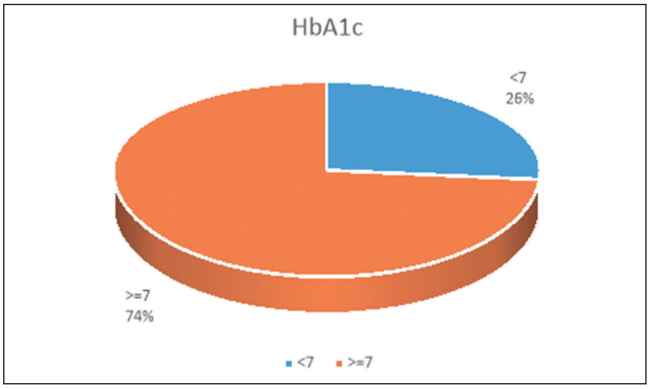

Fig. 3 : Frequency of diabetic patients with controlled and uncontrolled diabetic state

Table 1 : Comparison between glycaemic control (HbA1c) and corneal morphological parameters

Corneal Morphological Parameters	Mean ± SD (95% CI)		F - value	P-value
	HbA1c <7	HbA1c >=7		
CCT	493.39 ± 48.15 (482.39 - 504.40)	510.91 ± 42.23 (505.19 - 516.62)	8.916	0.003*
Cell density	2787.13 ± 625.40 (2644.22 - 2930.04)	2622.77 ± 459.50 (2560.56 - 2684.98)	5.850	0.016*
Coefficient of variance	33.37 ± 5.45 (32.12 - 34.61)	36.54 ± 5.38 (35.81 - 37.27)	19.318	<0.001*
Hexagonality	48.61 ± 5.84 (47.27 - 49.94)	44.64 ± 7.69 (43.74 - 45.53)	30.282	<0.001*

The above table shows the mean CCT and mean coefficient of variance values increased in uncontrolled diabetes patients with p values 0.003 and <0.001 respectively as compared to the controlled diabetes mellitus patients, whereas the mean endothelial cell density and mean hexagonality decreased in

uncontrolled diabetes patients with p values 0.016 and <0.001 respectively as compared to the controlled diabetes mellitus patients.

Diabetic retinopathy staging of 288 eyes in 144 subjects

In our study out of 144 subjects with total of 288 eyes, 40 eyes (13.9%) had no diabetic retinopathy,231 eyes (80.2%) had NPDR and 17 eyes (5.9%) had PDR.

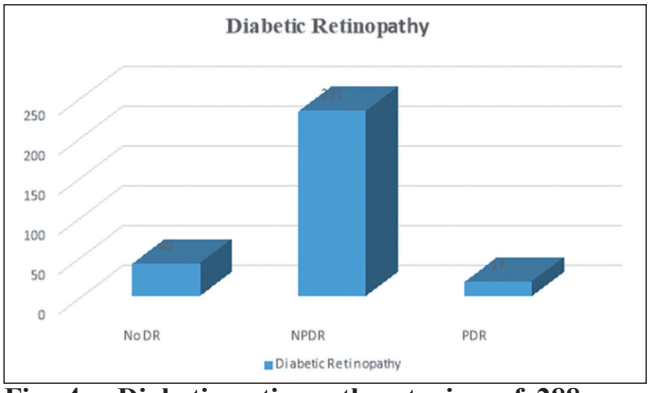

Fig. 4 : Diabetic retinopathy staging of 288 eyes in 144 subjects

Data showing different stages of DR in diabetic patients with controlled diabetes (Hb1Ac <7) and uncontrolled diabetes (HbA1c >=7)

The below figures (fig 5 and 6) show 6 patients (7.9%) had no diabetic retinopathy, 67 patients (88.2%) had NPDR,3 patients (3.9%) had PDR among controlled diabetes and 34 patients (16.0%) had no diabetic retinopathy, 164(77.4%) had NPDR and 14(6.6%) had PDR among uncontrolled diabetes.

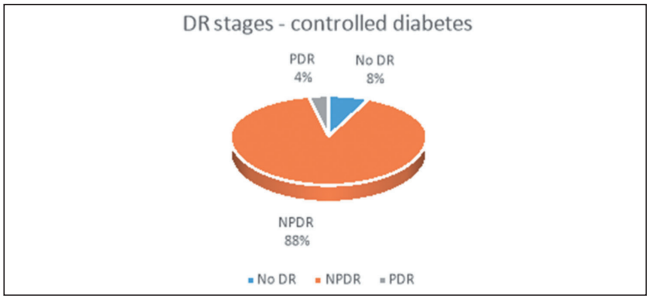

Fig. 5 : Distribution of diabetic retinopathy stages among controlled diabetes mellitus (HbA1c <7)

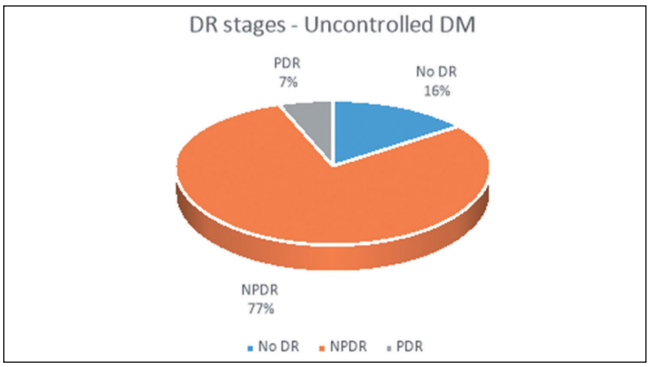

Fig. 6 : Distribution of diabetic retinopathy stages among uncontrolled diabetes mellitus (HbA1c >=7)

Table : 2 Association between diabetic retinopathy and corneal morphological parameters among controlled diabetic patients (Hb1Ac <7)

	Mean CCT	Mean ECD	Mean CV	Mean Hexagonality
NO DR	497.00 ± 16.61	3024.00 ± 437.80	32.33 ± 2.73	50.67 ± 4.27
NPDR	489.51 ± 47.85	2769.31 ± 626.47	32.93 ± 5.13	48.70 ± 5.70
PDR	573.00 ± 25.15	2711.33 ± 1024.18	45.33 ± 2.08	42.33 ± 9.45
Total	493.39 ± 48.15	2787.13 ± 625.40	33.37 ± 5.45	48.61 ± 5.84
F value	4.771	0.473	9.195	2.179
P value	**0.011***	0.625	**<0.001***	0.120

In patients with controlled diabetes (HbA1c <7), the mean CCT and mean CV values increased with the increase in the degree of diabetic retinopathy and is statistically significant with p value 0.011 and<0.001respectively. The mean ECD and mean Hexagonality decreased with the increase in the degree of diabetic retinopathy but are not statistically significant with p values 0.625 and 0.120 respectively.

Table 3: Association between diabetic retinopathy and corneal morphological parameters among uncontrolled diabetic patients (Hb1Ac >=7)

	Mean CCT	Mean ECD	Mean CV	Mean Hexagonality
No DR	473.85 ± 35.84	2809.74 ± 409.24	33.06 ± 3.79	49.00 ± 5.34
NPDR	516.15 ± 39.97	2623.92 ± 446.78	36.50 ± 4.89	42.82 ± 7.55
PDR	539.43 ± 29.06	2155.21 ± 414.85	45.50 ± 3.89	33.79 ± 3.40
Total	510.91 ± 42.23	2622.77 ± 459.50	36.54 ± 5.38	43.21 ± 7.79
F value	20.483	11.018	35.079	24.200
P- value	**<0.001***	**<0.001***	**<0.001***	**<0.001***

In patients with uncontrolled diabetes (HbA1c >=7), the mean CCT and mean CV values increased with the increase in the degree of diabetic retinopathy and is statistically significant with p values <0.001. The mean ECD and mean Hexagonality decreased with the increase in the degree of diabetic retinopathy and is statistically significant with p values <0.001.

Duration of Diabetes Mellitus

In our study, 39 patients (27.1%) had 0-5 years of duration of diabetes mellitus, 54 patients (37.5%) had 6-10 years of duration of diabetes mellitus and 51 patients (35.4%) had >10 years of duration of diabetes mellitus. The mean duration of diabetes mellitus among the study subjects was 9.70 ± 6.02 years.

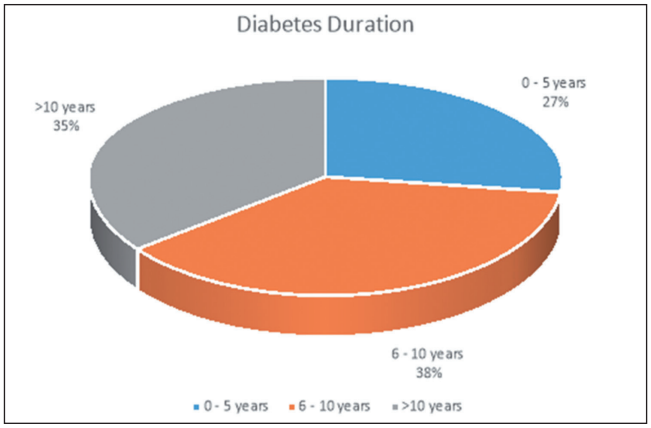

Fig. 7 : Duration of Diabetes Mellitus

Table 4 : Association between duration of diabetes mellitus and HbA1c levels

Duration of Diabetes Mellitus	HbA1c		P- value
	<7 (%)	>=7 (%)	
0 – 5 years	12 (30.8)	27 (69.2)	0.739
6 – 10 years	14 (25.9)	40 (74.1)	
>10 years	12 (23.5)	39 (76.5)	

The above table shows 12 patients (30.8%) had a duration of 0-5 years, 14 patients (25.9%) had a duration of 6-10 years and 12 patients (23.5%) had >10 years of duration of diabetes among the controlled diabetes mellitus (HbA1c<7). While 27 patients (69.2%) had a duration of 0-5 years, 40 patients (74.1%) had a duration of 6-10 years and 39 patients (76.5%) had >10 years of duration of diabetes among uncontrolled diabetes mellitus (HbA1c>=7). There was no statistical significance between the duration of diabetes mellitus and the control of diabetes i.e., HbA1c levels in our study.

Table 5: Association between duration of diabetes mellitus and central corneal thickness in patients with controlled diabetes mellitus (HbA1c <7)

DIABETES DURATION (YEARS)	Mean CCT ± SD	Mean ECD	Mean CV	Mean Hexagonality
0 – 5 YEARS	487.33 ± 43.35	2793.13 ± 609.46	30.79 ± 4.34	49.58 ± 4.83
6 – 10 YEARS	481.61 ± 43.31	2650.93 ± 555.25	31.86 ± 5.93	48.36 ± 5.25
>10 YEARS	513.21 ± 53.48	2590.04 ± 684.70	34.71 ± 5.65	47.92 ± 7.34
TOTAL	493.39 ± 48.15	2787.13 ± 625.40	33.37 ± 5.45	48.61 ± 5.84
F value	3.244	4.223	4.917	0.522
P value	**0.045***	**0.026***	**0.019***	0.596

In our study, the mean CCT and mean CV showed an increasing trend with the increase in the duration of diabetes mellitus with statistically significant p-values of 0.045 and 0.019. Whereas mean ECD and mean Hexagonality showed a decreasing trend with an increase in the duration of diabetes mellitus with a statistically significant p value of 0.025 was noticed with mean ECD and mean Hexagonality was statistically not significant among the controlled diabetes mellitus (HbA1c<7).

Table 6 : Association between duration of diabetes mellitus and central corneal thickness in patients with uncontrolled diabetes mellitus (HbA1c >=7)

DIABETES DURATION (YEARS)	Mean CCT ± SD	Mean ECD	Mean CV	Mean Hexagonality
0 – 5 YEARS	503.37 ± 45.17	2658.09 ± 399.34	36.04 ± 5.06	42.91 ± 7.24
6 – 10 YEARS	501.75 ± 37.74	2697.08 ± 423.36	35.35 ± 3.98	44.64 ± 7.62
>10 YEARS	525.51 ± 40.96	2522.10 ± 517.73	38.12 ± 6.42	41.96 ± 8.17
TOTAL	510.91 ± 42.23	2622.77 ± 459.50	36.54 ± 5.38	43.21 ± 7.79
F value	7.888	3.140	5.783	2.417
P value	**0.000***	**0.045***	**0.004***	0.092

In our study, the mean CCT and mean CV showed an increasing trend with the increase in the duration of diabetes mellitus with statistically significant p-values of 0.000 and 0.004. Whereas mean ECD and mean Hexagonality showed a decreasing trend with an increase in the duration of diabetes mellitus with a statistically significant p value of 0.045 was noticed with mean ECD and mean Hexagonality was statistically not significant among the uncontrolled diabetes mellitus (HbA1c<7).

