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CHANGES OF THE AIRWAY REACTIVITY IN PATIENTS WITH RHINOSINUSITIS

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Abstract

Rhinosinusitis is one of the most common conditions in primary and secondary care all over the world. Rhinosinusitis together with asthma and gastroesophageal reflux disease represent the most common causes of chronic cough. The relationship between rhinosinusitis and cough is still not completely understood, however, direct stimulation of nasal mucosa, upper airway cough syndrome, inflammation of the airways, and cough reflex sensitisation play the crucial role in the pathogenesis of chronic cough.

Keywords
Rhinosinusitis, Cough, Upper Airway Cough Syndrome, Cough Reflex Sensitisation

INTRODUCTION

Cough is the most common symptom of respiratory diseases. Under normal conditions cough is beneficial for the organism, however, long lasting non-productive cough considerably reduces the quality of life.

Acute cough is defined as lasting up to 3 or 4 weeks in adults and is usually self-limited. Cough persisting for 8 weeks in adults or 4 weeks in children is defined as chronic cough. Upper and lower airway etiologies of chronic cough are collectively termed upper airway cough syndrome (UACS) and lower airway cough syndrome (LACS), respectively. Other important etiologies include extraoesophageal reflux disease, gastroesophageal reflux disease (GERD)-related cough and laryngeal hyperresponsiveness (LHR), obstructive sleep apnea (OSA), COVID-19, tumors, and drugs (ACE inhibitors, opioids) (1). This review article provides an overview of pathomechanisms causing cough in patients with rhinosinusitis.

RHINOSINUSITIS

Acute and chronic rhinosinusitis are common primary care diagnoses in most of the world. They are caused by mucosal inflammation, which inhibits mucociliary function of the nose and paranasal sinuses (2).

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Rhinosinusitis in adults is defined as an inflammation of the nose and the paranasal sinuses characterised by two or more symptoms, one of which should be either nasal blockage / obstruction / congestion or nasal discharge (anterior / posterior nasal drip). Other symptoms include facial pain/pressure, reduction or loss of smell and either endoscopic signs of nasal polyps, mucopurulent discharge primarily from middle meatus, oedema or mucosal obstruction primarily in middle meatus and/or CT changes – mucosal changes within the ostiomeatal complex and sinuses (3). According to the duration of the disease, acute rhinosinusitis lasts for less than 12 weeks. Recurrent acute rhinosinusitis occurs at least 4 times per year with symptom free intervals.

The spectrum of acute rhinosinusitis (ARS) includes the common cold (acute viral rhinosinusitis), post-viral ARS, and acute bacterial rhinosinusitis. Post-viral ARS is defined by an increase of symptoms after 5 days, or a persistence of symptoms after 10 days. It is estimated that less than 2% of episodes of viral upper respiratory tract infections are complicated by bacterial transformation (2).

Chronic rhinosinusitis (CRS) is, characterized by persistent symptomatic inflammation of the nose and paranasal sinus mucosa lasting for more than 12 weeks (3).

The EPOS2020 steering group has chosen to look at CRS in terms of primary and secondary one and to divide each into localized and diffuse disease based on anatomic distribution. In primary CRS, the disease is divided by endotype dominance, either type 2 or non-type 2.

Localized primary CRS is then subdivided into two phenotypes – allergic fungal rhinosinusitis (type 2) or an isolated sinusitis (non - type 2). For diffuse CRS, the clinical phenotypes are predominantly eosinophilic CRS, CRS with nasal polyps (CRSwNP), allergic fungal rhinosinusitis, central compartment allergic disease and non-eosinophilic CRS, determined by the histologic quantification of the numbers of eosinophils.

Among the main causes of secondary chronic rhinosinusitis are cystic fibrosis, Churg-Strauss disease, Wegener's disease, primary ciliary dyskinesia, selective immunodeficiency, odontogenic sinusitis, tumor of the sinonasal complex, and mycosis.

CRS is a syndrome with a multifactorial etiology resulting from a dysfunctional interaction between various environmental factors and the host immune system. For the vast majority, etiology is uncertain although multiple environmental and host genetic factors have been implicated.

In healthy individuals, the mucosa serves as a relative barrier limiting and regulating environmental interaction with the host immune system. In cases of CRS, the current working hypothesis is that alterred barrier penetration results in a chronic inflammatory response (3).

Some evidence suggests genetic and epigenetic factors, early stage environmental insults including an abnormal microbiome or viral injury, and possibly systemic hormone signaling defects, which could all serve as permissive factors in CRS pathogenesis (4).

The microbiota may play a pathogenic role in the development of CRS. Virus and bacterial infection might contribute to the development and exacerbations of CRS. Staphylococcus aureus (SA) is a frequent colonizer in humans, and it is considered to be associated with chronic airway diseases including CRS and asthma (3). The presence of biofilms in CRS patients was first demonstrated in 2004 (5). A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface. Multiple bacterial organisms have been implicated including Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenzae, and Moraxella catarrhalis. Of these, SA biofilms have the greatest association with severely recurrent and recalcitrant cases of CRS possibly because of their potential to produce antigens (6). Other possible pathogenic mechanisms are viral and fungal infection, allergy, immunodeficiency, lower airway inflammation, GERD, and exposition to the tobacco smoke and other air pollutants (3).

Remodeling is defined as an abnormal restitution of damaged tissues. In CRS, remodeling also takes place and observed changes include fibrosis, basement membrane thickening

(BMT), goblet cell hyperplasia, epithelial barrier abnormalities and polyp formation, osteitis, and angiogenesis (7).

Although patients with CRS do not necessarily have higher rates of specific anatomic variations, it appears that they can affect the progression of the disease (8).

Although rhinosinusitis can constitute up to 20% of adult patients with chronic cough, coughing is not a major feature of CRS, cough aggravation has been associated with presence of bacterial biofilms (1).

COUGH

Cough is the most common symptom of respiratory diseases. Under normal conditions cough is beneficial for our organism serving the protection of the airways by removing inhaled irritants, particles, and accumulated secretion. However, in specific conditions cough can become damaging (9). In diseases such as asthma, chronic obstructive pulmonary disease (COPD), gastroesophageal reflux disease (GERD), and rhinosinusitis, cough may become excessive and harmful to the airway mucosa (10). Cough can also be a mechanism of spreading of the life – threatening respiratory infections (10). Furthermore, persistent coughing can substantially reduce health-related quality of life and is associated with increased levels of depression and anxiety (11).

Cough reflex begins with a deep inspiration, followed by expiration against a closed glottis which produces large increases in intrapulmonary pressures such that the final phase of opening of glottis evokes a large expulsive airflow for clearing the airways (12).

Cough can be induced either reflexively or voluntarily. Reflexive cough is generated on the level of the brainstem, voluntary cough originates from the activation of cortical mechanisms (13).

The respiratory tree and lung parenchyma are densely innervated by heterogeneous populations of sensory receptors that respond to a wide variety of chemical and/ or mechanical stimuli. The majority of the sensory receptors originate in either the jugular (superior) or nodose (inferior) vagal ganglia (14, 15).

The vagal afferents terminate particularly in the nucleus of the solitary tract (nTS) (10), substantial portion of afferent nerve fibers heads to paratrigeminal nucleus (Pa5) (13). Second order neurons project to the respiratory – related neurons in medulla, pons, and spinal cord (16). Cortical mechanisms are responsible for voluntary cough and inhibition of coughing (17).

Cough reflex is modulated by many afferent inputs within and besides the vagus nerve. This modulation significantly contributes to the cough plasticity (18).

Afferent neuronal subtypes can be divided into two classes of sensory receptors.

1. Myelinated mechanoreceptors, which consist of neurons that display limited sensitivity to a wide variety of chemical mediators but are exquisitely responsive to stretch, touch or other mechanical forces (19) and
2. unmyelinated chemoreceptors, (frequently termed nociceptors), characteristically sensitive to the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor agonist capsaicin (15).

Airway mechanoreceptors are thought to be derived exclusively from the nodose ganglia (20). Chemoreceptors (C fibers, Ad fibers) originating from both the nodose and jugular ganglia (15).

C-fibers, the most represented afferent nerves in the airways, mediate the recognition of various chemical substances and endogenous inflammatory mediators (21). Mechanoreceptors can be further subdivided into three functionally unique subgroups; rapidly adapting receptors (RARs), slowly adapting receptors (SARs), and touch sensitive receptors, also known as cough receptors (mechanosensitive Ad fibers) (22).

The cough receptors are localized exclusively in the extrapulmonary airways, RARs and SARs terminate in the intrapulmonary airways (17).

Although C-fibers and the cough receptors serve primary roles in cough initiation, other bronchopulmonary afferent nerve subtypes likely modulate cough pattern and sensitivity to tussive stimuli (17).

Based on its duration cough is defined as acute (less than 4 weeks), subacute (less than 8 weeks), and chronic (more than 8 weeks) (23).

The most common causes of chronic cough are asthma, gastro-oesophageal reflux disease and extraoesophageal reflux, upper airway cough syndrome, or a combination of these conditions.

The relationships between rhinosinusitis and cough are incompletely understood. Patients with rhinitis suffering from chronic cough report that coughing occurs in response to sensations of airway irritation and an associated urge-to-cough (24). There are many factors that facilitate cough in this group of patients, including direct stimulation of nasal mucosa, post nasal drip syndrome, microaspiration of the inflamed aerosol, nasobronchial reflex, the impact of the unmodified air inhaled through the mouth in patients with nasal obstruction, spreading of the inflammatory process by the systemic circulation and the central and peripheral neuroplasticity (25).

Direct stimulation of nasal mucosa

Nasal mucosa is inervated by trigeminal afferents. Direct stimulation of nasal mucosa does not initiate cough (17), however, cough seems to be both up regulated and down regulated by distinct populations of nasal afferents. Signaling from nociceptors may primarily up regulate cough, whereas the activation of other nasal nociceptors may down regulate it (26).

There is a hypothesis that cough reflex is up regulated during the stimulation of nasal afferents in order to minimize the spreading of the pathological process from the nasal cavity to other parts of the respiratory tract (27).

The stimulation of trigeminal terminals in the nose by the TRPV1 agonists (capsaicin and histamine) significantly enhances cough response induced in laboratory animals. Such stimulation also up regulates cough responsiveness in human healthy subjects and patients with allergic rhinitis (28, 29).

These findings suggest cough is enhanced by an increased afferent drive form the nose to the sensory trigeminal nuclei and then by cooperation with the brainstem neuronal circuits modulating cough. Although the primary sensory fibers from the nasal cavity are interpolated to second order neurons mainly in the sensitive nucleus of trigeminal nerve, these afferents have connections to the chemosensitive areas such as the area postrema, and also nTS, which may interfere with modulation of inputs to “cough generator” (27). Hypothetically, the interpolation of nasal trigeminal sensory afferents in the paratrigeminal nucleus and also nTS, which represents the termination of vagal afferents, can feature interactive activity of both afferent nerves (30). Not every activator of nasal sensory nerves will enhance cough reflex. For example, nasal stimulation with water was reported to inhibit cough in anesthetized rabbits (31).

Cold temperatures, menthol, eucalyptol, or camphor activate neurons through gating of TRPM8. Intranasal application of menthol inhibited cough in guinea pigs (32).

Upper airway cough syndrome (UACS)

Postnasal drip syndrome (PNDS) refers to the sensation of nasal secretions at the back of the throat (or of a ‘drip’), often resulting in the need to clear the throat and is associated with nasal stuffiness or nasal discharge. Patients with PNDS often have features of rhinosinusitis, however, only a small fraction of patients with chronic rhinosinusitis present with cough (33). Nowadays, the term UACS is used instead of PNDS. In 2006, the American College of Chest Physicians (ACCP) defined UACS as one of several critical pathogeneses of chronic cough. In the past, chronic cough from UACS/PNDS was considered to result from

postnasal drip-inducing mechano- or chemostimulation of the afferent nerves innervating the pharynx, larynx, or lower airways (34). This includes sensory fibers of the trigeminal nerve and superior laryngeal and pharyngolaryngeal branch of the vagus nerve (35). However, postnasal drip and transport of nose and paranasal sinus mucous secretions to the pharynx or larynx are normal physiological processes (35). Patients with PNDS do not account for a homogeneous group with respect to etiology and PNDS is not a consistent complaint in patients with chronic cough (36). Furthermore, the united airway theory assumes a hematogenous or neural spread of inflammatory mediators between upper and lower airways (35).