Table 7 : Association between corneal morphological parameters and HbA1c levels among patients with duration of diabetes for more than 10 years

Corneal Morphological Parameters	Mean ± SD (95% CI)		F value	P value
	HbA1c <7	HbA1c >=7		
Mean CCT	477.92 ± 21.10	544.59 ± 33.52	41.986	**0.000***
Mean Cell density	3104.33 ± 613.79	2588.67 ± 561.04	7.424	**0.009***
Mean Coefficient of Variance	33.92 ± 6.38	39.13 ± 6.99	5.293	**0.026***
Mean Hexagonality	48.17 ± 4.95	41.85 ± 7.77	6.993	**0.011***

In this study, we found that the mean CCT and mean CV significantly increased in those with uncontrolled diabetes mellitus with a duration of DM of more than 10 years. This was statistically significant as well with p-values 0.000 and 0.009 respectively. While mean ECD and mean hexagonality were found to be decreased among those with uncontrolled DM with a duration of more than 10 years. This difference was also found to be statistically significant (p values 0.026 and 0.011).

DISCUSSION

In our study, there were 82 (56.9%) males and 62 (43.1%) females. In the study conducted by Nargis et al., the study population constituted more males 62% than females 38% .[9] In a study conducted by Papadakou et al., there were more females 61.1% than males 38.9%. [10] Majority of the study subjects belonged to the age group of 51 – 60 years. The mean age of the study population was found to be 54.85 ± 7.56 years. In the study conducted by Qu et al., the mean age of the study population was 62.30 ± 9.93 years.[11] The mean age of the study population was 62.17 ± 9.49 years in the study conducted by Jha et al[12]. The mean age in the study conducted by Papadakou et al was 67.1 ± 10.7 years[10].

Looking at HbA1c levels in our study, we found that, 38 (26.4%) of the study subjects had diabetes mellitus under control (HbA1c levels less than seven) while the rest 106 (73.6%) of them had uncontrolled diabetes mellitus (HbA1c levels of more than or equal to seven) and the mean HbA1c level of the study subjects was 8.32 ± 1.84. The mean HbA1c level in the study conducted by Storr-Paulsen et al was 7.3 ± 0.2[13]

In the study conducted by Kumari et al., the mean central corneal thickness increased in study subjects with poor glycemic control[14] but didn't show a statistically significant association(p=0.231). **In our study, the central corneal thickness increased with an increase in**

HbA1c levels and the difference were statistically significant(p=0.003).

In the study conducted by Shukla et al., lower endothelial cell density was associated with higher HbA1c levels and showed statistically significant association ($p<0.05$).[15] In Durukan et al study showed decrease in endothelial cell density with poor glycemic control but couldn't find statistically significant association due to small number of patients.[16] **In our study the mean ECD values decreased with uncontrolled blood sugars and the difference was statistically significant(p=0.016).**

In the study conducted by Shukla et al the coefficient of variance increased with an increase in HbA1c values and was statistically significant ($p<0.05$).[15] In our study the coefficient of variance increased in uncontrolled diabetes patients with statistically significant p value <0.001.

In the study conducted by Meenakshi Sundaram et al the hexagonality decreased with increase in HbA1c values but was not statistically significant (p=0.6).[17] **In our study the hexagonality deceased in the uncontrolled diabetes patients with significant p value <0.00**.

In the study conducted by Nargis et al the mean central corneal thickness increased with the severity of the diabetic retinopathy, showed a statistically significant positive correlation between the two parameters (Pearson r = 0.933, p = 0.001). While the endothelial cell density decreased with the severity of diabetic retinopathy, Analysis of the relationship between ECD and severity of DR showed a statistically significant negative correlation between the two parameters (Pearson r = -0.872, p = 0.001).[9] **In our study mean CCT showed increase with statistically significant p value 0.002, while mean ECD showed decreased values with statistically significant association (p value 0.014) with severity of diabetic retinopathy**.

In the study conducted by Parekh et al they found that the duration of diabetes was significantly correlated with decrease in endothelial cell density and increase in central corneal thickness ($p<0.000$).[18] **In our study statistically significant association was found with increase in duration of diabetes, increase in mean CCT and decrease in mean ECD ($p=0.000$, $p=0.009$)**.

CONCLUSION

As type II diabetes mellitus has become epidemic in our country and diabetes affects the various organs of our body. Our research found that type II diabetes mellitus has an effect on corneal morphology. The central corneal thickness and coefficient of variance increases with increases with poor glycaemic control i.e., >=7 HbA1c levels. Thus, uncontrolled type II DM patients will have thick central cornea and increase in coefficient of variance compared to those with controlled type II DM. In our study, corneal endothelial cell count or cell density as well as hexagonality shows an inverse relation with HbA1c levels it implies that both the cell density and hexagonality decreases with increase in HbA1c levels. Duration of diabetes also plays a significant role in the changes of endothelial cell characteristics with increased duration of DM, it is associated with increased central corneal thickness and coefficient of variance while decreased endothelial cell density and hexagonality.

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CLINICAL STUDY OF COVID-19 ASSOCIATED MUCORMYCOSIS INFECTION

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ABSTRACT

Introduction : The unprecedented increase of COVID-19-associated Mucormycosis (CAM) during the 2nd pandemic wave was a perfect Tsunami in India! A review article published in 2021 surprisingly reported that 81 % of the total cases of CAM worldwide were from India. Hence there is a need to understand the biological behaviour of this unholy combination to identify risk factors and establish standard protocols to reduce morbidity and mortality in India. AIM- To study the epidemiological profile of COVID -19 associated Mucormycosis during 2nd pandemic wave. **Materials and Methods** : This retrospective observational study included 57 cases of CAM reported to the Department of Otorhinolaryngology at our tertiary care center from May 2021 to July 2022. **Results :** Out of 57 CAM cases 75.4% were males. The youngest patient was of 12 years and the oldest 75 years. Pre-existing Diabetes Mellitus was present in almost 79% of patients. Corticosteroid intake was recorded in 52.63% of patients of which 70% received steroids for 10 days and 30% for 5 days. Mucormycosis involving the Nose and sinus was seen in all patients with palatal and alveolar involvement in 19.3%. Rhinoorbital Mucormycosis was seen in 12.2% and Rhinocerebral type was seen in 3.51%. Oxygen therapy was given in 45.61% and 7% needed ventilator support during the treatment of COVID-19 infection. All patients underwent endoscopic debridement. FESS with maxillary sinus debridement was done via total maxillectomy in 8.77%, partial maxillectomy in 10.53%, modified Denker's approach in 0.57% and Caldwell Luc approach in 0.57%. All patients received Amphotericin-B emulsion in the range of 1gm to 5.7gm. The drug was stopped when the histopathological report of debrided tissue was negative for Mucormycosis. All patients were put on gastro-resistant Posaconazole tablets for 21 days on discharge. Mortality was seen in only 1 patient (0.57%) and survival rate was 99.43%. **Conclusion :** Diabetes Mellitus, steroid therapy, and oxygen support during COVID-19 treatment were the risk factors for the sudden spike in cases of Mucormycosis during the 2nd wave. Prompt diagnosis, repeated debridement, and optimal systemic and oral antifungal therapy markedly reduced the mortality to a much lower rate than expected in our study.

Keywords : COVID-19 associated Mucormycosis, Diabetes Mellitus, steroid, oxygen therapy, Amphotericin-B.

INTRODUCTION

A rare and life-threatening fungal infection "Mucormycosis" had a drastic rise during the second wave of COVID pandemic worldwide. India, with a vast burden of SARS-CoV-2 affected population i.e

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32,036,511 cases as reported on August 11th 2021, also had the highest number of Covid-19 associated Mucormycosis cases.[17,7]. According to reports from the Union Health Ministry, India reported 40,845 cases of Covid Associated Mucormycosis(CAM) as on June 28, 2021.[18] The impact of the disease was such that the government declared "Mucormycosis", a notifiable disease under the Epidemic Diseases Act, 1897. [19] Interestingly, Mucormycosis cases were already higher in the Indian subcontinent as compared to other parts of the globe even before the pandemic. This variation has been explained by the high diabetic population in our country. [20]

In a recent systematic review of the CAM cases in India and worldwide during the pandemic, 82 % have been reported from India against only 18% from other parts of the world[7].

This sudden increase in the number of COVID19-associated mucormycosis (CAM) cases has been explained by the combination of hyperglycemic state induced by the SARS-CoV-2 as well as drugs used during therapy (especially steroids), hypoxic conditions, oxygen therapy, and ventilator in affected patients. Hyperglycemia, the single most important risk factor may either be due to pre-existing or new-onset Diabetes Mellitus or deranged glucose metabolism as reported in a recent review article. [21]

Mucormycosis is an angioinvasive opportunistic infection caused by order Mucorales with a worldwide distribution**.** [12] The genera responsible for human infection are Rhizopus, Mucor and Rhizomucor, Cunninghamella, Lichtheimia and Apophysomyces. [13] These ubiquitous filaments normally occur in soil, manure, fruits, and decaying matter. [12]

These fungal spores can cause aggressive and life-threatening disease in immunocompromised hosts but is harmless in healthy individuals. They can provoke infections in immunosuppressed cases like uncontrolled Diabetes mellitus (DM) hematological malignancy, chronic malnutrition, chronic liver diseases and hematopoietic stem cell transplantation patients. [14] The rapid progressive nature of Mucorales entails its early and prompt diagnosis followed by efficient management with a multidisciplinary team, which is one of the major limitations in resource-limited countries.

However, even with the best management, high mortality has been reported in the past. Clinically, CAM can be categorised into Rhino-Orbital, Paranasal Sinus, Rhino-Cerebral, Rhino-Orbital-Cerebral, Oral, Pulmonary, Gastrointestinal, Cutaneous, and disseminated type. [15] The most common presentation being palatal ulceration or necrosis and later palatal perforation due to the spread of infection from the nasal cavity or paranasal sinuses via palatal vessels. [16] Hence the dental surgeon's need has aroused to be able to identify the oral manifestation at an early stage to plan the treatment protocol to prevent its rapid spread leading to fatality. However, a link between COVID-19 and Mucormycosis needs to be unearthed. With this background, this study was specifically designed to present the epidemiological profile of the CAM cases admitted and managed in our tertiary care centre during the second wave of the COVID-19 pandemic in India.

MATERIAL AND METHODS

This retrospective observational study included 57 cases of CAM reported to Department of Otorhinolaryngology at our tertiary care hospital from May 2021 to July 2022. Clinical diagnosis of

Mucormycosis was made followed by contrast magnetic resonance imaging, then functional endoscopic sinus surgery and finally histopathology confirmation of Mucormycosis.

Data about demographics, clinical manifestations, associated comorbidities, hematological investigations, medical management, surgical management and prognosis was collected after obtaining informed written consent from all patients and approval from the institutional Ethical committee.

STATISTICAL ANALYSIS

Data and master chart was prepared in MS EXCEL 2007. Percentages of different groups were compared by using the chi-square test. $P<0.05$ was considered statistically significant and $P<0.0001$ was considered statistically highly significant. To test the association of organ involvement and Inj. Amphotericin-B dose chi-square test was used.

RESULTS

Table 1 : Demographic profile of patients with Covid-19 associated Mucormycosis.

RISK FACTORS		No. of patients affected	Percentage	X^2	P value
Age in years	0-20	2	3.5%		
	20-40	7	12.28		
	40-60	36	63.15		
	>60	12	21.05	48.00	<0.0001
Sex	Males	43	75.43		
	Females	14	24.56	14.75	<0.0001
Diabetes Mellitus		45	78.94		
Covid-19		57	100		
Steroids	<5 days	9	15.79	4.8	0.0255
	>5 days	21	36.83		
Inj.Remdisivir		37	64.9		
O2 Therapy		26	45.61		
Ventilator support		4	7.01		
Vaccination		15	26.31		
Total No. of patients		57	100		

During the study period, 57 cases of mucormycosis were admitted at our tertiary care hospital. All 57 cases of mucormycosis were earlier confirmed as covid-19 positive by RT-PCR or antibody detection. Out of 57 patients (Table.1), 75.43%(n=43) were males and 24.56%(n=14) were females. We had one 12 years old paediatric patient of Type I Diabetes and one 18 years old adolescent in age of 18 years. 12.28%(n=7) were in the age group of 20-40 years. The majority of patients were in the age group of 40-60 years which accounted for 63.15% (n=36) and 21.05% (n=12) patients were above 60 years of age.