Airway inflammation

UACS can also be influenced by a chronic inflammation in the pharynx or larynx, such as inflammations resulting from allergic pharyngitis and chronic tonsillitis. Such inflammations may result from a long-term contact with nasal or sinus secretions (37). Cough can mechanically damage airway mucosa and either cause or aggravate airway inflammation (38).

Some studies have demonstrated that patients with rhinitis have increased numbers of neurons capable of generating large amounts of neurogenic inflammatory mediators in their nasal mucosa (39). The diseases of the nose and paranasal sinuses have a significant impact on the function of the lower airways.

Patients with UACS showed a remodeling of the airways, characterized by increased sub-basement membrane thickness, vascularity, vessel size, and signs of goblet cell hyperplasia (40). Numerous studies have shown that airway inflammation in patients with non – asthmatic chronic cough, including patients with UACS, is mainly due to an infiltration of mast cells, neutrophils, and lymphocytes, which is different from the aetiology associated with cough-variant asthma and eosinophilic bronchitis (41). The mechanical stimulation by repeated cough leads to the epithelial transformation causing an increased cough sensitivity (35).

Lower airway inflammation is commonly associated with chronic cough (35). The main causes of lower airway inflammation resulted in chronic cough are either the postnasal drip or aspiration, mainly in older individuals and patients with cerebrovascular disease, (35).

Cough reflex sensitization

The term ‘cough hypersensitivity syndrome’ has been used in an attempt to encompass the clinical picture of patients suffering from chronic cough and reporting cough in response to a wide range of activities and exposures (42).

Cough reflex sensitization (CHS) can be demonstrated as a decreased intensity of a stimulus required to trigger cough or increased coughing in response to a stimulus with a constant intensity. In patients with sensitized cough reflex, the endogenous and environmental stimuli are expected to be more effective to initiate coughing and thus these patients cough in the situations when their healthy counterparts do not (43). Cough hypersensitivity underlies the aetiology of chronic cough in the majority of patients (44).

Patients with CHS usually present one of three different phenotypes: (1) patients with a predominant phenotype of rhinal symptoms (such as UACS), (2) patients with a Th2-cell dominant phenotype (cough variant asthma or nonasthmatic eosinophilic bronchitis), and (3) patients with a predominant phenotype characterized by acid reflux and heartburn (chronic cough caused by gastroesophageal reflux) (42).

The activation of sensory nerves in the nose and oesophagus leads to an increase in cough reflex sensitivity (43).

Chronic nasal symptoms attributable to sensory nerve activation in patients with rhinitis implicate that the inflammation leads to a repeated activation of sensory nerves. The repeated activation and mediators associated with inflammation in patients with rhinitis can induce sensitization at multiple levels of sensory pathways (25) and may lead to an altered central neural processing in cough control (45).

Stimulation of nasal sensory nerves also affected the urge-to-cough - an irritation or tickle in the back of the neck and is associated with >90% of coughs in patients with chronic cough (46).

Central mechanisms of cough sensitization from the nose are poorly understood. There is some evidence that suggests that the sensitivity of the cough reflex is higher in patients with UACS (34). Inflammatory mediators, neurotrophic factors, and other signals emanating from the nose during symptomatic period could, in theory, initiate long-lasting neural plastic changes in the circuits regulating the cough reflex (25). Allergic rhinitis is connected with the sensitization of the cough reflex, persisting also off pollen season (29,34). It is suggested that allergic rhinitis modulates both distinct types of cough–either cough induced by stimulation of TRPV1-expressing capsaicin-sensitive fibers, or cough initiated by capsaicin- insensitive mechanosensitive A nodose fibers (27).

CONCLUSION

Cough is a very important defensive reflex, essential for normal function of the airways. Rhinosinusitis represents one of the most common causes of chronic cough. We tried to clarify the most significant factors leading to excessive coughing accompanying rhinosinusitis. Although, direct stimulation of nasal mucosa does not initiate cough, cough is supposed to be up and down regulated by distinct populations of nasal afferents. Postnasal drip induces the stimulation of the afferents in pharynx, larynx, and lower airways, moreover, hematogenous or neural spread of inflammatory mediators between upper and lower airways is possible. Among other important pathomechanisms there are the airway inflammation with remodeling of the airways, epithelial transformation, and cough reflex sensitization. Further studies are needed to recognize the exact mechanism of the connection of these two entities.

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INFLUENCE OF COLIFORM BACTERIA INFECTION ON INTESTINAL GOBLET CELLS SECRETORY ACTIVITY OF GERM-FREE PIGLETS

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Abstract

Recently, influence of bacteria colonization on development and maturation of gut wall is getting more into the focus of gastrointestinal research. For years, the main interest and research were aimed to the development and maturation of gut wall and its functional properties in normal conditions, less attention has been paid on the germ-free animals. Germ-free (GF) piglets have clear microbiological background and are reared in sterile environment. GF piglets are regarded as clinically relevant models for studying of human diseases, as these piglets' manifest similar clinical symptoms to humans. In this study we briefly summarised the main characteristics in the distribution of goblet cells in the wall of jejunum and colon of GF piglets as healthy control (HC) group and piglets, which were experimentally infected by *E. coli* O149:K88 as ECK group. Neutral mucins were stained with periodic acid-Shiff (PAS) whereas acidic mucins are stained with Alcian blue. Numbers of goblet cells containing total acidic mucins in both, the jejunum and colon, differed significantly between HC and ECK piglets and in the colon, a similar trend was also observed. In the ECK piglets, jejunal goblet cells exhibited decrease in neutral mucins. This change in mucin profile in response to bacterial colonization suggests a potential role as a protective mechanism against pathogenic invasion of the intestinal mucosa during of gut mucosa development in piglets.

Key words: germ free environment, piglet, Escherichia coli, intestine, goblet cells

INTRODUCTION

The pig is very similar to humans in terms of anatomy, genetics and physiology. Choosing the right breed and age allows various surgical and non-surgical procedures typically used in human medicine, including catheterization, heart surgery, valve manipulation, endoscopy and broncho-alveolar lavages (1, 2). These procedures are particularly difficult or impossible to perform in many animal models including rodents. In terms of genetics, the size and the composition of the porcine genome are comparable to those of humans. During the past half century, even though the germ-free (GF) pig has been increasingly recognized as a very valuable experimental animal model system in the inves-

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tigation of pathogens (1), the number of functional GF pig facilities remains low, mainly because of the high costs and the technical complexity in establishing and maintaining the facility (2). Despite this financial difficulty, the advantages of the piglet's models highlighted by these studies include physiological similarity to the human gut as well as to the pathological mechanisms of human diseases. Piglets, have also proven usefulness in the study of intestinal barrier function, surgical manipulation, and tissue intervention, as well as in biomaterial implantation and tissue transplantation (3). Available literature sources show that microbiota is necessary for the normal postnatal development of the structures of the gut wall. It has been suggested that pigs possess a "developmental window" in which the developing host–gut microbiota interactions are the easiest to manipulate and during which time the gut is the most susceptible to major disturbances (4). Therefore, the examination of GF animals before introduction of bacterial colonization, may provide us with better understanding of the communication that occurs between the host and its bacterial residents in the future.

Escherichia coli is an important enteric pathogen of weaned pigs, causing postweaning diarrhoea and edema disease worldwide (5). Despite the progress that has been observed in modern pig farms during the last decade to prevent infectious diseases and improve global animal health, postweaning diarrhoea remains a problem that causes significant economic losses in pig production. The age of affected pigs depends on the age at weaning. Disease normally occurs within the first 14 days after weaning, but can also be encountered after transfer to fattening premises (6). Pathogenic strains colonize the small intestine by means of specific adhesion factors (fimbriae) and produce one or several exotoxins responsible for disease (6, 7). The fimbria types as (*E. coli* O149: K88) are commonly found in pathogenic *E. coli* isolated from weaned pigs (6, 8). These strains produce an outer membrane protein (intimin) which is involved in the intimate attachment of the bacteria to enterocytes (9).

Nowadays, the main focus of attention has been directed to study distribution of bacteria and their influence on development and maturation of piglet´s gut, but a smaller number of information is obtained from GF environment. Our study is focused on investigation of *E. coli* influence on distribution and secretory activities of goblet cells (GCs) in epithelial lining of jejunum and colon of GF piglets as healthy control group (HC) and GF piglets in which at 5th day their gut was colonized with *E. coli* bacteria as ECK group. In this study, we briefly evaluated the differences in distribution of neutral and acidic mucins in the goblet cells in the wall of jejunum and colon in HC piglets and ECK piglets, which were infected by *E. coli* O149:K88.

METHODS

The structure of the small intestine is very similar in humans and piglets, including macroscopic features such as the ratio of intestinal length *per* kg bodyweight (10). Therefore, the experiment was carried out on group (n=4) of clinically healthy GF piglets (cross-bred – Yorkshire × Pietrain) – healthy control group (HC). The piglets were obtained by the open hysterotomy method, kept in sterile insulators and fed with a sterilized milk replacer (Sanolac, Ferkel, Sano, Germany). The piglets were sacrificed using T61 (Intervet International B.V., Boxmeer, The Netherlands, dose: 0.3ml/kg body weight) intracardially on 5th day. All piglets were fed every 4 hours, i.e. 6 times daily. On the day 5 after birth piglets in the infected experimental group (ECK group, n=5) were infected by the dose 2 ml of prepared culture of *E. coli* O149: K88ac (1×10^4 CFU/ml). The *E. coli* O149: K88ac strain without enterotoxin production was obtained from the Danish Institute of Agricultural Science, Denmark. Overnight culture of *E. coli* (1 ml) was inoculated into 50 ml Trypticase soy broth (Oxoid Unipath, Ltd., Basingstoke, UK) and cultivated at 37 ºC in a water bath shaker (JULABO SW 2C, Labor Technic GMBH Selbach, Germany) for approximately 2 h to optical density 0.5 at 640 nm (corresponded to 1×10^8 CFU/mL). Subsequently, the bacterial

culture was diluted in isotonic saline solution to obtain a final concentration of 1×104 CFU/ml. The purity of the broth culture was verified by spread plating on MC agar and TSA agar with sheep blood respectively (Oxoid Unipath). Piglets were examined daily for clinical signs, including lethargy, pyrexia, diarrhoea and anorexia. The infected experimental piglets were sacrificed using T61 (Intervet International B.V., Boxmeer, The Netherlands, dose: 0.3ml/kg body weight) intracardially on 8th day. The presented experiment with protocol number 3629/05-221, was approved by the State Veterinary and Food Administration of the Slovak Republic. The animals were handled and sacrificed in a human manner in accordance with the guidelines established by the relevant Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice.

Gastrointestinal (GI) tract was removed from the sacrificed piglets immediately. Small intestine (jejunum) and large intestine (colon) 1–2 cm long bioptic samples were obtained and washed with cold saline and fixed in 4% paraformaldehyde. Histological sections (4–5 μm) were deparaffinized and rehydrated. The population of GCs present in the intestinal mucosa of jejunum and colon was detected using the PAS - reaction and Alcian blue histochemical staining method. For neutral mucosubstances and mucins detection the PAS-reaction modified according to McManus was used. Fresh-made 1% solution of PAS was prepared for the PAS-reaction. For further differentiation of cell nuclei, Mayer hematoxylin staining was performed. The goblet cells and glycocalyx were stained magenta by PAS-reaction, and cell nuclei were stained dark blue by Mayer's hematoxylin (SIGMA-ALDRICH, Co). Alcian blue 8GX solution (pH 2.5) (Sigma–Aldrich, St. Louis, MO, USA) stains both sulfated and carboxylated acidic mucopolysaccharides and sulfated and carboxylated sialomucins (glycoproteins). Excessive amounts of non-sulfated acidic mucosubstances were visible in the cytoplasm of secretory GCs. Strongly acidic mucosubstances in the cytoplasm were stained blue, while nuclei were counterstained pink to red by nuclear red stain. Alcian blue/nuclear red stained tissues were acquired and the number of Alcian blue positive GCs was determined in 10 intestinal villi and corresponding intestinal crypts in each sample. All histochemically stained tissue sections were cover-slipped with Pertex (Histolab Products AB; Göteborg, Sweden).