Type II Diabetes mellitus was present in 44 patients and Type I in 1 patient which accounted for 78.94%. Corticosteroids were given for less than 5 days in 16% (n=9) patients and more than 5 days in 36.8% (n=21) patients during their Covid-19 treatment. 64.9%(n=37) patient received Inj. Remdesivir, 45.61%(n=26) took oxygen therapy and 7%(n=4) needed ventilator support during their COVID-19 treatment. 26.3%(n=15) of patients had received 1 dose of vaccination against COVID-19,

Fig. 1 : Clinical Presentation of Patients with Mucormycosis (a) Left-sided Facial swelling with Numbness (b) Right eye involvement in Mucormycosis patient.

All the 57 patients reported to our tertiary care center presented with (Fig.1) swelling numbness over one side face, nasal obstruction, loosening of tooth, diplopia, facial swelling, headache and generalized weakness After magnetic resonance imaging, functional endoscopic sinus surgery and confirmed Histopathology report of mucormycosis diagnosis was made according to organs involved and categorized into Sino-nasal type of mucormycosis, Rhino-orbital and rhino-cerebral mucormycosis.

Mucormycosis involving the nose and sinuses was seen in all patients. Hence all patients underwent endoscopic debridement surgery (Fig.2.A). The modified Denkers procedure was done in one patient and the Caldwell luc approach was done in one patient. Palatal and alveolar involvement was seen in 19.3% (n=11) out of which 10.5%(n=6) underwent partial maxillectomy surgery (Fig.2.B) and 8.77%(n=5) underwent total maxillectomy surgery

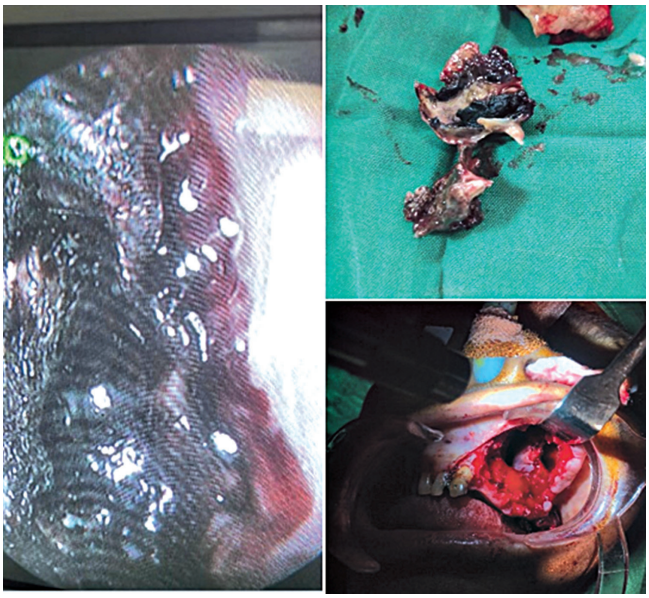

Fig. 2 : Intra-operative findings of Mucormycosis Patients (a) Blackish ischemic necrotic tissue seen on Endoscopy (b) Eschar (c) Partial maxillectomy.

(Fig.2). Rhino-orbital mucormycosis was seen at 12.2%(n=7) for which TRAMB (transcutaneous retrobulbar Amphotericin-B) was done. None of the patients required orbital exenteration. The rhino-cerebral type was seen in 3.5%(n=2) of patients. One presented with meningitis and the other with frontal lobe abscess who underwent craniotomy abscess drainage followed by FESS.

Inj Amphotericin-B emulsion (LFAB) was given to all 57 patients after renal function assessment. Prior infusion antacids and antiemetics were given. Emergency medications like Inj. Avil and Inj. Hydrocort was kept ready in case of adverse reactions. Inj. Amphotericin -B was administered in 500ml of 5% dextrose with pre and post-flushing with 100ml normal saline. The whole assembly was wrapped up in black polythene wrap as Amphotericin-B is photosensitive. A test dose of 2mg was given to every patient prior starting the full dose. A renal function test was done every 72 hours to look for any nephrotoxicity. Inj. Amphotericin was given till debrided tissue histopathology report was negative for Mucormycosis.19.2%(n=11) of patients received less than 2g of Inj. Amphotericin-B, 43.8%(n=25) received 2-4 grams, and 36.8%(n=21) received more than 4 grams of inj. Amphotericin-B (Table.2). On discharge all patients were put on gastro-resistant Tab. Posaconazole for 21 days and alkaline nasal washes. Mortality was seen in only one female patient who died due to cardiac arrest.

To improve the quality of life in patients who underwent maxillectomy, oral rehabilitation was done with Interim (Fig.3.A) and Definitive Obturators. Interim obturators were given after 7-10 days of surgery and Definitive obturators was given 3 months post-operatively to 11 patients.

DISCUSSION

The second wave of the COVID-19 pandemic had a great impact on uncontrolled diabetic patients with severe complications and high fatality rates. Its association with mucormycosis was a perfect Tsunami in India. India being one of the most affected countries by Covid-19 witnessed a rapid surge of Mucormycosis during the 2nd wave. A distinctive feature of Mucormycosis is angioinvasion followed by thrombosis and tissue necrosis. Various contributing factors during the treatment of Covid-19 have been suggested for CAM. Hence to study the demographic profile, staging of the disease and outcome of management of CAM patients we conducted this study.

In our study 63% of patients were from the age group 40-60 years of age similarly studies by (Table.3) Manjunath Vijapur et al [4], SS Chavan et al [22], Ravani SA et al [23] W. Jeong et al [9] showed a maximum number of patient were in the age group of above 40 years. In our study out of 57 patients 75.43%(n=43) patients were Male similar the studies by Sen et al[2], Kamleshun et al [3], SS Chavan et al [22], Ravani SA et al and W. Jeong [9] also shows the male preponderance accounting for 61%.

A study by Anson. [1] et al, Prakash et al, Vanghan et al[10] and Yohai et al [11] suggested 7.5 times higher chances of mucormycosis infection in pre-existing Type-2 diabetes mellitus patients and similar study by Singh et al [7] showed 93% patients having pre-existing diabetes mellitus. These above studies correlate with our study which shows 79% patients were having diabetes mellitus.

In our study 53% patients received corticosteroid therapy during their Covid-19 treatment, similarly, a study by Akhil Pratap Singh et al [8] and W. Jeong et al [9] showed 54% and a study by Mona G Alshahaway et al [5] shows 76% patient received Steroid therapy during their Covid-19 treatment. Our 45.6% of patients had received oxygen therapy, similarly, a study by Sen et al[2] showed 47% of patients receiving oxygen support during Covid-19 treatment.

A study by Smile Kajal et al showed patients receiving 2-4 grams of Inj. Amphotericin-B and aggressive endoscopic mucormycosis debridement had excellent results in the treatment of Mucormycosis, which was similar to our study where in maximum patients, 44% received Inj. Amphotericin-B in the range of 2-4 grams. In our study Palate involvement was seen in 19.30% out of which 10.5% underwent Partial Maxillectomy and 8.75 underwent Total maxillectomy, similar to a study done by SS Chavan et al [22] showed 17.5% Palate involvement and 6.76% underwent partial maxillectomy and 5.67% underwent Total maxillectomy.

CONCLUSION

Diabetes mellitus, steroid therapy and oxygen support during COVID-19 treatment were the risk factors for sudden spike in cases of mucormycosis during the 2nd wave. Prompt and repeated debridement with optimal systemic and oral antifungal therapy reduced the mortality to a much lower rate than expected.

FUNDING

Nil

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ORIGINAL ARTICLE

STUDY TO COMPARE ACCEPTANCE AND ADVERSE EFFECTS OF EXTENDED WEAR SOFT CONTACT LENSES FOR FOOD AND DRUG ADMINISTRATION (FDA) GROUP 1 AND FDA GROUP 4 SUBJECTS

Kamaxi Panchal, **Milind Sabnis*****

ABSTRACT

Introduction- Contact lenses offer a good alternative to eyeglasses depending on your eyes. Like eyeglasses, contact lenses help to correct refractive errors. Contact lenses provide a safety and effective way to correct vision when used with proper care and supervision. **Methodology-** All patients coming to Ophthalmology OPD of Tertiary care hospital who met the inclusion criteria for FDA Group 1 and FDA Group 4 respectively were taken up for the study. Detailed anterior segment evaluation was done using slit lamp biomicroscope. Schirmer's I test was done. Fluorescein staining of cornea was done under topical anaesthesia under aseptic precautions. Dilated fundoscopy was performed to rule out any posterior segment pathology. Follow up was taken at 1st month, at 3rd month and 6th month. **Results-** The incidence of right and left eye complication complications were comparable when compared between FDA group 1 patient and FDA group 4 patients (P>0.05). The most common complication in both group patients was mild to moderate dry eye and discomfort. A significant association was found between discomfort and dry eye in FDA group 1 as well as in FDA group 4 when assessed in right (FDAG1-P=0.004 and FDAG4-P=0.04) and left eye (FDAG1-P=0.0.002 and FDAG2-P=0.01). **Conclusion-** The incidence of adverse experience in FDA group 4 patients was more compared to FDA group 1 patients, however, the difference was statistically insignificant. A significant association was found between dry eye and discomfort.

Keywords- Contact lenses, microbial keratitis, dry eye, discomfort

INTRODUCTION

The use of contact lenses is very common and constitutes a profitable industry. Worldwide there are 140 million contact lens wearers as per estimate with year on year increase.[4] For treatment of refractive errors that cannot be corrected by spectacles, such as keratoconus, aphakia, irregular cornea, and high anisometropia, contact lenses are prescribed. As an alternative to glasses, they can also be used to treat simple refractive problems.[3] Types of contact lenses are rigid gas permeable, Semi-soft, Soft contact lenses.[1] Contact lenses interacts with ocular surface. There is necessity to stay updated regarding contact lens associated complications. Contact lens complications are frequent. As per one study survey up to 3rd of contact lens wearers needed medical guidance for complications related to contact lens use.[4] Therefore, contact lens complications are a significant aspect of ophthalmology practise. Simple allergic conjunctivitis

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to microbial keratitis that threatens vision are only a few examples of complications.[5] Complications associated with CL use is multifactorial. Which is related to material of CL, types of CL, wearing schedule, patient compliance, CL cases and solutions.[6]

Dryness and discomfort are reported by 30 to 50% of CL users despite introduction of many new lens materials and care systems mainly at end of the day. Discomfort leads to 30-50% of users to lapse from CL wear and 25% will permanently stop using. Long term wearing success depend upon Tear exchange improvement and flushing beneath lenses.[7]

Mechanical interactions of CL to eyeball disrupts Ocular surface – posterior surface of CL interacts with anterior surface of cornea, bulbar conjunctiva, limbus, anterior surface of CL is in contact with palpebral conjunctiva and both lid margins. As these lenses cannot imitate ocular surface truly. These mechanical interactions occurs continually with CLs and leads to mechanically driven complications like mucin balls, corneal erosions, papillary conjunctivitis, superior epithelial arcuate lesions. After the discovery of silicone hydrogel CL, CL complications related to RGP lenses have been eliminated like hypoxia related complications.[8] Oxygen Permeability (Dk) of Soft CL related complications include stromal straie, micro cysts, neovascularization, limbal hyperemia, peripheral infiltrates, limbal epithelial hypertrophy and polymegathism. Hypoxia related complications are common in patient requiring thicker lenses to neutralize their refractive error or with overuse of CL.[9] Clinical studies have demonstrated that this hypoxia related problems have been addressed when high Dk silicone hydrogel CL has been used. Dk value of contact lens is measure of their oxygen permeability. The oxygen permeability property is expressed by the DK/L value of the material where

D = Diffusion coefficient

K= Oxygen solubility

L= Lens thickness[2]

CL wear and sleeping in contact lenses are major risk factor for bacterial keratitis, which if left untreated leads to perforation of cornea and endophthalmitis. Etiology of corneal ulcer is related to defective normal eye's natural resistance to infection from either trauma or CL use. Microbial keratitis is 90% bacterial in nature. Staphylococci and pseudomonas are the most prevalent pathogens. In developing nations, trauma is the main cause of corneal ulcers, whereas in North America, most of these lesions are bacterial in origin and connected to using contact lenses.[10] The objective of our study is to compare acceptance and adverse experience of extended wear soft contact lens with FDA group 1 to FDA group 4.