All measurements were performed in order to ensure objectivity in blind conditions, by two observers (unaware of the experimental groups) for all experimental groups and methods, carrying out the measures of control and experimental samples of each segment of the gut under the same conditions. For the quantitative analyses of PAS positive and Alcian Blue positive GCs, we used five sections from both gut segments in all animals' groups. All measurements were done using magnification 200x. For quantitative and qualitative analyses of histochemical and histological methods for detection of mucin in GCs, light microscope OLYMPUS BX50 with a digital camera OLYMPUS SP350 (Olympus; Tokyo, Japan) and Quick PHOTO Industrial 2.3 image analyser software (Promicra; Prague, Czech Republic) were used. The statistical analysis was performed in GraphPad InStat ver. 3.10 for Windows (GraphPad Software Inc., San Diego, CA, USA). Quantitative evaluation of studied markers is expressed as mean ± SEM (standard error of the mean). The significance of the differences between experimental groups was analysed using one-way analysis of variance ANOVA test followed by a Tukey-Kramer multiple comparison test. The value of $p<0.05$ was considered to be statistically significant.

RESULTS

Mucins possess potential binding sites for both commensal and pathogenic organisms and may perform a defensive role during establishment of the intestinal barrier. In this study, the effects on intestinal GCs mucin production in the gut of HC piglets and ECK piglets were examined. Numbers of GCs containing total acidic mucins in both, the jejunum and colon, differed significantly between HC and ECK piglets (Fig. 1).

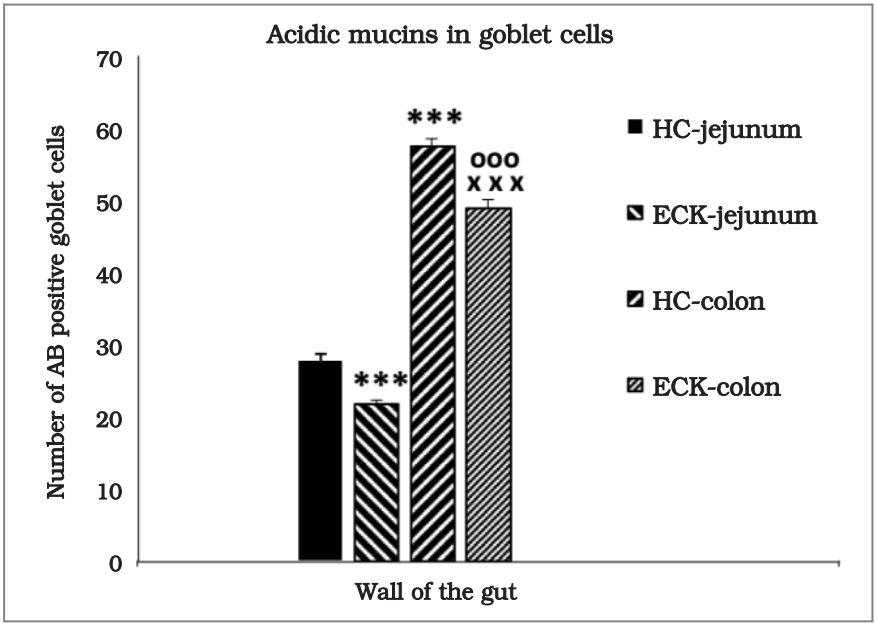

Fig. 1 Graph illustrating the average number of alcian blue (AB) positive GCs in gut for each condition. *Note that AB positive GCs were decreased in the jejunum of ECK piglets as well as in the colon of ECK piglets (***indicates the values differ significantly from the from the jejunum of HC piglets at p<0.001; [xxx] indicates the values differ significantly from the colon of HC piglets at p< 0.001). Greater numbers of AB positive GCs were observed in colon of both groups of piglets (***indicates the values differ significantly from the jejunum of HC piglets at p< 0.001; [ooo]indicates the values differ significantly from the jejunum ECK piglets at p< 0.001). Data are expressed as mean ± S.E.M., ANOVA and Tukey–Kramer tests were used.*

In the epithelium of jejunum of ECK piglets, the epithelial lining exhibited a marked decrease in number of GCs which produced acidic mucins (***indicates the values differ significantly from the jejunum of HC piglets at $p<0.001$) and they were positive for Alcian blue (AB) histochemically. In the colon, a similar trend was also observed, occurring ECK piglets ([xxx]indicates the values differ significantly from the colon of HC piglets at $p<0.001$). Overall, there were greater numbers of GCs containing acidic mucins in the colon compared with the jejunum in HC piglets (***indicates the values differ significantly from the jejunum of HC piglets at $p<0.001$) and also in the ECK piglets ([ooo]indicates the values differ significantly from the jejunum ECK piglets at $p<0.001$). Representative microphotographs show distribution of AB positive GCs in jejunum (Fig. 2. A_1, A_2) and colon (Fig. 2. B_1, B_2) of both group of piglets.

Number of PAS positive GCs (detection of neutral mucins in goblet cells) in the HC piglets was significantly different in comparison to the number of PAS positive cells in the ECK piglets (Fig. 3).

In the ECK piglets, jejunal GCs exhibited decrease in neutral mucins (**indicates the values differ significantly from the jejunum HC piglets at $p<0.01$). Further analysis of numbers of PAS positive GCs showed a marked increase in neutral mucins in the epithelium of colon in the HC piglets (***indicates the values differ significantly from the jejunum HC piglets at $p<0.001$) and also in the ECK piglets ([ooo]indicates the values differ significantly from the jejunum ECK piglets at $p<0.001$) in comparison to jejunum of both group of piglets. Significant increase of PAS positive GCs was observed between colon of HC and ECK group of piglets ([xxx]indicates the values differ significantly from the colon of HC piglets at $p<0.001$). Representative microphotographs show distribution of PAS positive GCs in jejunum (Fig. 4. A_1, A_2) and colon (Fig. 4. B_1, B_2) in both group of piglets.

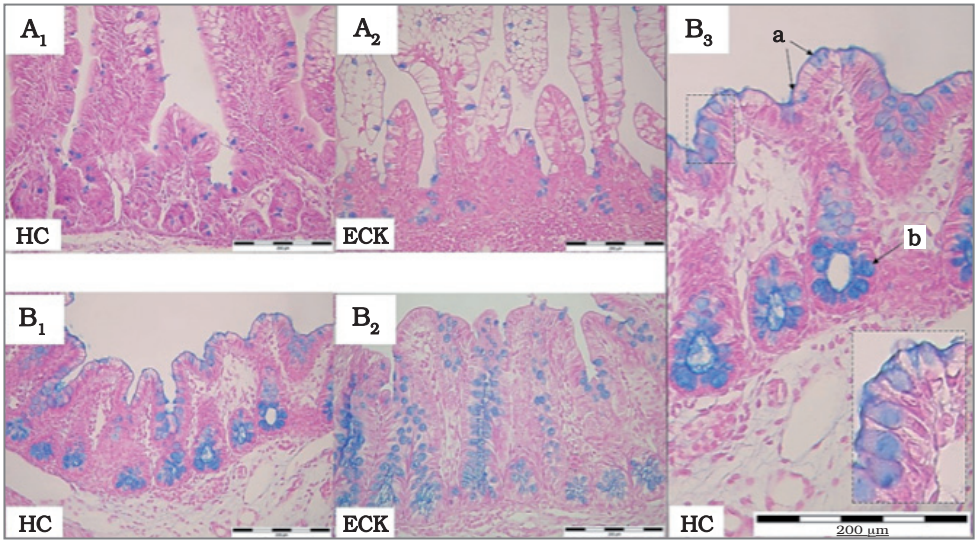

Fig. 2 Representative microphotographs of histochemical analysis of GCs producing acidic mucins by Alcian blue staining in the gut for each condition. *(HC – healthy control A_1 –jejunum and B_1 - colon; ECK – infected experimental group, A_2 – jejunum and $B_{2,3}$ – colon, a – positive thin mucus layer, b – strongly positive cytoplasm of GCs). Scale bar = 200 µm.*

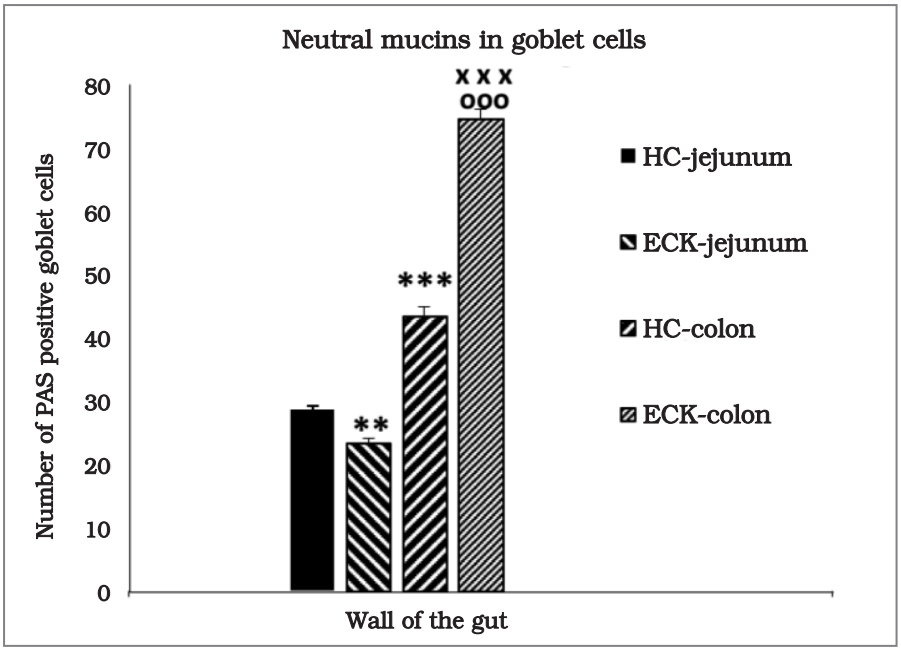

Fig. 3 Graph illustrating the average number of PAS positive GCs in gut for each condition. *Note that PAS positive GCs were decreased in the jejunum of ECK piglets (**indicates the values differ significantly from the from the jejunum of HC piglets at p<0.01). Greater numbers of PAS positive GCs were observed in colon of both groups of piglets (***indicates the values differ significantly from the jejunum of HC piglets at p< 0.001; ^{ooo}indicates the values differ significantly from the jejunum ECK piglets at p<0.001). Significant increase of PAS positive GCs was detected also in colon of ECK piglets in comparison to HC piglets (^{xxx}indicates the values differ significantly from the colon of HC piglets at p<0.001). Data are expressed as mean ± S.E.M., ANOVA and Tukey–Kramer tests were used.*

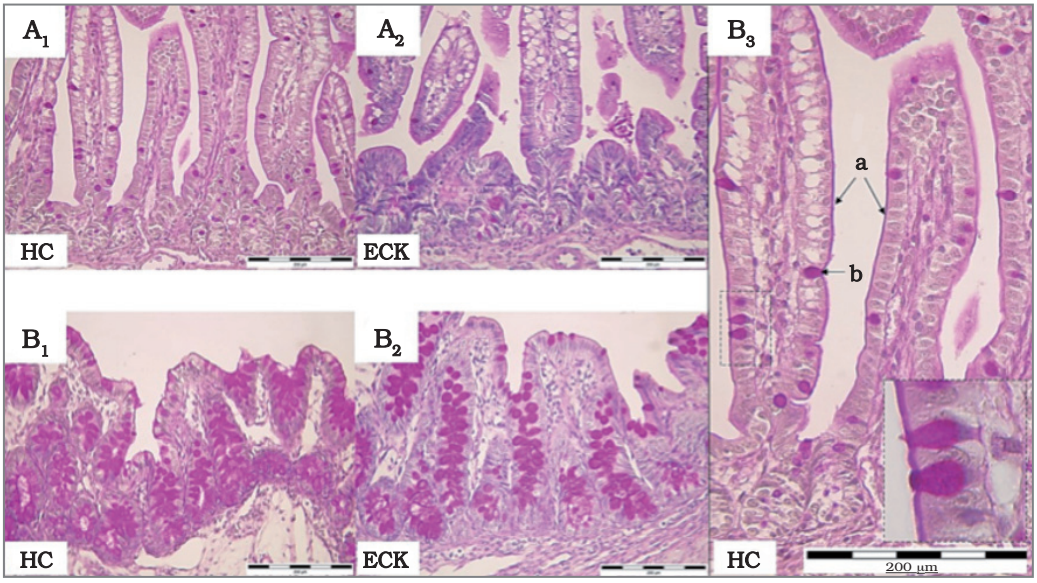

Fig. 4 Representative microphotographs of histochemical analysis of GCs producing neutral mucins by PAS-reaction in the gut for each condition. *(HC – healthy control, A_1 – jejunum and B_1 – colon; ECK – infected experimental group, $A_{2,3}$ – jejunum and B_2 – colon a – positive glycocalyx, b – strongly PAS positive cytoplasm of GCs). Scale bar = 200 µm.*

DISCUSSION

Enteric infections with pathogenic bacteria play an important role in animal health with the initiation and perpetuation of diseases such as diarrheal disease caused by enterotoxigenic *Escherichia coli* in new born pigs, calves, and lambs (11) and are responsible for reducing growth rates and consequent economic losses in animal production (5, 11).