MATERIALS AND METHODS

The study was conducted at Tertiary care hospital, in the Department of Ophthalmology after getting all the ethical clearance from the Institutional Ethics Committee. The study type was Prospective Comparative Study. Written informed consent in patient's understandable language was taken. All consenting individuals with stable refractive error for at least 6 months who opt for extended wear soft contact lens as treatment of their refractive error, belonging to both the genders were included for the study. Patients with past history of corneal and conjunctival pathology, with previous history of ocular trauma, ocular surgeries, with all types of glaucoma, and with pre-existing dry eye, lid abnormalities were excluded from the study. Sample size taken for the study was 60 cases, 30 in each group. Detailed history was taken and visual acuity tested on

a standard Snellen's chart and refraction was done with help of Streak Retinoscope. Detailed anterior segment evaluation was done using slit lamp biomicroscope. Fluorescein staining of cornea was done by instillation of 0.5%w/v Proparacaine Hydrochloride eye drops as topical anaesthesia then a blotting paper containing the dye (fluorescein strips) is touched to the surface of eye and asked patient to blink. Schirmer I Test was done using Whatman no.41 filter paper being placed in inferior cul-de-sac from outer one-third and inner two-third and the amount of wetting of filter paper after 5 mins was measured. Normal value of Schirmer I test is more than 10 mm. Wetting of 10mm is mild, 5-10 mm is taken as moderate and <5 mm is severe dry eye. Special care is taken to prevent alteration of test results caused by evaporation of tear from the film etc. Dilated fundoscopy was performed to rule out any posterior segment pathology. Contact lenses selected for study were as below.

FDA GROUP 1- Characteristics of this lens are –

LOTRAFILCON B material 67%

WATER 33%Non-ionic, Low water content

Silicone hydrogel polymer

Dk value – 110

FDA GROUP 4

Characteristics of this lens-

OCUFILCON D material 45%

WATER 55%

Ionic, High-water content

HEMA (Hydroxyethyl methacrylate)

Dk value – 14.8 to 16.7

Patients are advised to wear contact lenses daily for 12 to 16 hours and to remove before bedtime. Each pair of contact lenses should be discarded on 30th day. A fresh pair should be worn from the next day. Patients are educated about care of contact lenses. Follow up was taken at 1st month, at 3rd month and 6th month. In follow up history and detailed examination which included – visual acuity, refraction, slit lamp bio microscope examination, schirmer's I test and fluorescein staining. Patients were enquired extensively regarding contact lens discomfort at every follow up.

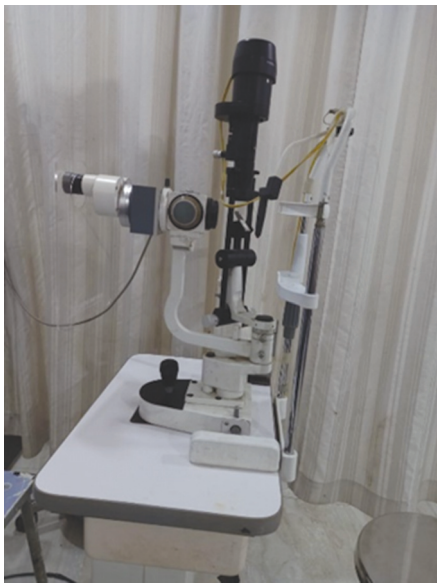

Fig. 1 : Slit Lamp

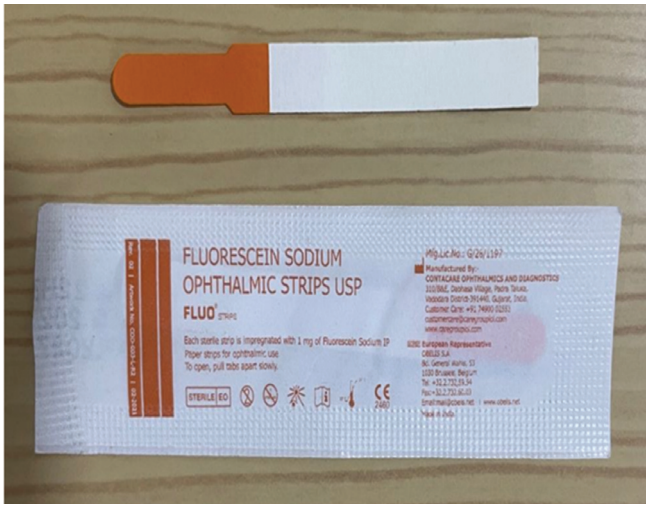

Fig. 2 : Fluorescein strips

STATISTICAL ANALYSIS

The data recorded will be analysed by z test for proportion. Data analysis and master chart will be prepared by using MS-Excel 2013 [SPSS (23.0)].

RESULTS

Age

The mean age of the FDA group 1 and 4 patients was 26.13±3.03 years and 27.13±3.55 years respectively. In FDA group 1 patient, most of the patients (53.33%, n=16) belong to the 21 – 25-year age category followed by 33.33% (n=10) and 13.34% (n=4) of patients belonging to >25 - 30 years and >30 – 35 year age category respectively. In FDA group 4, the majority of participants (46.66%, n=14) belonged to the >25 - 30 year's age category followed by 40% (n=12) and 13.34% (n=4) of the patients belonged to 21 - 25 years and >30 - 35 years' age category respectively. The detailed distribution of subjects according to age categories is illustrated in Fig.1

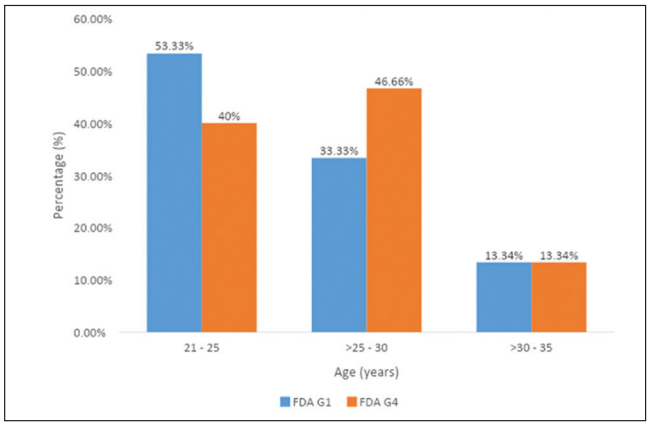

Fig. 3 : Distribution of subjects according to age categories

Sex

In FDA groups 1 and 4, the majority of participants were female (60% and 66.67%) followed by males (40%, and 33.33%). The detailed distribution of subjects according to sex is depicted in Fig. 2.

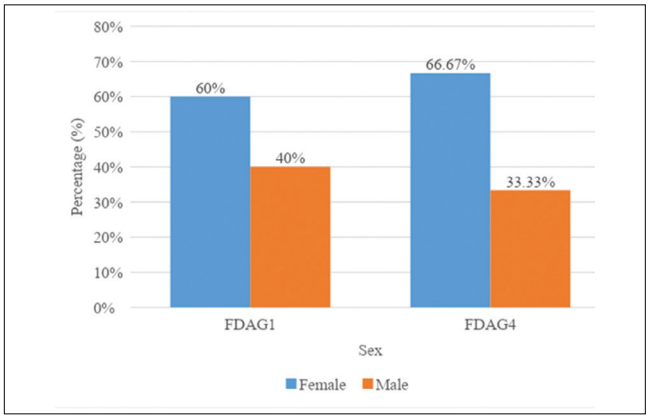

Fig. 4 : Distribution of subjects according to sex

Incidence of right eye adverse experience in FDA group 1 and FDA group 4 patients

In FDA group 1 patients, n=9 (60%) out of n=15 subjects had right eye adverse experience at first follow-up this including CL discomfort (n=5), Dry eye (n=3), and CLARE (n=1). Whereas, incidence of Allergic conjunctivitis, SPK's, Staining, Corneal abrasion, GPC, Corneal edema, and Corneal ulcers were not seen at first follow up.

At 2nd follow up the incidence of right eye adverse experience was noted in n=7 (40%) patients had complications such as CL discomfort (n=3), Dry eye (n=1), Allergic conjunctivitis (n=1), SPK's (n=1), and Corneal abrasion (n=1). There was no incidence of CLARE, staining, GPC, Corneal edema, and Corneal ulcer at 2nd follow up.

At 3rd follow up out of n=15 only n=1 (6.67%) subjects had Dry eye. No incidence of CL discomfort, CLARE, Allergic conjunctivitis, SPK's, Staining, Corneal abrasion, GPC, Corneal edema, and Corneal ulcer was seen at 3rd follow up.

In the FDA group 4 patients, out of 15, n=9 (60%) subjects had right eye complications such as CL discomfort (n=4), Dry eye (n=3), CLARE (n=1), and SPK's (n=1) at first follow-up. Whereas, no patient presented with Allergic conjunctivitis, Staining, Corneal abrasion, GPC, Corneal edema, and Corneal ulcer at 1st follow up.

At 2nd follow up n=8 (53.33%) patients had CL discomfort (n=3), Dry eye (n=3), CLARE (n=1), and Allergic conjunctivitis (n=1). No incidence of SPK's, Staining, Corneal abrasion, GPC, Corneal edema and Corneal ulcer.

At 3rd follow up only n=2 (13.33%) patients had CL discomfort (n=1), and Corneal abrasion (n=1). There were no incidences of Dry eye, CLARE, Allergic conjunctivitis, SPK's, staining, GPC, Corneal edema and Corneal ulcer.

The incidence of right eye complications was comparable when compared between FDA group 1 patient and FDA group 4 patients (P>0.05). The detailed distribution of subjects according to the type of right eye adverse complication in FDA group 1 patients and FDA group 4 patients are shown in Table.1

Table 1 : Distribution of subjects according to the type of right eye adverse experience in FDA group 1 patient and FDA group 4 patients

Adverse experience	FDA Group 1, % (n=15)			FDA Group 4, % (n=15)		
	1st	2nd	3rd	1st	2nd	3rd
CL discomfort	33.33 (5)	20 (3)	0	26.67 (4)	20 (3)	6.67 (1)
Dry eye	20 (3)	6.67 (1)	6.67 (1)	20 (3)	20 (3)	0
CLARE	6.67 (1)	0	0	6.67 (1)	6.67 (1)	0
Allergic Cong.	0	6.67 (1)	0	0	6.67 (1)	0
SPK's	0	6.67 (1)	0	6.67 (1)	0	0
Corneal abrasion	0	6.67 (1)	0	0	0	6.67 (1)

Incidence of left eye adverse experience in FDA group 1 and FDA group 4 patients

In FDA group 1 patients n=2 (13.33%) out of n=15 had left eye adverse experience including CL discomfort (n=1) and Corneal abrasion (n=1) at first follow-up. No incidence of Dry eye, CLARE, Allergic conjunctivitis, SPK's, Staining, GPC, Corneal edema, and Corneal ulcer were seen.

At 2nd follow up the incidence of left eye adverse experience was noted in n=6 (40%) patients including CL discomfort (n=2), Dry eye (n=2), and CLARE (n=2). There was no incidence of Allergic conjunctivitis, SPK's, and Corneal abrasion, Staining, GPC, Corneal edema, and Corneal ulcer at 2nd follow up.

At 3rd follow up out of n=15 only n=2 (13.33%) subjects presented with left eye adverse complications such as CL discomfort (n=1), Dry eye (n=1). There was no incidence of CLARE, Allergic conjunctivitis, SPK's, Corneal abrasion, Staining, GPC, Corneal edema, and Corneal ulcer.

In the FDA group 4 patients out of 15, n=6 (40%) subjects had left eye complications such as CL discomfort (n=3), Dry eye (n=1), CLARE (n=1), and Corneal abrasion (n=1) at first follow-up. Whereas, no incidence of Allergic conjunctivitis, SPK's, Staining, GPC, Corneal edema, and Corneal ulcer was seen.

At 2nd, n=3 (20%) had CL discomfort (n=1), Dry eye (n=1), and Allergic conjunctivitis (n=1). No patient presented with CLARE, Corneal abrasion, SPK's, Staining, GPC, Corneal edema, and Corneal ulcer.

At 3rd follow up n=5 (33.33%) patients had complications namely CL discomfort (n=1), Dry eye (n=2), CLARE (n=1), and Allergic conjunctivitis (n=1). No patient

presented with SPK's, Staining, Corneal abrasion, GPC, Corneal edema, and Corneal ulcer.

The incidence of left eye complications was comparable when compared between FDA group 1 patient and FDA group 4 patients (P>0.05). The detailed distribution of subjects according to the type of left eye adverse complication in FDA group 1 patients and FDA group 4 patients are shown in Table 2.

Table 2 : Distribution of subjects according to the type of left eye adverse experience in FDA group 1 patient and FDA group 4 patients

Adverse experience	FDA Group 1, % (n=15)			FDA Group 4, % (n=15)		
	1st	2nd	3rd	1st	2nd	3rd
CL discomfort	6.67 (1)	13.33 (2)	6.67 (1)	20 (3)	6.67 (1)	6.67 (1)
Dry eye	0	13.33 (2)	6.67 (1)	6.67 (1)	6.67 (1)	13.33 (2)
CLARE	0	13.33 (2)	0	6.67 (1)	0	6.67 (1)
Allergic Cong.	0	0	0	0	6.67 (1)	6.67 (1)
SPK's	0	0	0	0	0	0
Corneal abrasion	6.67 (1)	0	0	6.67 (1)	0	0

Severity of the dry eye

The most common complication in both groups of patients was dry eye. In FDA group 1 the severity of the right dry eye in 20% (n=3) of the patients was mild, at 2nd follow up 1 patient had moderate right dry eye whereas at 3rd follow-up in 1 patient mild right eye dryness was observed. In the FDA group 4 patients, the severity of right eye dryness at 1st follow-up in 2 and 1 patients was mild and moderate respectively. Whereas, at 2nd follow up mild and moderate right eye severity was found in 1 and 2 patients respectively. The detailed distribution of subjects according to right eye dryness severity is depicted in Table 2.1.

Table 3 : Distribution of FDA Group 1 and FDA Group 4 subjects according to right eye dryness severity.

Severity of dry eye (right)	FDA Group 1 (follow-up)			FDA Group 4 (follow-up)		
	1ST % (n)	2nd% (n)	3rd % (n)	1st % (n)	2nd % (n)	3rd% (n)
MILD	20(3)	0	6.67 (1)	13.33(2)	6.67 (1)	0
MODERATE	0	6.67 (1)	0 0	6.67 (1)	13.33 (2)	0
SEVERE	0	0	0	0	0	0

In FDA group 1, at 2nd follow up the severity of the left dry eye in 1 patient was mild and moderate respectively, at 3rd follow up 1 patient had a mild left dry eye. In FDA group 4 patients the severity of left eye dryness at 1st follow-up in 1 patient was mild. Whereas, at 2nd follow up 2 patients had mild left eye dryness severity. The detailed distribution of subjects according to right eye dryness severity is depicted in Table 2.2.

Table 5 : Distribution of FDA Group 1 and FDA Group 4 subjects according to left eye dryness severity

The severity of dry eye (left)	FDA Group 1 (follow-up)			FDA Group 4 (follow-up)		
	1st, % (n)	2nd, % (n)	3rd, % (n)	1st, % (n)	2nd, % (n)	3rd, % (n)
Mild	0	6.67 (1)	6.67 (1)	6.67 (1)	13.33 (2)	0
Moderate	0	6.67 (1)	0	0	0	0
Severe	0	0	0	0	0	0

Association between sex and discomfort

There was no significant association found between discomfort and sex in FDA group 1 as well as in FDA

group 4 when assessed in right (FDAG1-P=0.67 and FDAG4-P=0.44) and left eye (FDAG1-P=0.11 and FDAG4-P=0.7).

Association between dry eye and discomfort

There was a significant association between discomfort and dry eye in FDA group 1 as well as in FDA group 4 when assessed in right (FDAG1-P=0.004 and FDAG4-P=0.04) and left eye (FDAG1-P=0.0.002 and FDAG2-P=0.01).

DISCUSSION

The current observational study was undertaken to compare acceptance and adverse experiences of wearing soft contact lenses with FDA group 1 to FDA group 4.

In this study, the average age of FDA group 1 and 4 patients was 26.13±3.03 years and 27.13±3.55 years respectively. Average was comparable between groups. Here the majority of participants in both groups were female 60% and 66.67%). In the study of Reindel W. et al. the average age of the subjects of the samfilcon A group and balafilcon A group was 29.8±6.2 years and 29.2±6.1 years respectively. In their study female participants were predominantly present which is similar to the present study (FDAG1 - 18/15, FDAG4 – 20/10).[11] In the US, FDA regulates contact lenses as class II and III medical devices that necessitate added supervisory and professional oversight to keep subject safe.[12] compared to rigid CL, the incidence of complications is more in SCL wearers due to lens material and wear modality.[13] Multipurpose solutions are a consisted preservative, disinfectants, surfactants, buffers, and other substances to clean and avoid contamination without affecting CL and eye. Based on ionicity and water content, the FDA categorizes hydrophilic CL materials (Group 1 through Group 4).

The incidence of CL associated ocular complications was reported to be 39% irrespective of type of CL.[14] In this study, we compared the incidence of adverse outcomes of wearing a soft contact lens with FDA group 1 to FDA group 4 at various time intervals. In FDA right eye group 1 (n=15), 60% of patients had complications (CL discomfort, dry eye, and Clare) at the first follow up which was reduced to 40% (CL discomfort, dry eye, allergic conjunctivitis, SPK's, and corneal abrasion) and 6.67% (dry eye) by the 2nd and 3rd follow up respectively. In FDA right eye group 4 (n=15), 60% of the patients had complications (CL discomfort, dry eye, Clare, and SPK's) at 1st follow up which further reduced to 53.33% (CL discomfort, dry eye, Clare, and allergic conjunctivitis), and 13.33% (CL discomfort, and corneal abrasion) by the 2nd and 3rd follow up respectively. Furthermore, the incidence of left eye adverse experience in FDA group 1 at 1st follow-up was found to be 13.33% (CL discomfort, and corneal abrasion) which was in 40% (CL discomfort, dry eye, and Clare) and 13.33% (CL discomfort, and dry eye) of patients at 2nd and 3rd follow-ups. In FDA group 4, 40% of subjects had left eye complications (CL discomfort, dry eye, CLARE) at the first follow-up. Whereas at 2nd and 3rd follow up it was present in 20% (CL discomfort, dry eye, and allergic conjunctivitis) and 33.33% (CL discomfort, dry eye, CLARE, and allergic conjunctivitis) of patients respectively. The incidence of adverse experience in FDA group 4 patients was more compared to FDA group 1 patients, however, the difference was statistically insignificant. These findings suggested that both contact lenses have minimal adverse experiences. To the best of our knowledge, the present study is the first of its kind, therefore, no similar studies reported. A 3 year follow up study conducted

on Chinese population to assess the complications associated with CL, complication in SCL wearers such as dry eye (38.79%), SPK (37.93%), blepharitis and MGD (36.21%), GPC (17.24%), microbial keratitis (6.03%), allergic conjunctivitis (12.07%), CLPUs (5.17%), neovascularization (5.17%), infiltrative keratitis (4.31%), follicles (4.31%), Clare (2.59%), injection of conjunctiva (1.72%), corneal abrasion (0.86%), corneal edema (0.86%), and SLK (0.86%).[5] Moreover, Forister JF. et al. and Cunha et. al. suggested incidence of SCL associated complication in 50% of study subjects over 3.5-year duration. These findings are in contrast with present study due to type of study, sample size, and follow up. [13,15] Peterson RC. et al. conducted a study to assess the clinical performance of daily disposal soft contact lenses using sustained release technology suggesting that the initial comfort was better for Aqua Release compared to ocufilcon B lenses. [16] "According to the Tear Film & Ocular Surface Society (TFOS), CL discomfort is defined as a condition characterized by episodic or persistent adverse ocular sensations related to lens wear, either with or without visual disturbance, resulting from reduced compatibility between the contact lens and the ocular environment". This complication can lead to decreased wearing time or even discontinuation of contact lens wear".[17] The most common complication in both group patients was mild to moderate dry eye and discomfort. Similarly, in the study of Lie W et al. and Mc Monnies CW. the most common complication was discomfort and dryness[5,18] Furthermore, it was suggested that dry eye due to lens is observed in >50% of CL wearers which was significantly more compared to spectacle wearers and clinical emmetropes using the same criteria.[19] In this study we found a significant association was found between discomfort and dry eye in FDA group 1 as well as in FDA group 4 when assessed in right (FDAG1-P=0.004 and FDAG4-P=0.04) and left eye (FDAG1-P=0.0.002 and FDAG2-P=0.01). The possible explanation of CL associated eye dryness and discomfort that lenses can disrupt normal tear physiology through thinning and break-up of the tear film, with consequent increases in tear film evaporation.[20]

The limitations of the study such as investigator was not blind and the study was single centered all together could have led to some bias. The sample size was relatively small and does not fulfil required criteria as the study was part of a postgraduate thesis and the time was a limitation. Randomization of choice would ideally have been a simple stratified random sampling since our resources were limited thus, we had to limit ourselves to a single study centre so we proceeded by convenient sampling. The follow up was relatively small thus cannot produce long-term insights. A multicentre study with large sample size and long follow-up is the further recommendation of the study.

CONCLUSION

In FDA group 1 wear soft contact lens patients, the most common adverse experience was dry eye and discomfort. In FDA group 4 wear soft contact lens patients, the most common adverse experience was dry eye and discomfort. The incidence of adverse experience in FDA group 4 patients was more compared to FDA group 1 patients, however, the difference was statistically insignificant. A significant association was found between dry eye and discomfort. Further studies are warranted to confirm the present study findings.

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ORIGINAL ARTICLE

CONGENITAL ANOMALIES AND SOCIO-DEMOGRAPHIC FACTORS AFFECTING THEM IN A TERTIARY HOSPITAL, INDIA

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ABSTRACT

Background : Congenital anomalies (CAs) are responsible for most neonatal morbidity and mortality, particularly in developing nations. Data regarding CAs are not properly maintained in such nations. The present study aimed to assess the socio-demographic factors affecting in congenital anomalies. **Material and Methods :** A cross-sectional prospective study was performed at the tertiary Care center. A total of 3549 pregnant women between the gestational age of 18-20 wks coming to OPD were registered and all of them underwent anomaly scans. the study was conducted over a 9-month period from January 2022 to September 2022. **Results :** During the study period, 3549 babies were delivered in our institute; among which 47 had CAs suggesting a prevalence rate of 1.32%. The most common defects were craniospinal (21.28%) and renal (21.28%). The prevalence of CAs was more in multipara (3.56%) than in primipara (0.84%) mothers. There was a significant association between parity and incidence of CAs (P<0.0001). The prevalence of CA in ≤20 years, 21-25 years, 26-30 years, and > 30 years age groups was 0.14%, 0.59%, 0.47%, and 0.11% respectively. There was a significant correlation between maternal age and CAs (P=0.0001). A total of 16.66% of congenitally anomalous babies were predominantly seen in consanguineous couples. There was a significant association between consanguineous marriage and CAs (P<0.001). A total of 55% (n=11 out of n=20) of mothers delivered preterm babies with very low birth weight. Among these males were predominantly present (54.54%). In the terminated pregnancies (n=27), 59.25% (n=16) foetuses were male. **Conclusion :** Enhancing public awareness of preventable risk factors of CAs should be promoted and early prenatal diagnosis and management of common anomalies are highly recommended for a better outcome.

Keywords: Congenital anomaly, Consanguinity, Prevalence, Risk factors

INTRODUCTION

Congenital anomalies (CA) are defined as “structural or functional anomalies that occur during intrauterine life”. It is also known as “birth defects, congenital disorders, or congenital malformations”, these conditions develop prenatally and may be identified before or at birth, or later in life. It is estimated that globally, out of total births, every year 6% of babies are born with CA. It is associated with 240000 mortalities within 28 days of birth and 170000 under-five children every year. [1] The global neonatal mortality rate due to CA has increased from 3% in 2008 to 4.4% in 2013. 2-3 Moreover, the majority of

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incidences of CA (>90%) are seen in low and middle-income countries. [4]

In India, CAs are the fifth most common cause of neonatal mortality in the year 2010, contributing to 9% of total deaths. 3 A meta-analysis showed the pooled prevalence of CA is 184.48 per 10,000 births among 802,658 births. [5] However, due to a lack of nationwide birth defects surveillance, it is uncertain how many babies in India are affected by congenital malformations. Thus, there is a need for data regarding the impact of CA on maternal, neonatal, and health care facilities utilization. Also, the data can be useful to predict the rate of childbirth with CA in the region. Moreover, data on the extent of congenital defects is also required, since some of the risk factors can be avoided by primary care treatments directed at women throughout the preconception, intraconception, and prenatal periods. [6] Along with various strategies, prevention of CAs involve minimizing the risk factors to decrease reproductive waste and improve pregnancy outcomes. Therefore, the present study was undertaken to assess the incidence of CA and the factors affecting it in the Kolhapur region of Maharashtra.

MATERIAL AND METHODS

This prospective cross-sectional study was performed at the tertiary care center. The study was conducted over a 9-month period from January 2022 to September 2022. Ethical approval was obtained from the institutional ethical committee before the initiation of the study.