Goblet cells are important cells of intestinal epithelial lining because they play important role in synthesis and secretion of mucus. Its major function is to protect the intestinal epithelium from damage caused by food and digestive secretions. The overlying mucus gel layer is the first line of defence that foreign bacteria and other pathogens encounter when they attempt to traverse the intestinal mucosa (12). However, simultaneously, mucin provides a desirable environment for proliferation of specific microflora due to its high carbohydrate content (13). Thus, the true chemical composition of mucus is essential for establishment of the intestinal barrier. Mucins can be classified into two broad categories: neutral and acidic depending on sugar type in the chains. These terms are derived from the chemical nature of the oligosaccharide sugar moieties (14).

Currently, small number of information is available describing the effects of bacterial colonization on the secretory histochemical pattern of intestinal mucins in piglets. Reference to numbers of GCs containing acidic mucins compared with neutral mucins in conventionally reared poultry has been reported (15). However, it has not been described in piglets from GF environment. Thus, the aim of the current study was to investigate the effects of bacterial colonization on mucin production in jejunal and colon´s GCs of piglets.

In the current study, we found that piglets infected with *E. coli* (ECK piglets) showed a significant increase in the production of neutral mucins by GCs in the colon compared to HC piglets, whereas we obtained the opposite results in the production of acidic mucins

by colon GCs in the ECK piglets compared to HC. Mucus gel is in a dynamic balance between mucin synthesis and secretion from GCs and erosion on the luminal surface. An increase in GCs number in the epithelial lining of villi and crypts in the gut may potentially increase the mucin secretion capacity of the mucosa and, consequently, improve gut health. Goblet cells are the most abundant secretory epithelial cells in the gastrointestinal (GI) tract. Their primary function involves the secretion of mucins that self-assemble into a protective mucus layer, coating the apical surface of epithelial cells. This mucus layer not only limits commensal microbe contact with the epithelium but also reduces mechanical stress on the epithelium through lubrication of the luminal bolus comprised of food contents (16). The change in mucin profile in response to bacterial colonization suggests a potential role as a protective mechanism against pathogenic invasion of the intestinal mucosa during early development. The goblet cells play role as key regulators of intestinal homeostasis at the host-microbe interface within the GI tract (17). Once released into the lumen, these mucins expand to form a dense, carbohydrate-rich matrix, assembling into homo-oligomers that give mucus its viscous properties. The mucus barrier is dynamically regulated, not only by continuous low-level mucin release by GCs but also by its continuous degradation via the mucinolytic actions of commensal, as well as pathogenic, bacteria. The oligosaccharide content in the mucus layer can influence the microbiota of the GI tract by the enhancement or inhibition of adherence of specific bacteria (18, 19, 20). *E. coli* uses fimbrial adhesion factors to bind to the mucus layer and colonize the intestine. *E. coli* fimbriae has affinity for sialic acid residues on mucin glycopeptides and glycolipids found in the pig small intestine (21). Our results support the idea that GCs play key role in host defence by responding to their luminal environment through the composition of their secretory granule (the production ratio of neutral to acidic mucins) during exocytosis. We supposed that decrease of AB positive GCs (secretory granules rich in acidic mucins) may lead to prepare small number of opportunities to colonise the gut with *E. coli* by fimbriae, which has high affinity for sialic acid residues.

This change in mucin profile in response to bacterial colonization suggests a potential role as a protective mechanism against pathogenic invasion of the intestinal mucosa during of gut mucosa development in piglets.

CONCLUSION

It can be concluded that colonization by *E. coli* generates morphological alterations reflected in changes of GCs population and distribution in the intestine. The results of current study help to understand the role of mucins in maintenance of intestinal integrity during early life period of piglets. These findings underline the need of further studies to understand the mechanisms involved in the regulation of intestinal mucin secretion. The mucus-pathogen interactions are complex and depend on microbe and host species; therefore, in order to achieve such high advancements of understanding, more knowledge is required in this topic. It warrants further investigation of gut development of immunity and its interactions with mucin dynamics and bacterial colonization.

Therefore, using of piglets from GF environment as animal models may contributed to the acquisition of new knowledge to improve both animal and human health.

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ADAPTIVE MORPHOFUNCTIONAL REARRANGEMENTS IN THE ADULT RATS ADENOHYPOPHYSIS AFTER LONG-TERM EXPOSURE OF HEAVY METAL SALTS

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Abstract

Important environmental problem of some northern regions of Ukraine is the accumulation of heavy metal salts in the soil, water and air, which is observed in various combinations depending on the region and causes adverse effects on population's health. The central link, in particular the pituitary gland, is involved in triggering a stress response, limiting its further development preventing adverse effects on the body. The study of morphofunctional rearrangements in the pituitary gland of adult male rats under the influence of heavy metal salts complex on the body remains a relevant aspect of modern morphology. The experiment was performed on 12 white mature male rats weighing 200-250g at the age of 7-8 months, which were divided into 2 groups (control and experimental). The experimental group included rats, which after 90 days of use of a heavy metals salts complex (zinc, copper, iron, manganese, lead and chromium) for 30 days used ordinary drinking water. General morphological (histological, morphometric), mmunohistochemical, biochemical and statistical research methods were used. Results showed a decrease in the linear parameters of the adenohypophysis, there was a significant increase in the value of fibrous connective tissue component in the stroma, increased collagenization of large vessels, significant diffuse stromal edema and edematous processes in the gland parenchyma persisted. Measurements revealed an increase in the number of chromophobes and at the same time a decrease in the number of chromophilic basophils compared to control animals, the presence of cysts in the parenchyma was found out. number of morphological features still indicated the development of adaptive and compensatory processes in the adenohypophysis (vascular plethora decreased, significantly improved the rheological properties of blood, an increase in the number of adenocytes with increasing expression of hsp90 in their cytoplasm). However, despite the positive dynamics of adaptive processes, it should be noted that the 30-day period of adaptation is insufficient for complete recovery of the organ.

Keywords: pituitary gland, heavy metals, hypoxia

INTRODUCTION

Environmental contamination with some metalloids and heavy metals raises concern due to well known adverse effects on health (1). There is growing interest in the possible health threat posed by endocrine-disrupting chemicals (EDCs), which are substances in our environment, food, and consumer products that interfere with hormone biosynthesis, metabo-

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lism, or action resulting in a deviation from normal homeostatic control or reproduction. Endocrine disruptors have effects on neuroendocrinology (2). They mimic natural hormones, inhibit the action of hormones, or alter the normal regulatory function of the endocrine system (3).

Anterior pituitary hormones are central for the body homeostasis (4), are involved in triggering a stress response, limiting its further development preventing adverse effects on the body. In the modern scientific literature there is evidence of the effects on the pituitary gland of certain heavy metals salts. The effect of cadmium Cd, chromium Cr VI and arsenic As through drinking water on the hypothalamic-pituitary system of rats was studied.

Cabilla et al. in their study found out that heavy metals accumulated in hypothalamus and pituitary gland and decreased pituitary cell viability and prolactin release mostly by generation of reactive oxygen species, since it was partially prevented by antioxidant treatment. In the pituitary, they increased lipid peroxydation and the expression of several oxidative stress markers. Cell death was mainly due to caspase-dependent apoptosis. Cd stimulated the production of low levels of nitric oxide which exerted cytoprotective actions. These results showed that these heavy metals display deleterious actions in hypothalamic-pituitary physiology by altering hormone release and promoting cell death (1). he anterior pituitary gland can be a target of Cr VI toxicity in vivo and in vitro, thus producing a negative impact on the hypothalamic-pituitary-gonadal axis and affecting the normal endocrine function (4). ther US data showed a positive association between lead and testosterone levels in males, and cadmium and FSH levels in perimenopausal women (5,6). In China, a recent study found positive associations between lead and testosterone levels in men and between lead and FSH and LH levels in postmenopausal women (7). Lead exposure can affect the FSH level in postmenopausal women. Further studies are needed to evaluate the effects of low-dose long-term exposure to heavy metals on sex hormones (8).

Today, an important environmental problem of some northern regions of Ukraine is the accumulation of heavy metal salts (zinc, chromium, lead, manganese, copper and iron) in the soil, water and air, which is observed in various combinations depending on the region and causes adverse effects on population's health (9).

The purpose of the study was to elucidate the morphofunctional rearrangements the structural component changes in the pituitary gland of mature rats after the long-term influence of heavy metal salts complex (zinc, copper, iron, manganese, lead and chromium) on the body.

MATERIALS AND METHODS

The experiment was performed on 12 white mature male rats weighing 200–250 g at the age of 7-8 months, which were divided into 2 groups (control and experimental). The control group included rats that were kept in vivarium conditions similar to those of experimental animals. At the same time, a constant temperature regime was observed, as well as a natural day/night regime. As food, the control animals received granular mixed fodder, drinking – ordinary drinking water. The study was conducted in the autumn-winter period. Animals were kept and manipulated in accordance with national and international bioethics standards. The experimental group included rats, which after 90 days of use of heavy metals salts complex: zinc (ZnSO4 7H2O) – 5 mg / l, copper (CuSO4 5H2O) – 1 mg / l, iron (FeSO4) - 10 mg / l, manganese (MnSO4 5H2O) – 0.1 mg / l, lead (Pb (NO3) 2) – 0.1 mg / l and chromium (K2Cr2O7) – 0.1 mg / l, for 30 days used ordinary drinking water.

Groups of experimental animals were removed from the experiment after previous thiopental anesthesia (at the rate of 30–40 mg / 10 g of body weight) on the 120th day of the experiment (Protocol No. 8 of 17/11/2020 of the Bioethics Commission of Sumy State University). The subject of the study was the pituitary gland of experimental and control animals. For morphological studies of the pituitary gland, the organ was removed, histological preparations were made, stained with hematoxylin-eosin and Mason-Goldner were stained according to the original method (10). The absolute number of different types of adenocytes was counted in a grid by experimenters in various random fields of the adenohypophysis view (at least 5 fields from each control and experimental animal).

To calculate the level of expression of receptors for antibodies, used a semi-quantitative method. Determination of the expression of the heat shock protein marker 90 (Hsp90) was performed using an antibody panel "Thermo scientific", USA: rabbit polyclonal antibodie to the Hsp90 protein with a titer of 1:200 according to the manufacturer's recommendations. Evaluation of Hsp90 marker expression was performed by the number of stained nuclei and cytoplasm of gland cells. The result was expressed as a percentage and evaluated on a scale in the case of a positive reaction: low positive (1 point), moderately positive (2 points) and strongly positive (3 points) reaction, taking into account the number of cells and the intensity of their color. The number of HSP90-positive cells was counted in a grid by experimenters in various random fields of view of the adenohypophysis (at least 10 fields from each control and experimental animal).