In this study, CAs were defined as structural abnormalities present at birth or diagnosed during the neonatal period. Complications were defined as pregnancy-associated adverse maternal events such as antepartum haemorrhage and pregnancy-induced hypertension.

Neonatal data including sex, age at presentation, gestational age at birth, birth weight, type of gestation, and mode of delivery was obtained. Whereas, maternal age, parity, antenatal care, folic acid use, illness during pregnancy, self-medication, history of consanguinity, and socioeconomically data were also noted.

STATISTICAL ANALYSIS

Data were analyzed using SPSS V 21 software. Categorical variables were expressed in percentage and frequency whereas, continuous variables were expressed in terms of mean±SD. The prevalence of CAs was calculated as the number of neonates with CA present in the total number of neonates admitted during the study period. A Chi-square test was used to find the association between CAs and maternal factors. Binary logistic regression was used to assess the association between risk factors and CAs. $P<0.05$ was considered statistically significant.

RESULTS

During the study period, 3549 mothers visited our institute; among which 47 had CAs suggesting a prevalence rate of 1.32%. The most common defects were craniospinal (21.28%) and renal (21.28%) followed by cardiovascular (19.15%), central nervous system (14.90%) abdominal (10.64%), musculoskeletal (6.38%), and other (6.98%) (table 1).

Table 1 : System-wise distribution of congenital anomalies.

System	Frequency (n)	Percentage (%)
Craniospinal	10	21.28
Renal	10	21.28
Cardiovascular	9	19.15
Central nervous	7	14.90
Abdominal	5	10.64
Musculoskeletal	3	6.38
Other	3	6.38

In this study, 1065 mothers were multiparas and 2484 were primiparas. The prevalence of CAs in multipara and primipara mothers were found to be 3.56% and 0.84% respectively. There was a significant association between parity and incidence of CAs (P<0.0001).

The mean age of the mother who gave birth to the anomalous babies was 25.38±3.70 years. Most mothers belong to the 21-25 years (44.69%) and 26-30 years (36.17%) age groups (table 2). The prevalence of CA in ≤20 years, 21-25 years, 26-30 years, and > 30 years age groups were 0.14%, 0.59%, 0.47%, and 0.11% respectively. There was a significant correlation between maternal age and CAs (P=0.0001).

Table 2 : Distribution of subjects according to age categories

Age (years)	Frequency (n)	Percentage (%)
≤20	5	10.63
21-25	21	44.69
26-30	17	36.17
>30	4	8.51

A total of 16.66% of congenitally anomalous babies were predominantly seen in consanguineous couples. The prevalence of consanguineous marriage-associated congenital anomalies was found to be 0.22%. There was a significant association between consanguineous marriage and CAs (P<0.001). Hypothyroidism was found in n=4 (8.51%) mothers, increased HbA1c was noted in n=25 (53.19%) moreover, vitamin B12 and vitamin D deficiency was noted in n=10 (21.47%), and n=8 (17.02%) cases respectively. Whereas, n=4 (8.51%) mothers had a previous history of anomalies. Other complications including gestational hypertension (n=1, 2.12%) and infertility treatment (n=1, 2.12%) were seen. There was no association between maternal history and CAs (P>0.05). Among 47 CAs cases, 42.56% (n=20) pregnancies were of non-endangering risk thus were continued. A total of 55% (n=11) of the mothers delivered preterm babies with very low birth weight. Among these delivered infants males were predominantly present (54.54%). In the terminated pregnancies (n=20), 59.25% (n=16) foetuses were male (table 3).

Table 3 : Sex distribution of aborted fetuses.

Sex	Frequency (n)	Percentage
Male	16	59.25
Female	11	40.75
Total	27	100

DISCUSSION

The characteristics and incidences of CAs vary according to various factors such as geographical, genetic, environmental, socioeconomic, racial, and ethnic. [7] Early identification and management (CAs due to infection or malnutrition or modifiable risk factors) have importance in the prevention of maternal and neonatal mortality and morbidity in India.

In this study the prevalence of CAs was 1.32% similarly studies conducted within or outside of the Maharashtra state reported the incidence ranging from 0.9% to 2.72%. [8-11] However, reported incidences by previous studies include only live births thus the prevalence may be more than the reported rate. In our study, we included antenatal CAs. Here, the most common system associated with CAs was craniospinal (21.28%) and renal (21.28%) followed by cardiovascular (19.15%), central nervous system (14.90%) abdominal (10.64%), and musculoskeletal (6.98%). These findings are similar to the findings of Kokate P, and Bang R. 8 Some studies showed higher incidences of the musculoskeletal system, CNS, gastrointestinal tract, genitourinary, and cardiovascular system. [9, 12] Whereas, the more incidences CAs of CNS followed by GIT and the musculoskeletal system were reported by Khatemi F et al. and Dutta V. et al. [13-14]

It has been suggested that the incidence of CAs is more in multiparas. 15 Similarly, the present study findings suggest a higher prevalence of CAs in multipara mothers (3.56%). Moreover, we found a significant association between parity and the incidence of CAs. These findings are following the findings of Bhalerao A, and Bhalerao K. [9] Furthermore, the mean maternal age was 25.38±3.70 years, which falls within the active reproductive year. Various studies had showed that as the maternal age increases, the incidence of CAs increases particularly in mothers >30 years of age. [16-19] However, Ajao AE. et al. and Dutta V. et al. suggested no significant association between CA and maternal age. [14, 20] In the present study, the odds of birth with CAs was more in mother younger than 30 years. The association of CAs with older maternal age may not have been found significant in this study due to the relatively small number of women >35 years of age. However, we found a significant correlation between maternal age and incidence of CAs as here the majority of babies with CAs were reported to mothers with ages ranging from 20-30 years which was similar to the study of Bhalerao A, and Bhalerao K. 9 We found various cases with the deficiencies of vitamin B12, and vitamin D, hypothyroidism, and increased HbA1c which might be also correlated with the CA. However, we did not assessed correlation due to paucity of data in records. Prospective assessment of laboratory parameters and their correlation with CA can be the further recommendation of the study.

Consanguinity has a vital role in the occurrence of CAs. [21] In this study, the prevalence of CAs was more in consanguineous couples with a significant association. These findings are similar to the previous reports. [8-9] The association between low birth weight and CAs is well documented. [16-17, 22, 24] Ajao AE. et al. reported an association of LBW with a 30% high risk of CAs but fail to prove statistically significant. [14] However, Bhalerao A and Bhalerao K. suggested a significant association. [9] which was also similar to the present study findings. The difference in the results may be due to the difference in the sample size, geography, inclusion criteria, etc. Furthermore, the male gender is reported to be predominantly associated with CAs which is similar to the present study findings. [9-10, 15] This might be because the female babies were affected with more lethal congenital malformations and so could not survive to be born with signs of life. [9]

The strength of the study was the uniform application of protocol and we included antenatal cases of CAs. The study suggests that health education, antenatal care, prenatal diagnostics, and strong preventative

actions are required to reduce the prevalence of different malformations. To reduce the occurrence of CAs, it is necessary to enhance knowledge about maternal care during pregnancy, educational programs on CAs, and the effects of consanguineous marriages. Moreover, primary healthcare centers often serve as broad areas and receive complicated treatment cases. Thus, prevalence determined in hospital-based studies cannot be projected to the total population. A community-based large sample study could be ideal for the determination of incidences of CAs in the population.

CONCLUSION

The study showed the predominance of craniospinal and renal anomalies in the Kolhapur region. CAs were more commonly associated with multiparity, maternal age, consanguinity, LBW, and male gender. Antenatal use of folic acid, regular hospital visits, and prenatal diagnosis are recommended for the prevention and early diagnosis of CAs. Stringent preventive measures should be taken to enhance the knowledge of society regarding CAs.

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ORIGINAL ARTICLE

A COMPARITIVE STUDY OF EARLY VERSUS LATE LAPROSCOPIC CHOLECYSTECTOMY FOR CHOLECYSTITIS

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ABSTRACT

Introduction : Inflammation of the gall bladder is known as acute cholecystitis. Sudden pain in the upper right of the abdomen, vomiting, fever, tenderness are symptoms of acute cholecystitis. Laproscopic cholecystectomy is considered to be the gold standard for the treatment of acute cholecystitis. **Objective :** To compare operative and post-operative outcomes like operating time, injury to bile ducts, postoperative pain, total length of hospital stay, need for conversion to open cholecystectomy between immediate and late LC. **Methodology :** Sixty patients aged between 18 to 60 years having acute cholecystitis admitted for laparoscopic cholecystectomy were included. Patients were categorized and analyzed based on length of time from presentation to surgery. We defined operation of cholecystectomy within 3 days of presentation as 'early' laparoscopic cholecystectomy and anywhere after 3 days as 'delayed' laparoscopic cholecystectomy. **Results :** The p value obtained for ROFA is 0.042. and that for Pain scale is 0.027. Since the p value is less than 0.05, the null hypothesis is rejected and we can conclude that there is a statistically significant difference between the means of two groups with respect to these factors. **Conclusion :** Both early and delayed laparoscopic cholecystectomy is possible and safe in the treatment of acute cholecystitis but return to full activity is early and pain scale is less in cases of early cholecystectomy.

Keywords : Acute cholecystitis, Laparoscopic cholecystectomy, pain, abdomen, gallstones.

INTRODUCTION

Acute cholecystitis (AC) is inflammation of the gallbladder that occurs due to occlusion of the cystic duct or impaired emptying of the gallbladder". The most common reason for impaired emptying is stones or biliary sludge. It is found in both men and women but may have a propensity for certain populations.[1] The risk of formation of gallstones is high in women, obese patients, pregnant women, and persons ≥40 years of age.[3] The overall global prevalence of cholecystitis is estimated to be around 20% with higher incidences in developed nations. In the United States, it is estimated to affect about 20 million people. [4] In 90% of the patients, AC results from gallstones. It is predicted that 20-40% of subjects with gallstones will grow symptoms and 12% will result in AC.[5] Laparoscopic cholecystectomy (LC) is considered the gold standard for the treatment of AC.[6] However, there is disagreement regarding the ideal time of LC in AC patients. There are two categories of LC including early and delayed cholecystectomy. Recent evidence showed that early LC can be performed before 72 hours from the onset of symptoms, defining a rigid 72- hours boundary.[2,7-9] The

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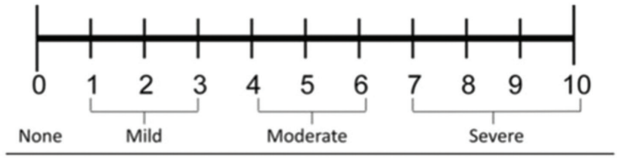
advantage of early LC including ultimate treatment throughout the same admission, decreases the chances of unsuccessful treatment, empyema, gangrene, and perforation.[2] Moreover, early LC is associated with reduced hospital stay, and expenditure compared to delayed LC.[10-12]

The present study was undertaken to compare the incidence of postoperative complications of early versus delayed laparoscopic cholecystectomy.

MATERIALS AND METHOD

The present prospective observational study was performed at Tertiary care hospital and for 2 years after the institutional ethics committee approval. A total of 68 patients who fulfilled inclusion criteria such as the patients aged between 18 to 60 years and those diagnosed with acute cholecystitis presenting within seven days after the presentation were included in the study. Whereas, patients presenting with acute cholecystitis more than seven days duration, those having common bile duct stones or "ductal dilatation", patients with significant medical disease that extracted them unfit for "laparoscopic surgery", and patients, who rejected to undertake "laparoscopic surgery", patients with coagulopathy,severe chronic obstructive pulmonary disease, end-stage liver disease, congestive cardiac failure, obstructive jaundice, patients of acute cholecystitis with moderate to severe pancreatitis and pregnant women" were omitted from the study. A total of n=68 patients were included in the study and a detailed medical history was obtained with a specific focus on symptoms such as pain in "right hypochondrium", "fever", and "vomiting". Physical examination was done to correlate and confirm the diagnosis and assess the patient for operation. Routine investigations in all cases including blood counts, blood sugar level, serum creatinine, liver function tests, chest X-ray, electrocardiogram, HIV, HBsAg, and routine ultrasound were done in all patients. Intraoperative/ postoperative pain was assessed by using "numeric pain rating scale". Patient was requested to make three pain readings equivalent to "present, best and worst pain" experienced immediately after the operation upto 24 hours. The patients 24 hours pain score was calculated using the average of three readings. Patients were instructed to indicate the severity of discomfort on range of "0 (no pain) to 10 (worst pain imaginable)".