The functional state of the pituitary was evaluated by determining the adrenocorticotropic hormone ACTH (pg / ml) in the serum of peripheral blood of experimental animals (by the ELISA method). A set of reagents from the Siemens was used on the Immulite 1000 Siemens Healtheare Global Immune Chemiluminescent Analyzer. General morphological analysis was performed using a light optical microscope Zeiss Primo Star with lenses x4, x10, x40, glasses 7 and 10. For the morphometric study of micropreparations, the “SCPR-2017-Zen 2 lite” software was used with photo-documentation of the results by means of “axiocam ERC 5S Zeiss” digital camcorder. Statistical processing of the obtained data was performed by parametric method of variation statistic using the software package STATISTIKA v. 10 (Stat Soft Inc., USA). Data are presented as the mean (X) ± standard deviation (SD), using the Student's t test. The error probability of less than 5% ($p \leq 0.05$) was considered sufficient.

RESULTS

Thirty days of adaptation to long-term exposure to heavy metals salts complex did not cause significant positive changes in most structural components of the experimental animals. The length and width of the pituitary gland decreased by 16.2% ($P > 0.05$) and 15% ($P < 0.05$, $t = 2.28$), respectively, compared with control animals. The thickness of the pituitary capsule significantly decreased compared to control animals by 59.6% ($P < 0.001$, $t = 6.36$), but significantly increased the value of the fibrous component of connective tissue in the stroma of the gland (Table 1).

Significant diffuse stroma edema (Mason-Goldner trichrome staining) and edematous processes in the gland parenchyma continued. The intertrabecular spaces were sharply expanded. Venous plethora was preserved, and adventitia of large vessels underwent collagenization processes (Fig. 1). At the same time, the state of rheological properties of blood has significantly improved. Blood coagulation processes have practically disappeared. Capillaries remained significantly full-blooded, in the peripheral areas of the gland they were still visualized violations of the rheological properties of blood and formation of "coin

Table 1 Indicators of experimental and control rats adenohypophysis after adaptation to long-term exposure to heavy metal salts complex (X ± SD).

Indicator	Series of animals	
	Control rats (n=6)	Experimental rats (n=6)
Pituitary length, mm	7.92±0.83	6.64±0.59
Pituitary width, mm	4.26±0.19	3.63±0.2*
Capsule thickness, μm	3.52±0.27	1.42±0.19***
Vessels area, μm²	215.83±5.96	224.3±7.96
Chromophobic cells	57.46 ± 2.58	65.15 ± 0.43 *
Chromophilic acidophiles	31.28 ± 1.71	30.2 ± 0.59
Chromophilic basophils	11.26 ± 0.06	4.65 ± 0.18 ***
ACTH (pg / ml)	591.0±1.83	319.5 ± 3.17***

Note: * p≤0.05, *** p≤0.001

columns". Vessels with local disorders (increased permeability) of the vascular wall and infiltration of a small number of erythrocytes into the extravascular space were found in some areas, with the formation of single small hemorrhages. At the same time, perivascular and pericellular edema are absent. There was an increase in the area of vascularization of the adenohypophysis compared with animals in the 90-day period of the experiment.

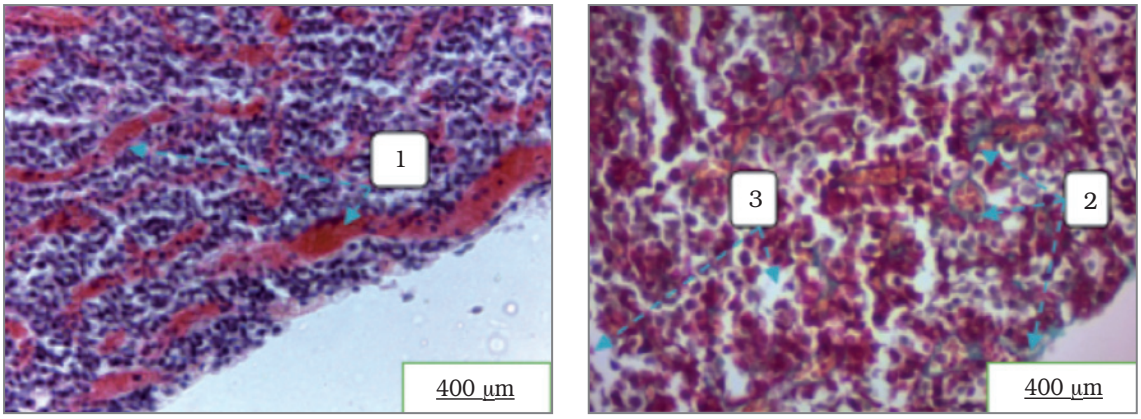

Fig. 1. Morphological rearrangements in the experimental rat pituitary gland under the condition of 30-days adaptation to heavy metals salts: 1 – venous pleural effusion; 2 – edema tissue trabeculae; 3-collagenization of the vessel walls. Staining: A – by hematoxylin-eosin; B – by Mason-Goldner.

As in the previous period of the experiment, significant edema of connective tissue trabeculae and vascular plethora led to further discomplexation of epithelial trabeculae, violation of their histoarchitectonics. Endotheliocytes continued to show signs of significant edema, their hyperchromic and hypertrophied nuclei protruding sharply into the lumen of full-blooded vessels. The area of the lumen of the vessels increased by 3.9% (P> 0.05) relative to the control animals, which can be considered a compensatory phenomenon (Table 1). In our opinion, the most obvious feature of this term of the experiment is cyst formation. Subcapsular and peripheral areas of the gland had a moderate number of cysts of various sizes (from small multiple to single large and very large). The shape of the cysts varied from round to oval. The wall of large cysts consisted of flat adenocyte cells and was filled with light oxyphilic content (Fig. 2).

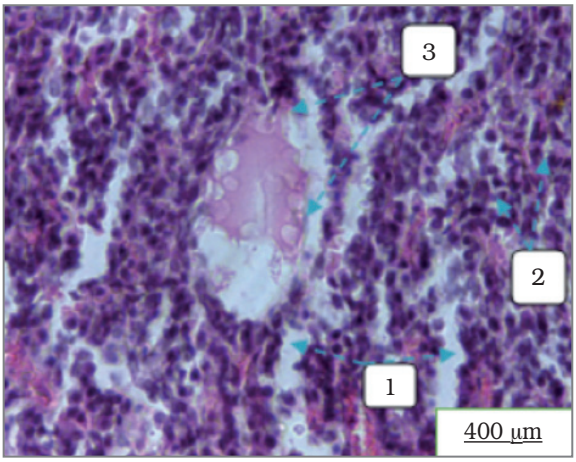

Fig. 2. Morphological rearrangements in the experimental rats pituitary gland under the condition of 30-days adaptation to heavy metals salts: 1 – edema of connective tissue trabeculae; 2 – discomplexation of cellular trabeculae; 3 – cyst. Staining: by hematoxylin-eosin.

Despite the 30-day recovery period after exposure to heavy metal salts, no significant recovery of adenohypophysis cell composition was observed in experimental rats. Thus, in the epithelial trabeculae of experimental animals there was an increase in the number of chromophobes and at the same time a decrease in the number of chromophilic basophils compared with control animals. However, groups of basophilic adenocytes were found in some isolated areas of the adenohypophysis (Fig. 3).

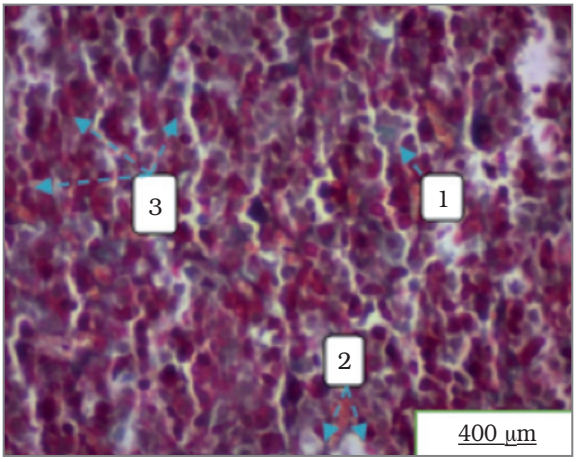

Fig. 3. Cellular composition of the experimental rats adenohypophysis after the condition of 30-day readaptation to heavy metal salts: 1 – chromophilic basophils; 2 – chromophobes; 3 – chromophilic acidophiles. Staining: by Mason Goldner.

The number of basophils decreased by 58.7% (P <0.001, t = 34.84), the number of acidophiles was lower by 3.4% (P> 0.05) compared with control animals. The number of chromophobes in males was higher by 14.4% (P <0,05, t = 2,94) compared with control animals (Table 1).

Thirty days after the end of exposure to heavy metal salts, morphologically, 2 types of cells were detected in the parenchyma. In the peripheral area, subcapsular groups of adenocytes with signs of vacuolation of the cytoplasm, balloon dystrophy. The nuclei of such cells were often enlightened and with chromatin margination. Often such groups of cells were located together with cysts. However, the vast majority of parenchymal cells had hypochromic cytoplasm, homogeneous hyperchromic elongated nuclei, with increasing amounts of heterochromatin in them. The nucleoli in the nuclei of cells of both types were not contoured. A small percentage of cells showed signs of apoptosis.

Adaptive changes in the adenohypophysis were characterized by an increase in the level of Hsp90 expression in the cytoplasm of cells to moderate (2 points) and strongly positive (3 points) levels. Hsp90-positive cells were diffusely located in the parenchyma, had a high level of staining (++ and +++). The number of low positive 1 adenocytes decreased by 69.05% (P <0.001, t = 431.562) in the adenohypophysis of experimental animals compared to control animals. The number of adenocytes moderately positive 2 HSP90 increased by 34% (P <0.001, t = 7.21), in the adenohypophysis of experimental rats compared to control animals. The number of strong positive 3 HSP90 adenocytes increased by 57.14%(P <0.001, t = 35.49) in the adenohypophysis of experimental rats compared to control animals (Tabl.2).

Table 2 Expression level of HSP90 in adenohypophysis adenocytes of control and experimental rats after adaptation to long-term exposure to heavy metal salts complex (X ± SD).

HSP90 expression	Control group total number 6 animals	Experimental group total number 6 animals
Low positive 1	69.05±0.16	–
Moderately positive 2	31.91±0.82	42.75±1.26***
Strong positive 3	–	57.14±1.61***

Note: * p≤0.05, *** p≤0.001

Particularly high levels of expression to Hsp90 were found in the cytoplasm of cells located perivascularly (Fig. 4).

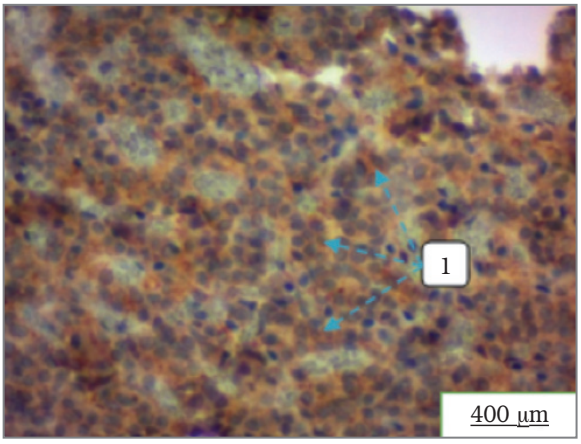

Fig.4. Expression of hsp90 in the cytoplasm of experimental animals adenocytes under the condition of 30-day readaptation to 90-day exposure to heavy metal salts: 1 – high level of hsp90 expression in the adenocytes cytoplasm. Immunohistochemical study of hsp90 expression.

The level of ACTH in the serum of experimental animals decreased by 46% (t = 74,17424, p <0,001) relative to control animals (Table 1).

DISCUSSION

Thirty days of readaptation to long-term exposure to heavy metals salts did not cause significant positive changes in most structural components of the experimental animal adenohypophysis. There were signs of weakening of the compensatory adaptation processes functional activity in the body, as evidenced by mutually conditioned morphological changes of the stromal and parenchymal components. At these period of adaptation to the exposure of heavy metals salts we revealed a decrease in the linear parameters of the adenohypophysis, but there was a significant increase in the value of fibrous connective tissue component in the stroma, increased collagenization of large vessels. According to the authors, this indicated the development of alternative changes due to organ hypoxia. Hypoxic phenomena accompanying vascular disorders caused an increase in the proliferative activity of stromal fibroblasts and their production of fibrous connective tissue component (11,12).

As in the previous period of the experiment (90th day of the experiment), significant diffuse stromal edema and edematous processes in the gland parenchyma persisted, which undoubtedly negatively affected the trophism and the course of regenerative processes in the adenohypophysis and histoarchitectonics of the gland. Morphological signs of adenocytes indicated slow recovery processes in their structure: the cytoplasm of some adenocytes had signs of vacuolation, balloon dystrophy, which indicated their vacuolar degeneration. There was no significant improvement in the functional activity of adenocytes: cells with enlightened nuclei, chromatin margination, nucleoli were absent and a small number of cells with signs of apoptosis. ACTH levels decreased compared to control animals.

Despite the thirty days adaptation period after exposure to heavy metal salts, no significant recovery in the cellular composition of the experimental rats adenohypophysis was observed. An increased number of chromophobes was accompanied by the decreased number of chromophilic basophils compared to control animals. Phenomenon of a decrease in the number of chromophilic cells can be explained by the occurrence of an "emergency" in the body due to the increased level of tropical hormones secretion by the body in the previous terms of the experiment. Conclusion correlates with the opinion of a number of authors (13,14).

However, groups of basophilic adenocytes were found in some isolated areas of the adenohypophysis. This can be explained by the mosaic of morphological changes of the gland – the alternation of dystrophic, necrotic areas of the parenchyma with intact, which can be explained by the manifestations of the law of intermittent activity of functioning structures (13). It can be assumed that the complex of salts of heavy metals negatively affected the morphofunctional parameters of the adenohypophysis, in particular by activating the processes of lipid peroxidation and oxidative stress. This, in turn, led to a change in hormone release and contributed to cell death by caspase-dependent apoptosis, which correlates with the opinion of some authors. (1, 4).

One of the clear morphological features of this term of adaptation is the presence of cysts in the parenchyma, the formation of which can be explained by delayed excretion of hormones from the adenohypophysis due to disruption of transmembrane transport and insufficient time of adaptation.

However, a number of morphological features still indicated the development of adaptive and compensatory processes in the adenohypophysis aimed at leveling the stress response and a number of hypoxic phenomena caused by the previous exposure to heavy metal salts (15). Thus, vascular plethora decreased, morphometric indicators of vascular area approached those of control animals. There was an increase in the area of vascularization of the

adenohypophysis and significantly improved the rheological properties of blood. However, the condition of the vascular wall in terms of its permeability and the condition of endothelial cells improved only partially. The rather bright adaptive rearrangements in the adenohypophysis of experimental animals include an increase in the number of adenocytes with increasing expression of hsp90 in their cytoplasm. This fact can be considered one of the mechanisms of cellular and organ defense. According to the literature, the production of heat shock protein (HSP) cells make these cells more resistant to further extreme conditions, developing resistance to further stress (16). After all, the renaturation of proteins damaged during stress is an integral part of stress resistance. Hsp90 has been shown to play an important role in protein quality control by directing damaged proteins to 26S proteosomes for degradation or to other chaperones (particularly Hsp70) for renaturation (16). Under stressful conditions, leading to the accumulation of proteins in the cells with disturbed conformation, Hsp90, it is believed, partially switch to their refolding (17). This fact indicates the participation of Hsp90 in the active processes of adenohypophysis cells adaptation to prolonged exposure to heavy metal salts.

CONCLUSIONS

Thus, a comprehensive study of the adenohypophysis structural components of experimental animals with thirty days of adaptation to long-term consumption of heavy metal salts indicate a number of adaptive and regenerative morphofunctional changes aimed at reducing the stress of adaptive processes on the part of the adenohypophysis. However, despite the positive dynamics of adaptive processes, it should be noted that the thirty days period of adaptation is insufficient for complete recovery of the organ.

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OMICRON SARS-COV-2 VARIANT: AN OBSERVATIONAL STUDY FROM A HOSPITAL IN SOUTHERN INDIA

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Abstract

Introduction: The Omicron variant rapidly outpaced Delta with documented community transmission in most countries and has led to an upsurge in cases in most regions. Since its initial detection from a specimen collected on November 8th 2021, Omicron amounted to 74.0% of the genome sequenced in South Africa and more than 99.0% in rest of the world.

Objectives: 1. To describe the socio-demographic and clinical profile of Omicron cases treated at our tertiary care institution. 2. To assess the factors associated with the vaccination status of such Omicron cases.

Methods: This observational study was conducted at a 500 bedded hospital in southern India from 15th of December 2021 to 5th of February 2022. Of the 333 COVID-19 patients who were registered with Reverse Transcription-Polymerase Chain Reaction (RT-PCR) positive result along with S Gene Target Failure (SGTF), 203 patients were included and were interviewed using a pre-designed semi-structured questionnaire. With prior approval from the Institutional Ethics Committee (IEC) data was collected and statistically analyzed with descriptive statistics and inferential statistics using SPSS software trial version 28.0 and OpenEpi software. At 95% confidence level, a P value of < 0.05 was considered as statistically significant.

Results: Of the 203 cases studied, majority 149 (73.4%) were symptomatic, of which almost 114 (76.5%) had fever, 72 (48.3%) had cough and 29 (19.5%) had myalgia. 193 (95.1%) cases were categorized as mild, 8 (3.9%) as moderate and 2 (1.0%) as severe cases of COVID-19 with SGTF. Only 10 (4.9%) patients received supplementary oxygen support. Almost 158 patients (77.8%) were vaccinated against COVID-19 of which 106 (67%) were vaccinated with Covishield vaccine followed by 50 (31.7%) of them with Covaxin. 126 (79.7%) patients were completely vaccinated with two doses of any COVID-19 vaccine and 32 (20.3%) were partially vaccinated with a single dose of any COVID-19 vaccine. Among those who required supplemental oxygen ($n = 10$), the proportion of those vaccinated (40.0%) was lower compared to those who were unvaccinated (60.0%). This association was statistically significant ($P = 0.003$, OR = 0.169, 95% CI of OR = 0.045, 0.628). Among the completely vaccinated subjects ($n = 125$), there was a statistically significant difference in mean (95% CI) interval between the last dose of vaccine taken and date of RT-PCR positivity with SGTF ($P < 0.001$). It was 186 (162, 210) days for Covaxin and was 131 (114, 148) days for Covishield vaccine.

Conclusion: Omicron (SGTF) cases manifests mostly as mild cases with symptoms like fever, cough, myalgia and majority were independent of oxygen supplementation and had good prognosis. Omicron infection was delayed over six months among completely vaccinated subjects especially those who were vaccinated with Covaxin.

Key words: COVID-19, Omicron, SGTF, Vaccination, Covaxin, Covishield

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INTRODUCTION

The COVID-19 pandemic has entered its third year of occurrence. COVID-19 was first reported in Wuhan, China in December 2019 and then on has marched relentlessly throughout the globe assuming many forms and names. It has so far affected 332 million people as on 19th January 2022 and has led to 5.5 million deaths worldwide (1). India has faced two waves of COVID-19 pandemic and witnessed the third wave from the last week of December 2021. In India 38.2 million people were affected due to COVID-19 and 0.49 million have died (2). The B.1.1.529 variant was first reported to World Health Organization (WHO) from South Africa on 24th November 2021. It was subsequently named as Omicron and was declared as a Variant of Concern (VoC) by WHO (3). This variant has a large number of mutations and the points of concern are increased risk of re-infection as compared to other VoCs, increased risk of transmissibility and a probable immune escape and questionable susceptibility to monoclonal antibody treatment (4).

Omicron has over 50 mutations including 32 on the spike protein. Since its initial detection from a specimen collected on November 8th 2021, Omicron replaced delta as the dominant variant amounting to 74% of the genome sequenced in South Africa and more than 99% in rest of the world (4). The SARS-CoV-2 Omicron variant harbors 37 Amino acid substitutes in the spike protein, 15 of which are in receptor binding domain. The gold standard for testing is the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) from the nasopharyngeal and oropharyngeal secretions collected with the swab. Ideally COVID-19 is diagnosed if 3 out of 4 genes namely the N gene, E gene, RdRp gene and the S gene are detected. However, the characteristic S Gene Target Failure (SGTF) due to deletion at Spike position 69 – 70 leading to failure of detection of S gene also called the S gene dropout is 98.9% sensitive and 99.9% specific in diagnosis of the Omicron variant and this is being used as a surrogate marker in the diagnosis of Omicron variant infection (5).

The Incidence of Omicron variant cases was confirmed in 149 countries by the WHO as of 6 January 2022. The global weekly incidence of COVID-19 had increased by 71.0% compared with the previous week. The Omicron variant rapidly outpaced Delta with documented community transmission in most countries and had led to an upsurge in cases in most regions. The WHO regions especially the Americas and the South-East Asia Region reported the highest increases of 100% and 78.0%, respectively (4).

India has two major vaccines which are approved and used in the COVID-19 Vaccination program namely the Covishield (ChAdOx1 nCov-19) and Covaxin (BBV152). The Multicentric Case Control study done in India indicated that the overall vaccine effectiveness was 80.0% with two doses of ChAdOx1 nCov-19/Covishield and 69.0% with BBV152/Covaxin against severe COVID-19. The vaccine effectiveness was highest for a 6 to 8 week interval between two doses of the both the vaccines. The study also indicated a substantial reduction in the risk of severe COVID-19, particularly against the Delta strain (6). Similarly, an interim analysis of a multicenter randomized control trial on ChAdOx1 nCoV-19 vaccine in four trials across three continents, showed a significant vaccine efficacy of 70.4% after two doses and protection of 64.1% after at least one standard dose, against symptomatic disease, with no safety concerns (7).

The immune protection offered by vaccines available at present against the Omicron variant is a point of concern. A research study in Denmark done in December 2021 showed majority 76% of the Omicron cases were fully vaccinated while 7.1% had their booster dose. 4.3% of the cases had a previous SARS-CoV-2 infection and almost 76% were symptomatic and community transmission was present (8). The reason for immune evasion, increased transmissibility and escape from neutralizing antibodies of already vaccinated individuals might be due to several mutations, specifically on the S-protein of the Omicron variant. Omicron variant also had a high reinfection capacity and may affect previously infected COVID-19 patients (9).

As of 23 January 2022, a total of 9.62 billion vaccine doses were administered worldwide with 52.5% of global population being completely vaccinated as per WHO (10). As on 25th January 2022, India's Cumulative COVID-19 Vaccination Coverage exceeded 1.63 billion doses with 67.4% of population being vaccinated with at least one dose of vaccine and 49.6% being completely vaccinated and 0.6% has received its precautionary dose (11). In spite of a robust COVID-19 vaccination programme being carried out in India since January 2021, Omicron cases surged in early part of January 2022. Not many studies have been carried out in this part of southern India related to clinic-epidemiological profile of omicron variant and also related to the vaccination status of such omicron cases. Hence the present study was conducted with the following objectives:

1. To describe the socio-demographic and clinical profile of Omicron (SGTF) cases treated at our tertiary care institution.
2. To assess the factors associated with the vaccination status of such Omicron (SGTF) cases.

METHODS

This prospective observational study was conducted in the southern region of India at a hospital in Chennai city which is a 500 bedded public health facility which mainly caters to the healthcare needs of people around the city as well as to the people within the state of Tamil Nadu. This study was conducted for a period of two months from 15th of December 2021 to 5th of February 2022. All the omicron suspect cases with SGTF registered at the COVID-19 clinic of the Hospital who consented were included as the study subjects. Patients with hearing, speech and metal impairment and those outpatients on home isolation who could not be contacted through telephone were excluded. There were 333 patients who were registered at the outpatient COVID-19 clinic of the hospital who were tested positive for COVID-19 by RT-PCR along with SGTF (using TaqPath™ COVID-19 RT-PCR Kit (Thermo Fisher Scientific, California, USA)) were considered as omicron suspect cases.