Clinical criteria used to define acute cholecystitis are pain in the Right upper quadrant, tenderness in right hypochondrium (Murphy's sign), and fever (temperature >100 degrees F), whereas sonological findings show Cholelithiasis (GB Calculi, single / multiple/sludge), thickened GB wall (>3 mm), sonographic Murphy's Sign, peri-cholecystic collection. Subjects were categorized into two groups as 'early group' and 'delayed group' each with n=34 patients depending on the "length of time from presentation to surgery". Operation of cholecystectomy within 3 days of the presentation was defined as "early laparoscopic cholecystectomy (early group)" and anywhere after 3 to 7 days was considered as delayed laparoscopic cholecystectomy (delayed group). Data were collected and entered into a Microsoft excel sheet. Using the SPSS IBM 20 version categorical variables were evaluated in terms of frequency and percentages, and the distribution was illustrated using pie charts. Independent sample T test and Mann Whitney u test were used to find the significant difference between the groups. $P<0.05$ was considered statistically significant.

RESULT

Table 1 : Distribution of subjects according to age categories

Age (years)	Early group		Delayed group	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
18-32	6	18	7	20
32-46	13	38	7	20
46-60	15	44	20	60

Age distribution

The mean age of the "early and delayed group" patients was 46.64±12.76 years and 44.76±12.18 years respectively. The patients of both groups were categorized according to age groups such as 18-32, 33-46 years, and 47-60 years. Most of the participants in the "early and delayed groups" were belonging to the 46-60 year's age group (44% and 60% respectively) (Table 1)

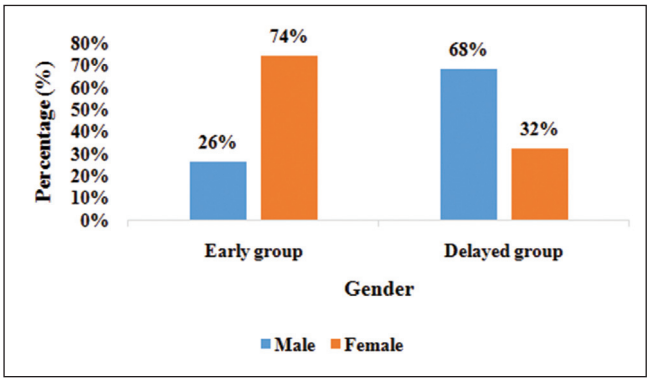


Fig 1 : Distribution of subjects according to gender

Gender distribution

In the early group, females were predominantly present (74% vs 26%) whereas, in the delayed group males were predominantly present (68% vs 32%) (Fig 1).

Table 2 : Comparison of duration of surgery

Group	Duration of surgery (min)		T value	P value
	Mean	SD		
Early	66.47	10.190	1.254	0.214
Delayed	70.44	15.392		

Duration of surgery (DS)

The mean DS in the "early group and the delayed group" was 66.47±10.19 min and 70.44±15.39 min respectively. There was no statistically significant difference in DS when compared amongst the groups (P=0.214) (Table 2).

Table 3 : Comparison of duration of hospital stay

Group	Duration of Hospital stay (days)		T value	P value
	Mean	SD		
Early	4.78	1.447	6.210	0.00968
Delayed	7.44	1.211		

Duration of hospital stay (DHS)

A significant difference in DHS was observed when compared between the groups (4.78 ±1.44days vs 7.44±1.21 days, P=0.00968) (Table 3).

Table 4 : Comparison of return of full activity

Group	Return of full activity (days)		T value	P-value
	Mean	SD		
Early	15.82	2.48	2.078	0.042
Delayed	16.97	2.05		

Return of full activity (RFA)

In early group patients, the mean duration required for

RFA was significantly less compared to delayed group patients (15.82±2.48 days vs 16.97±2.05 days P=0.042) (Table 4 and Fig. 2).

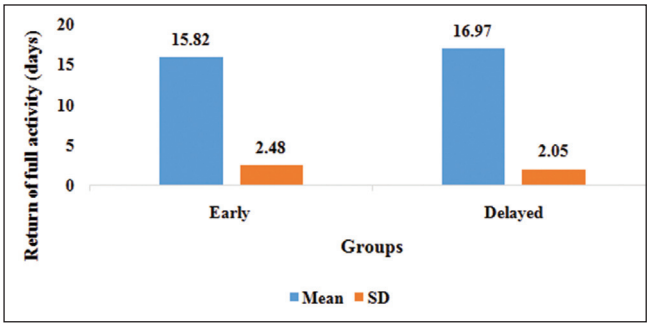


Fig. 2. Distribution of return of full activity

Table 5 : Comparison of pain score

Group	Pain scale		T value	P value
	Mean	SD		
Early	4.823	0.968		
Delayed	6.08	0.753	4.106	0.0273

Pain scale

The mean pain scale score was significantly more in "delayed group patients" (6.08±0.75) than in "early group patients" (4.823±0.96) (P=0.027) (table 5).

DISCUSSION

The study aimed to perform a comparison between early and delayed laparoscopic cholecystectomy for acute cholecystitis in patients aged between 18 to 60 years. The significant findings of the study were the subjects treated with early laparoscopic cholecystectomy had lower hospital stay (P=0.00968) and postoperative pain scores (P=0.027) compared to a patient with delayed laparoscopic cholecystectomy. Moreover, in early group subjects, the postoperative RFA was rapid compared to delayed group subjects (15.82±2.48 days vs 16.97±2.05 days P=0.042). These findings suggested that early laparoscopic cholecystectomy has fewer postoperative complications compared to delayed laparoscopic cholecystectomy. This suggests that the prevalence of acute cholecystitis is more in subjects≥46 years of age. Moreover, out of 68 patients, 36 were female similarly, Lal S. et al. and Rather ZM also depicted female predominance.[13,14] The mean DS in early group patients was less (66.47±10.19 min) than in delayed group patients (70.44±15.39) however, the difference was statistically insignificant (P>0.05) . In this study no incidence of complications such as bile leak, bile duct injury, and complication associated open procedure in any patients of either group. The strength of the study was the adequate sample size and uniform application of the protocol. The study showed that early laparoscopic cholecystectomy was better than delayed laparoscopic cholecystectomy in terms of duration of surgery, duration of hospital stay, return of full activity, and pain. The limitations of the study were the investigator was not blinded during data collection, and the study was single centered, all together could have led to some bias. The other important limitation such as operation expenditure was not assessed in this study. Randomization was not performed due to the inadequate sample size. Further, a blind randomized study with an adequate sample size is required to approve the present study discoveries. Moreover, considering the variability in the incidence of complications in literature, we assume that there might be a correlation between the surgeon's experience and the incidence of complications that need to be evaluated.

CONCLUSION

The study was conducted to compare the incidence of postoperative complications of early versus delayed laparoscopic cholecystectomy. The duration of surgery was less in the early group than in the delayed group

however, the difference is statistically insignificant. Also, the duration of hospital stay was significantly more in the delayed group compared to early group patients. The time required to return to full normal activity was significantly less in early group subjects than in delayed group subjects. The mean pain scale score was significantly more in the delayed group patient. No incidence of postoperative complications was seen in either group. Thus, we can conclude that early laparoscopic cholecystectomy was better than delayed laparoscopic cholecystectomy in terms of duration of surgery, duration of hospital stay, return of full activity, and pain. Further studies large multicentre studies are required to confirm the present study findings.

Conflict of Interest- The author declares no conflict of interest. The study was conducted in accordance with the ethical standards of the relevant institutional or national ethics committee and the Helsinki Declaration of 1975 as revised in 2000.

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ORIGINAL ARTICLE

A STUDY OF EFFECT OF CARBON DIOXIDE PNEUMOPERITONEUM ON LIVER FUNCTION TESTS FOLLOWING LAPAROSCOPIC PROCEDURES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction : Laparoscopic surgeons should be aware of the clinical changes that occur during PNP (Pneumoperitoneum), as well as the fundamental physiologic changes that occur during PNP, and make the required intraoperative alterations to limit the deleterious consequences. Over the next ten years, we may expect laparoscopic surgery to be used for the majority of abdominal surgical procedures. Its numerous advantages are well documented, including reduced postoperative discomfort, a faster recovery, a shorter hospital stay and recuperation period, and fewer complications. LS (Laparoscopic surgery) is performed by insufflating gas into the peritoneal cavity. Because it does not burn, carbon dioxide (CO_2) is commonly employed as an ideal gas. After absorption from the peritoneum, it is easily removed through the lungs. The most significant hemodynamic alteration is a brief decrease in hepatic circulation as a result of "pneumoperitoneum." The extent and compression of the formed "pneumoperitoneum" were shown to influence the degree of "hepatic ischemia. **Materials and Methods :** Each participant in the study provided written informed consent after being fully explained the benefits and drawbacks of the trial. In this study, we included 70 subjects of 18 – 60 years of age, ready to give informed consent, and who were operated on by laparoscopic procedures during the study period. Patients with pre-operative irregularity in "liver enzymes, suspected chronic liver diseases (CLD), common bile duct pathology, and conversion to open cholecystectomy, recent elective retrograde cholangiopancreatography, extraperitoneal laparoscopic surgeries, and laparoscopic surgeries of >80 min duration" were omitted from the study. **Discussion :** The present study was undertaken to study the effect of CO_2 pneumoperitoneum on liver function tests following the laparoscopic procedure in tertiary care hospitals. The significant findings of the study were the "AST (Aspartate aminotransferase), ALT (alanine aminotransferase), ALP (Alkaline phosphatase), total bilirubin, and direct bilirubin" were significantly increased postoperatively on day 1 and day 2 compared to preoperative liver function test parameters. **Conclusion :** The present study aimed "to study the effect of CO_2 pneumoperitoneum on liver function tests following laparoscopic procedures in a tertiary care hospital". Postoperative AST was significantly increased on day 1 and day 3 compared to preoperative values. Compared to preoperative ALT values, postoperative ALT values were decreased on day 1 and day 3. The postoperative mean ALP value was higher than the preoperative value.

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INTRODUCTION

The key component of laparoscopic surgery (LS) is the pneumoperitoneum (PNP). Laparoscopic surgeons should be aware of the clinical changes that occur during PNP, recognize the fundamental physiologic changes that take place during PNP, and make the necessary intraoperative modifications to minimize the adverse effects. We may anticipate that over the next ten years, laparoscopic surgery will be employed for the majority of treatments involving abdominal surgery. Many benefits of LS are widely documented, including less postoperative pain, a speedier recovery, a short duration of hospital stay and recovery period, and fewer problems.[1] LS is carried out by the insufflation of gas into the peritoneal cavity. Carbon dioxide (CO_2) is largely used as an ideal gas since it lacks combustion. It is readily eliminated through the lungs after absorption from the peritoneum. The solubility of CO_2 is more than 20 times in serum than in atmospheric oxygen and is absorbed 32 times more rapidly as compared to room air when used for "double-contrast barium enemas". It's necessary to keep in mind, for instance, that the typical intra-abdominal pressure (IAP) of CO_2 used during LS is greater than (12–14 mm Hg of CO_2) the distinctive pressure values of the portal system (7–10 mm Hg of CO_2).[1] A temporary reduction in hepatic circulation as a result of "pneumoperitoneum" is the major important hemodynamic change. The compression of the developed "pneumoperitoneum" and its extent were shown to impact the grade of "hepatic ischemia". This marks an increase in liver enzymes such as "alanine aminotransferase (ALT), aspartate aminotransferase (AST), 'alkaline phosphatase, gamma-glutamyl transferase (GGT), bilirubin, and international normalized ratio" (INR). Even though "laparoscopic cholecystectomy" (LC) is related to small raise of liver enzymes, in patients with normal "liver function tests". The conflicts following the surgery are self-limited and not linked with any morbidity. LC affects the liver function parameters which was indicated by various investigations. The splanchnic microcirculation is altered by the pneumoperitoneum induced by carbon dioxide, which can also have an impact on the physiology of the heart, lungs, liver, and kidneys. There have also been changes in the blood acid-base balance, immunological system, and intracranial pressure.[2] So, this research work was done in tertiary care hospitals to "study the effect of pneumoperitoneum induced by CO_2 on liver function tests".

AIM AND OBJECTIVES

Aim : To study the impact of CO_2 Pneumoperitoneum on liver function tests (LFT) following laparoscopic procedures in a tertiary care hospital.

Objectives : To assess the variations in liver function tests in patients undertaking laparoscopic actions pre-operatively and post-operatively on day-1 and day-3. To compare the period of the laparoscopic procedure with araise of liver enzymes if any.