Response rate of the study participants was 60.96%. Of the 333 patients who were registered, 203 patients who consented for the study were included as per the inclusion criteria and were telephonically interviewed. For patients under supplemental oxygen support, socio-demographic details were collected from their family members and clinical data from their medical records respectively. Inclusion criteria was based on the Clinical and laboratorial parameters of COVID-19 as per the Indian Council for Medical Research (ICMR) guidelines. Patients were categorized into mild, moderate and severe cases accordingly. Mild cases were those COVID-19 cases who presented with upper respiratory tract symptoms and or fever without hypoxia while moderate cases defined as those COVID-19 cases with breathlessness i.e, a respiratory rate of ≥ 24/ minute and/or SpO2 of 90% to ≤ 93% on room air and severe cases were defined as those COVID-19 cases with breathlessness i.e, a respiratory rate of > 30/ minute and/or SpO2 of < 90% on room air. Accordingly, mild cases were sent for home isolation or those eligible were advised observation at a COVID-19 care center. Moderate and severe cases of COVID-19 were hospitalized and managed as per established protocols of the hospital in inpatient ward and intensive care unit respectively (12). Data was collected in a pre-designed semi-structured questionnaire and entered through google forms and recorded in google spreadsheet. Data on socio-demographic details, symptoms, vaccination status, travel history, past history of COVID-19 infection, co-morbidities, treatment details, duration of hospital stay and prognosis were collected by interview method as well from scrutiny of their medical records. Vital parameters including pulse rate, respiratory rate, blood pressure, temperature and oxygen saturation (SpO2) in blood and a baseline complete blood count were recorded. Data was collected after obtaining the informed consent and on prior approval from the Institutional Ethics Committee (IEC). Data was entered in google spreadsheet was statistically analyzed with descriptive statistics like mean, median, standard deviation, range, inter-quartile range and inferential statistics like

Mann Whitney U test, Chi-square test using SPSS software trial version 28.0 and OpenEpi software. At 95% confidence level, a P value of ≤ 0.05 was considered as statistically significant.

RESULTS

Table 1 Distribution of study subjects based on Socio-demographic characteristics

Characteristic (N = 203)	Frequency (%)
Age Group	
20 years and below	19 (9.4)
21 – 40 years	79 (38.9)
41 – 60 years	78 (38.4)
61 – 80 years	24 (11.8)
Above 80 years	3 (1.5)
Sex	
Male	129 (63.5)
Female	74 (36.5)
Comorbid status	
Comorbid	39 (19.2)
Non-comorbid	164 (80.8)
Travel History	
Present	29 (14.3)
Absent	174 (85.7)
Past History of COVID-19 infection	
Present	16 (7.9)
Absent	173 (85.2)
Not sure	14 (6.9)
Duration between past COVID-19 infection and current episode (n = 16)	
5 months and below	3 (18.8)
6 – 10 months	10 (62.5)
11 – 15 months	2 (12.5)
16 – 20 months	1(6.2)

(Note: figures in parentheses denotes percentages)

Age of the 203 study subjects ranged between 7 and 86 years with a mean age (95% CI) of 42 years (40, 45 years). Table 1 shows majority 79 (38.9%) and 78 (38.4%) of them were in the age group of 21 to 40 years and 41 to 60 years. Of the total 203 subjects with RT-PCR positivity with SGTF studied, 129 (63.5%) were males and 74 (36.5%) were females. 39 (19.2%) patients had comorbidities like Type II Diabetes Mellitus, Hypertension etc. 29 (14.3%) subjects had travelled to various national and international regions 14 days prior to their diagnosis of COVID-19 infection. Among the study subjects, 16 (7.9%) patients had past history of COVID-19 infection while 14 (6.9%) of them were not sure about it. The duration of past COVID-19 infection and the present infection ranged between 1 month to 18 months with a mean duration (95% CI) of 8 months (6, 10 months).

Table 2 Distribution of study subjects based on symptoms and treatment details

Characteristic	Frequency (%)
Symptom status (N = 203)	
Symptomatic	149 (73.4)
Asymptomatic	54 (26.6)
Type of Symptoms (n = 149)	
Fever	114 (76.5)
Cough	72 (48.3)
Myalgia	29 (19.5)
Running nose	28 (18.8)
Sore throat	26 (17.4)
Fatigue	20 (13.4)
Headache	20 (13.4)
Breathlessness	12 (8.0)
Sneezing	10 (6.7)
Chills	7 (4.7)
Diarrhoea	7 (4.7)
Course of Treatment	
Outpatient	114 (56.1)
In-patient	89 (43.8)
Case severity (as per ICMR)	
Mild case	193 (95.1)
Moderate case	8 (3.9)
Severe case	2 (1.0)
Supplemental Oxygen requirement	
Oxygen support required	10 (4.9)
Oxygen support not required	193 (95.1)
Mode of Oxygen delivery (n = 10)	
Simple face mask	8 (80.0)
CPAP	2 (20.0)
Outcome	
Treated in Home isolation	134 (66.0)
Discharged from IP ward	60 (29.6)
Referred to other Hospital	7 (3.4)
Died	2 (1.0)

(Note: figures in parentheses denotes percentages)

Of the 203 cases studied, majority 149 (73.4%) were symptomatic. As shown in table 2, among the 149 symptomatic cases, almost 114 (76.5%) had fever, 72 (48.3%) had cough and 29 (19.5%) had myalgia. Among the 203 subjects studied, 193 (95.1%) cases were categorized as mild, 8 (3.9%) as moderate and 2 (1.0%) as severe cases of COVID-19 with SGTF. Majority 114 (56.1%) were initiated treatment at the outpatient clinic and were moni-

tored under home isolation through teleconsultation services while 89 (43.8%) cases were admitted in In-patient ward and treated. Most of the patients were independent of supplementary oxygen support, while only 10 (4.9%) patients received supplementary oxygen support of which 8 (80%) received the support through simple face mask and 2 (20.0%) received through Continuous Positive Airway Pressure (CPAP). Of the 203 study subjects, majority 134 (66.0%) were monitored under home isolation following their treatment at outpatient clinic or in-patient ward, 60 (29.6%) were discharged following their treatment completion at in-patient ward, 7(3.4%) were referred to higher centers for further management and 2 (1.0%) had died.

Table 3 Distribution of Study subjects based on treatment details

Treatment details	Frequency (%)
Vaccination Status (N = 203)	
Vaccinated	158 (77.8)
Unvaccinated	45 (22.2)
Vaccine Type (n = 158)	
Covaxin	50 (31.7)
Covishield	106 (67.0)
Other vaccines	2 (1.3)
Dose of Vaccination (n = 158)	
One Dose (Partially vaccinated)	32 (20.3)
Two Doses (Completely vaccinated)	126 (79.7)

(Note: figures in parentheses denotes percentages)

Table 3 shows that almost 158 patients (77.8%) were vaccinated against COVID-19. Among the 158 subjects who were vaccinated majority 106 (67.0%) were vaccinated with Covishield vaccine followed by 50 (31.7%) of them with Covaxin. Of the 158 vaccinated subjects, 126 (79.7%) patients were completely vaccinated with 2 doses of any COVID-19 vaccine and 32 (20.3%) were partially vaccinated with a single dose of any COVID-19 vaccine. Among the 32 partially vaccinated subjects 6 (18.8%) had taken Covaxin, 25 (78.1%) had taken Covishield and 1(3.1%) had taken Pfizer vaccine. Among the 126 completely vaccinated subjects 44 (34.9%) had taken Covaxin, 81(64.3%) had taken Covishield and 1(0.8%) had taken Sputnik vaccine.

Table 4 Association between COVID-19 vaccination status of subjects and their symptom status, course of treatment and supplemental oxygen requirement (N = 203)

Variable	Vaccinated n = 158	Unvaccinated n = 45	P value	Odds Ratio [95% CI]
Symptom status				
Symptomatic	116 (73.4)	33 (73.3)	0.991	1.004
Asymptomatic	42 (26.6)	12 (26.7)		(0.475, 2.124)
Course of treatment				
Outpatient	99 (62.7)	15 (33.3)	**< 0.001***	3.356
In-patient	59 (37.3)	30 (66.7)		(1.669, 6.748)
Supplemental Oxygen				
Required	4 (2.5)	6 (13.3)	**0.003***	0.169
Not required	154 (97.5)	39 (86.7)		(0.045, 0.628)

(– Pearson Chi-square test)*

As shown in table 4, there was no statistically significant association between the vaccination status and symptomatic status of the study subjects (P = 1.004). The proportion of patients who received in-patient course of treatment was higher among the unvaccinated compared to the vaccinated. This association between the vaccination status and course of treatment was statistically significant (P < 0.001, OR = 3.356, 95% CI of OR = 1.669, 6.748). Among those study subjects who required supplemental oxygen (n = 10), the proportion of those vaccinated was lower compared to those who were unvaccinated. This association between supplemental oxygen requirement and vaccination status was statistically significant (P = 0.003, OR = 0.169, 95% CI of OR = 0.045, 0.628).

In India since Covaxin and Covishield vaccines are the two major vaccines under usage. Among the 158 vaccinated subjects, only 2 were vaccinated with other vaccines like Pfizer and Sputnik vaccines. Hence the 2 subjects were omitted in further analysis of data and results would limit to those 156 subjects who were vaccinated with either Covishield or Covaxin only. Therefore 125 completely vaccinated subjects and 31 partially vaccinated subjects would be considered for comparison between completely and partially vaccinated subjects.

Table 5 Comparison of interval between two doses of COVID vaccine taken among completely vaccinated RTPCR Positive SGTF subjects (n = 125)

Type of Vaccine	Mean (SD)	95% CI of Mean	Median (IQR)	Mean difference (95% CI of Mean difference)	P value
Covaxin (n = 44)	48 (25) days	40, 55 days	33 (30, 60) days	- 37 (-50, -24) days	**< 0.001***
Covishield (n = 81)	85 (39) days	76, 94 days	89 (51, 97) days		

(– Mann Whitney U test)*

Table 5 shows that among the 125 completely vaccinated subjects, the mean interval between two doses of Covaxin vaccine taken was 48 (95% CI = 40, 55) days [SD = 25; median (IQR) = 33 (30 - 60 days)] and mean interval between two doses of Covishield vaccine taken was 85 (95% CI = 76, 94) days [SD = 39; median (IQR) = 89 (51 – 97 days)]. This mean difference (95% CI) of 37 (24, 50) days between Covishield and Covaxin vaccine among the completely vaccinated subjects was statistically significant (P < 0.001).

Table 6 Comparison of duration of last dose of COVID-19 vaccine taken and RT-PCR Positivity with SGTF between partially and completely vaccinated subjects (n = 156)

Vaccination status	Type of vaccine	Mean (SD)	95% CI of Mean	Median	Mean difference (95% CI of Mean difference)	P value
Partially vaccinated (n = 31)	Covaxin (n = 6)	115 (72 days)	39, 191 days	121 days	-11 days (-75, 52 days)	0.960
	Covishield (n = 25)	126 (67 days)	98, 154 days	114 days		
Completely vaccinated (n = 125)	Covaxin (n = 44)	186 (82 days)	162, 210 days	195 days	55 days (25, 85 days)	**< 0.001***
	Covishield (n = 81)	131 (79 days)	114, 148 days	113 days		

(- Mann Whitney U test)*

As it is presented in table 6, it is inferred that among the partially vaccinated subjects (n = 31), the mean (95% CI) interval between the last dose of vaccine taken and date of RT-PCR positivity with SGTF was 115 (39, 191) days for Covaxin vaccine and was 126 (98, 154) days for Covishield vaccine. Among the partially vaccinated individuals, there was no statistically significant difference in mean interval between the last dose of vaccine taken and date of RT-PCR positivity with SGTF between Covaxin and Covishield vaccinated subjects (P = 0.960). It is also inferred that among the completely vaccinated subjects (n = 125), the mean (95% CI) interval between the last dose of vaccine taken and date of RT-PCR positivity with SGTF was 186 (162, 210) days for Covaxin vaccine and was 131 (114, 148) days for Covishield vaccine. Among the completely vaccinated individuals, there was a statistically significant difference in mean interval between the last dose of vaccine taken and date of RT-PCR positivity with SGTF between Covaxin and Covishield vaccinated subjects (P < 0.001).

DISCUSSION

According to the Directorate of Public Health of Tamil Nadu in India, the third wave of COVID-19 was predominantly due to Omicron variant which surged since 12th of December 2021 and lasted till the first week of February 2022. The transmission rate was higher but the disease was less severe leading decreased hospitalization, reduced supplemental oxygen utilization and deaths. The third wave predominantly due to Omicron variant took three weeks to attain its peak from onset compared to the second wave which was predominantly due to delta variant which took nine weeks to attain its peak (13). The mean age of study subjects was 42 years (range: 7 – 86 years) in our study. Similarly, Kim MK et al study had reported that the median age of omicron cases was 39.5 years [range: 2 – 69 years] (14).