Procedure of laparoscopy

Laparoscopic surgery begins with a little incision close to the belly button or pelvic bone. CO_2 gas is pumped into abdominal or pelvic cavities through this first incision. Trocars, which are small surgical tubes used in laparoscopic surgery, serve as ports for surgical

tools. The abdomen or pelvis will be inflated with gas when the surgeon inserts the first trocar and threads the gas tube through it. By separating the abdominal wall from the organs, the patient can more easily see their organs on the television monitor.[25]

MATERIALS AND METHOD

The present study is a "prospective, hospital-based observational study conducted in the department of surgery, tertiary care center, over 2 years from December 2020 to December 2022. This study was executed after approval from "Institutional Ethics Committee (IEC)". Each participant in this study provided written informed consent after being fully explained the benefits and drawbacks of the trial. In this study, we included 70 subjects 18 – 60 years of age, ready to give informed consent, and who were operated on by laparoscopic procedures during the study period. Patients with pre-operative irregularity in "liver enzymes, suspected chronic liver diseases (CLD), common bile duct pathology, and conversion to open cholecystectomy, recent elective retrograde cholangiopancreatography, extraperitoneal laparoscopic surgeries, and laparoscopic surgeries of >80 min duration" were omitted from the study. Detailed history, with clinical and laboratory evaluations, was performed on all the subjects. Pre-operative liver function tests such as liver enzymes "alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin (direct) and bilirubin (total)" were performed. These tests were again performed 24 hours after surgery and on the third day. Further, the length of hospital stay was recorded and the patients who established intra-abdominal problems were withdrawn from the study.

STATISTICAL ANALYSIS

Data were collected in approved proforma and entered into a Microsoft Excel sheet. Using the SPSS IBM 20 version "continuous variables were expressed in terms of mean and standard deviation, and the distribution was illustrated using a line with marker charts. Categorical variables were expressed in terms of percentage and frequency". One-way ANOVA was used to find the difference between AST, ALT, ALP, bilirubin (total), and bilirubin (direct) at different time intervals. Whereas, Pearson's correlation test was performed to find the correlation between LFT variables and the duration of surgery. "$P<0.05$ was considered statistically significant".

RESULT

The 'average age of the study subject was 36.68±17.54 years. Females were predominantly present (n=74, 67.14%) compared to male (n=23, 32.86%)

Table1 1 : Sex distribution

Gender	Frequency (n)	Percentage (%)
Female	47	67.14
Male	23	32.86

Duration of surgery

The mean "duration of surgery" was 60.64±6.68 min. In the majority of cases, the duration of surgery was 63-69 minutes (50%) followed by 58-62 minutes in 25.71% of cases.

Table 2 : Categorization of subjects basedon duration of surgery

Duration of surgery (min)	Frequency (n)	Percentage (%)
43-47	4	5.71
48-52	6	8.57
53-57	7	10
58-62	18	25.71
63-69	35	50

Comparison of AST values

AST was found to be significantly increased at day 1 (33.44±3.24) and day 3 (38.83±4.49) compared to the preoperative level (25.51±1.77) (P<0.00001).

Table 3 : Comparison of AST

AST	Mean	SD	P-value
Preoperative	25.51	1.77	< 0.00001
Day 1	33.44	3.24	
Day 3	38.83	4.49	

Comparison of ALT values

Compared to the preoperative ALT value (30.92±1.04), postoperative ALT values were significantly decreased at day 1 (42.21±5.85) and day 3 (50.3±7.61) (P<0.00001).

Table 4 : Comparison of ALT.

ALT	Mean	SD	P-value
Preoperative	30.92	1.04	< 0.00001
Day 1	42.21	5.85	
Day 3	50.3	7.61	

Comparison of ALP

The mean preoperative ALP was 112.39±2.44 which was found to be significantly increased by day 1 (130.69±14.41) and day 3 (147.14±13.79) (P<0.00001)

Table 5 : Comparison of ALP

ALP	Mean	SD	P-value
Preoperative	112.39	2.44	< 0.00001
Day 1	130.69	14.41	
Day 3	147.14	13.79	

Comparison of Total Bilirubin

Total bilirubin was significantly increased from a preoperative mean level of 0.6489±0.06 to 0.8796±0.09 and 1.0641±0.15 at post-operative day-1 and day-3 respectively (P<0.00001).

Table 6 : Comparison of Total Bilirubin

Total Bilirubin	Mean	SD	P-value
Preoperative	0.6489	0.06	< 0.00001
Day 1	0.8796	0.09	
Day 3	1.0641	0.15	

Comparison of direct bilirubin

At day 1 and day 2, direct bilirubin was found to be 0.3497±0.13 and 0.4799±0.21 respectively which was significantly more when compared with preoperative direct bilirubin value (0.2663±0.39) (P=0.00000408)

Table 7 : Comparison of Direct Bilirubin

Direct Bilirubin	Mean	SD	P-value
Preoperative	0.2663	0.39	0.00000408
Day 1	0.3497	0.13	
Day 3	0.4799	0.21	

Correlation between the duration of the procedure and increased liver enzymes

A significant positive correlation was found between the duration of the procedure and postoperative on day- 1 and day- 2 increased values of "AST, ALT, ALP, total bilirubin, and direct bilirubin (P<0.00001)"

Table 8 : Correlation between the duration of the procedure and increased liver enzymes

Parameters	Correlation Coefficient	P-Value
AST	0.7712	< 0.00001
ALT	0.7462	< 0.00001
ALP	0.5293	< 0.00001
Total Bilirubin	0.8599	< 0.00001
Direct Bilirubin	0.8815	< 0.00001

DISCUSSION

The present study was undertaken to study the effect of CO_2 pneumoperitoneum on liver function tests following the laparoscopic procedure in tertiary care hospitals. The significant findings of the study "AST, ALT, ALP, total bilirubin, and direct bilirubin" were significantly increased postoperatively on day 1 and day 2 compared to preoperative liver function test parameters. Moreover, there was a significant correlation between the duration of surgery and increased liver function test parameters. These findings suggested that prolonged CO_2 peritoneum has a significant effect on liver functioning.

In this study, the average of the subjects was 36.68±17.54 years. Here, the majority of the participants were female compared to male (67.14% vs 32.86%) which is similar to the previous reports.[2, 54] The higher incidences of acute cholecystitis in females may be due to lifestyle, hormones such as estrogen, gestation, and use of 'oral contraceptives or 'hormonal replacement therapy.[55-56] The average duration of surgery was 60.64±6.68 min. In the majority of cases, the duration of surgery was 63-69 minutes (50%) followed by 58-62 minutes in 25.71% of cases. Analogous results were stated by Sharma A. et al.[54] LC is the most commonly practiced technique due to its wide array of advantages compared to classical surgery. Despite these laparoscopic procedures are described to be related to altered liver function. However, the reason for this is not clear. Literature suggested that before laparoscopy, gas is insufflated at a flow rate of 1-2 L/min by using a Veress needle to produce pneumoperitoneum. This causes increased intra-abdominal pressure, hypothermia, and hormonal stress response. Moreover, intra-abdominal pressure >20 mmg can cause prograde flow with increased vascular resistance leading to an adverse effect on the abdominal vasculature. A significantly decreased circulation can be observed in the portal system of about 60% causing liver dysfunction with arise of IAP>20 mmHg which can persist postoperatively.[57] Furthermore, another possibility of an enzymatic modification of the liver is diathermy to the liver surface in LC and the propagation of heat to the liver parenchyma.[58] The squeeze pressure effect on the liver was mentioned by Tauro LF et al. as another potential reason for changes in blood liver enzymes following LC. The liver enzymes may be released into the bloodstream by the gall bladder's traction.[59] In this study, 'AST', 'ALT', 'ALP', 'total bilirubin', and 'direct bilirubin were assessed preoperatively and postoperatively on day 1 and day 2 in all patients. The findings of the study suggested that serum 'AST', 'ALT', 'ALP', 'total bilirubin', and 'direct bilirubin' were significantly increased postoperatively at day 1 and day 2 compared to basal values. These results are in line with the previous reports.[2, 60, 61]

Table 9 : Comparison between studies

Studies	LFT	Preoperative	Follow up 1	Follow up 2	P value
Bellad A. et al.[2]	AST	24.58±8.76	40.9±10.34	-	<0.0001
	ALT	30.9±7.63	53.5±17.13	-	<0.0001
	ALP	112.50±38.11	154.3±39.15	-	<0.0001
	TB	0.66±0.18	1.18±0.29	-	<0.0001
	DB	-	-	-	<0.0001
Reddy P. et al.[60]	AST	34.83±24.80	53.80±28.96	-	<0.001
	ALT	35.93±26.51	54.58±29.28	-	<0.001
	ALP	98.42±51.64	122.96±46.67	-	<0.001
	TB	0.62±0.31	0.82±0.36	-	<0.001
	DB	-	-	-	-
Avadhani GK. et al.[61]	AST	31.5±11.2	60.6±15.2	32.2±9.2	0.000
	ALT	34.8±10.9	61.1±14.1	34.8±10.9	0.000
	ALP	78.9±17.3	98.1±14.2	76.2±12.7	0.000
	TB	0.58±0.29	0.73±0.23	0.44±0.18	0.000
	DB	0.22±0.10	0.23±0.09	0.15±0.06	0.000
Present study	AST	25.51±1.77	33.44±3.24	38.83±4.49	<0.0000
	ALT	30.92±1.04	42.21±5.85	50.3±7.61	<0.0000
	ALP	112.39±2.44	130.69±14.41	147.14±13.79	<0.0000
	TB	0.6489±0.06	0.8796±0.09	1.0641±0.15	<0.0000
	DB	0.2663±0.39	0.3497±0.13	0.4799±0.21	<0.0000

In the current study, the 'mean duration of surgery' was 60.64±6.68 min which was 99±22 min in the study of Avadhani GK, and Dharanesh B.[61] The difference in the results may be due the patient-associated factors such as body mass index, male gender, and high ASA.[62] Here, we observed a significant positive association between the duration of surgery and postoperative increased values of 'AST', 'ALT', 'ALP', 'total bilirubin', and 'direct bilirubin at day- 1 and day- 2 (P<0.00001). Similar results were found by Reddy P. et al. and Hameed et al., who found a correlation between prolonged pneumoperitoneum and elevated bilirubin and liver enzymes. [60, 63] Moreover, Sharma A. et al. suggested that the duration of surgery with high IAP can alter the liver enzymes during LC. Also, less duration of surgery with pneumoperitoneum of 10 to 14 mmHg pressure was reported to be associated with minimally increased liver enzymes compared to long durational LC with high pneumoperitoneum pressure.[54] These findings suggested that as the duration of surgery increases, the duration of CO2 pneumoperitoneum increases hence the values of the hepatic enzyme also increase.

The strength of the present study was the adequate sample size and uniform application of the protocol. The study showed increased 'AST', 'ALT', 'ALP', 'total bilirubin', and 'direct bilirubin postoperative

day 1 and day 2 compared to preoperative values. Furthermore, a significant association was seen between the 'duration of surgery' and postoperative increased 'AST', 'ALT', 'ALP', 'total bilirubin', and 'direct bilirubin values. The limitations of the study were the investigator was not blinded during data collection, and the study was singly cantered, all together could have led to some bias. The other important limitation such as the correlation of indirect patient-related factors such as BMI (Body Mass Index), gender, and ASA grades that can affect the duration of surgery was not performed. Comparison between various CO_2 pneumoperitoneum pressures and their effect on the liver enzymes were not evaluated in the study. The clinical significance of elevated liver enzymes was not assessed. Lastly, postoperative follow-up was relatively small therefore the long-term effect of increased liver enzymes was not assessed in the study. A well-designed comparative study with an adequate sample size assessing the effect of various CO_2 pneumoperitoneum pressure on the liver enzymes with longer follow-ups is the further recommendation of the study.

CONCLUSION

The present study aimed "to study the effect of CO_2 pneumoperitoneum on liver function tests following laparoscopic procedures in a tertiary care hospital". Postoperative AST was significantly increased on day 1 and day 3 compared to preoperative values. Compared to preoperative ALT values, postoperative ALT values were decreased on day 1 and day 3. The postoperative mean ALP value was higher than the preoperative value. Total bilirubin was significantly increased postoperatively compared to preoperative values. At postoperative day 1 and day 2, direct bilirubin values were increased compared to preoperative values. A significant positive association was found between the duration of the process and postoperative on day 1 and day 2 increased values of 'AST', 'ALT', 'ALP', 'total bilirubin', and 'direct bilirubin'. The temporary rise of blood bilirubin and liver enzymes that result from all laparoscopic surgeries is closely proportional to the length of the procedure. Laparoscopic procedures should be completed rapidly and without undue delay. The choice to go from laparoscopic to open surgery should be made quickly.

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