The Kumar RM et al study showed that 19.9% had comorbidity which was in similar to our study where 19.2% of the patients had comorbidities (15). International travel of people during a Pandemic result in importing of cases while travelling within the country leads to spread of the disease across the states. In our study 14.3% subjects had travelled to various national and international regions 14 days prior to their diagnosis of COVID-19 infection. In contrast Kim MK et al reported 45% of subjects had travel history prior to their infection (14).

As per Meo SA et al study, infection due to Omicron variant mostly resulted in mild symptoms like mild cough, fever, generalized myalgia, malaise, a scratchy but not sore throat, headache, body ache, and moderate to severe fatigue (16). Kumar RM et al study done in Chennai reported that 64.5% had symptoms of which 43.2% had fever, 23.4% had body pain, 22.2% had running nose and 21.2% had cough as their predominant symptoms (15). Similarly in our study we found that majority 73.4% were symptomatic of which 76.5% had fever, 48.3% has cough and 19.5% had myalgia as their predominant symptoms. Several data sources on the Omicron variant suggest that the risk of hospitalization and the requirement for mechanical ventilation are lower than for the Delta variant. Research from, south Africa suggests that people infected with Omicron have 80% less likely to be admitted in the hospital than when they contract other strains (17). In similar to the study done by Sharma RP et al in Rajasthan (northern India) where almost 99.7% recovered, 33.0% had mild disease, 9.2% had moderate disease and 0.7% had severe disease requiring hospitalization, in our study we observed that majority 95.1% cases were categorized as mild, 3.9% as moderate and 1.0% as severe cases of COVID-19 with SGTF (18). This decrease in severity of Omicron variant of COVID-19 and reduction in hospitalization could be due to the repeated mutations of the virus strain resulting in its reduced pathogenicity. Also, this could be attributed to increase in herd immunity of the population due to COVID-19 immunization campaign and natural immunity gained from COVID-19 infection due to the previous variants.

Vaccination against COVID-19 and public health measures like social distancing, compliance to face mask and handwashing practices have played a major role in control of the COVID-19 pandemic. Vaccination has been proven to be the most effective means for COVID-19 prevention and control (19). WHO has indicated that a COVID-19 Vaccine with a minimum efficacy of 50% would be approved for emergency use in its target product profile (20). A study done by Bartsch SM et al found that a vaccine with efficacy of 60 – 80% could allow reduction in physical distancing measures, but this would still require high coverage (21).

WHO has defined breakthrough infection post vaccination as detection of SARS-CoV-2 RNA or antigen in a respiratory specimen collected from a person with or without COVID-19 like symptoms ≥ 14 days after completion of all recommended doses of the vaccine series (22). In our study we found that among the completely vaccinated subjects (n = 125), the mean (95% CI) interval between the last dose of Covaxin vaccine taken and date of RT-PCR positivity with SGTF was 186 (162, 210) days and for Covishield vaccine taken was 131 (114, 148) days. This suggests that booster doses to the vaccinated individuals could increase the duration of protection offered by these vaccines. Dejnirattisai W et al study reported that neutralization titers of Omicron by sera from vaccinees and convalescent subjects infected with early pandemic as well as Alpha, Beta, Gamma, Delta were substantially reduced or failed to neutralize. Although breakthrough infections could occur, vaccines would still offer protection from severe disease perhaps by T cells and complete failure of vaccines is unlikely. It was likely that the vaccine induced T cell response to SARS-CoV-2 would be less affected than the antibody response. Titers against Omicron were significantly boosted by third doses of vaccine and were high in cases both vaccinated and infected by Delta and hence booster vaccine campaign would add considerable protection against Omicron infection (23).

In our study we found that almost 77.8% were vaccinated against COVID-19 of which 67% were vaccinated with Covishield vaccine followed by 31.7% of them with Covaxin. Of the vaccinated subjects, 79.7% patients were completely vaccinated with two doses of any COVID-19 vaccine and 20.3% were partially vaccinated with a single dose of any COVID-19 vaccine. Similarly, Kumar RM et al study showed that 14.2% and 67.4% suspected omicron cases had received one and two doses of COVID-19 vaccines (15). In our study the proportion of patients who received in-patient course of treatment was higher among the unvaccinated compared to the vaccinated. This association was statistically significant ($P < 0.001$, OR = 3.356, 95% CI of OR = 1.669, 6.748). Also, we found that among those study subjects who required supplemental oxygen, the proportion of those vaccinated was lower compared to those who were unvaccinated. This association between supplemental oxygen requirement and vaccination status was statistically significant ($P = 0.003$, OR = 0.169, 95% CI of OR = 0.045, 0.628).

Similarly, Menni C et al study found that those who had received two or three vaccine doses had a lower risk of hospitalization during omicron prevalence which was statistically significant (OR = 0.75, 95% CI = 0.57 - 0.98; $P = 0.03$). Of the vaccinated individuals only 1.9% of them were hospitalized due to omicron and only 2.6% due to delta variant (24). Also, Karim SSA et al observed that vaccinated individuals were likely to have a much lower risk of severe disease from omicron infection. A combination of preventive measure such as vaccination and public health measures is expected to remain an effective strategy in control of the surge in cases due to Omicron variant (25).

CONCLUSION

Omicron (SGTF) cases manifests mostly as mild cases with symptoms like fever, cough, myalgia and majority were independent of oxygen supplementation and had good prognosis. Since majority of the Omicron cases were treated under home isolation, policies to strengthen the surveillance of home isolation cases and remote monitoring of cases through teleconsultation is necessary to prevent widespread transmission. Omicron infection has been delayed over six months among completely vaccinated subjects especially those who were vaccinated with Covaxin. Though the immune protection offered by the currently available vaccines against omicron variant remains to be a point of concern, vaccination would still play a major role in delaying the occurrence of a new episode as well as in minimizing the severity of illness. Improving the vaccination coverage and adherence to preventive public health measures like usage of face mask, hand hygiene and physical distancing would remain as the major strategy in control of COVID-19 pandemic amidst its periodic fluctuations due to variants of concern. Above all community participation is the keystone in successful implementation of public health measures for prevention and control of diseases, especially during a pandemic.

Limitations

The study was conducted among only 203 study subjects, a larger sample size could have given more information on the objectives of the study. Both univariate and bivariate analysis were used for statistical analysis. Multivariate analysis could have addressed the potential confounders in the study. Being a hospital based cross sectional study rather than a community-based field study, only vaccination status of patients who sought medical care from the hospital were studied.

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A RANDOMIZED CONTROLLED TRIAL OF INTRAVENOUS MAGNESIUM SULPHATE AS AN ADJUNCT TO STANDARD THERAPY IN ATRIAL FIBRILLATION

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Abstract

Background: Magnesium sulphate ($MgSO_4$) has been proven as an analgesic, neuromuscular blocker agent, and treatment of acute asthma.

Objective: The study aimed to assess the safety and efficacy of magnesium sulphate infusion for the treatment of patients with atrial fibrillation.

Methods: A prospective, randomized, double-blind, placebo-controlled study was conducted on 55 atrial fibrillation patients at the Emergency Department. The treatment group consisting of 41 patients received 20 mEq (2.5 g, 10 mmol) magnesium sulphate over a 20-minute period, followed by 20 mEq (2.5 g, 10 mmol) over a 2-hour period intravenously in addition to the standard treatment, and the control group consisting of 14 patients received placebo with a standard treatment. ECG was repeated and monitored upto 24 hours after the infusion.

Results: 41 patients received magnesium sulphate and 14 patients received a placebo. The heart rate was 127 bpm – 210 bpm at the presentation to the emergency department and it was reached <100bpm in 70%(n=31) patients after 150 minutes of $MgSO_4$ infusion. Two patients attained a heart rate of <100bpm in Placebo infusion. In the treatment group, 65% of subjects attained normal sinus rhythm at the end of 24 hours of infusion. Magnesium sulphate was more likely than placebo to achieve a heart rate of <100bpm and more likely to convert to sinus rhythm. ECG report reverted to normal in 74.5% in the treatment group and 25.5 % in the Placebo group within 24 hours. The patients in the treatment group stay a mean of 2.25 days in ICU, while the patients in the Placebo group stayed 4.25 days in ICU. Seven patients in the treatment group had minor side effects like flushing, headache, and nausea, which came to normal after 48hrs.

Conclusions: Magnesium sulphate has been shown a better efficacy to control the heart rate and conversion to sinus rhythm when used along with the standard management of Atrial fibrillation.

Keywords: Atrial fibrillation, Magnesium sulphate, heart rate, sinus rhythm.

INTRODUCTION

Atrial fibrillation is one of the common arrhythmia, reported in hemodynamically unstable patients. The loss of atrial contraction and the sequential atrioventricular contraction decrease of overall cardiac output results in a severe inadequate tissue perfusion. Drugs such as verapamil, diltiazem, amiodarone, and beta blockers compromise the patient's cardiac output by their negative inotropic effect [1,2,3]. Digoxin has a slow onset of action and

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is significantly less effective in states of increased sympathetic tone [4,5,6]. It also results in adverse effects with chronic dosing.

As per the American College of Emergency 2015, ventricular rate control is the primary therapeutic objective in atrial fibrillation [7].

Magnesium sulphate ($MgSO_4$) and its analgesic effect has been shown in animal models and humans. $MgSO_4$ competes with calcium ions in synaptic junctions and prevents the release of presynaptic acetylcholine, prolonging the effects of neuromuscular blocker agents [8-11]. It has been proposed as an effective and safe adjunct in the treatment of acute asthma [12]. Magnesium had an inherent physiological association because of its effects on membrane potentials and ion transport [13]. In a meta-analysis of 5 randomized trials (n=380), the patients who had received magnesium had 3 times more likely to reach a heart rate of <100 bpm compared to digoxin [14].

However, studies were scarce in India on the safety and efficacy of magnesium sulphate infusion in atrial fibrillation.

Hence, this study aimed to analyse the safety and efficacy of magnesium sulphate infusion in addition to conventional treatment modalities in patients with atrial fibrillation. The outcome of this study is to achieve a heart rate of <100bpm, and a conversion to sinus rhythm throughout the period of infusion of magnesium sulphate.

METHODS

Study Design: Prospective, randomized, double-blinded, and placebo-controlled trial.
Materials: Patients of adult age >18 years presenting with atrial fibrillation to the Emergency Department, Sri Ramachandra Medical College and Research Institute, Chennai, India were considered for enrolment.
The study was performed between Nov 2007 to Nov 2008.
Patients of age >18 years presenting to ED with atrial fibrillation and a ventricular response rate of >120 beats/min were included. The exclusion criteria were a requirement for cardioversion, a systolic blood pressure of <90 mm Hg, symptomatic hypotension, a history of atrioventricular node disease, patients on permanent pacemakers, an acute myocardial infarction eligible for thrombolysis.
Ethics statement
This study followed the tenets of the Helsinki declaration and the protocol was approved by the Institutional Ethical Committee. All the included patients and their relatives were informed about the procedures and their conditions. Informed consent was obtained from the patients (those that were able to provide it) or from their relatives if the patients' physical condition prevented them from signing it.
Randomization and Treatment Protocol
On arrival in the emergency room, a detailed history was taken, and a clinical examination was performed to rule out any associated illness. An informed consent was obtained from the patient's relatives.
After the enrolment the patients were randomly allocated to one of the two groups viz the $MgSO_4$ tratment group and the placebo group.
The inclusion criteria for both groups were similar. We used random number tables for the randomization. The individual random numbers were kept in separate envelopes so that the concealment could be maintained until the patient was included in the assigned group. The treatment group received $MgSO_4$ and the control group received 0.9% sodium chloride in a double-blind fashion. The solutions were prepared by the coordinator of the study and the ED physician in charge of the patients during the treatment was unaware of the study medication.
Treatment group: The treatment group patients were given 40 mEq (5 g, 20 mmol) of Magnesium sulphate in 100 mL of Normal saline [NS] 20 mEq (2.5 g, 10 mmol) was given intra-

venously diluted in 100ml NS over a 20-minute period followed by 20 mEq (2.5 g, 10 mmol) intravenously diluted in 100ml NS over the next 2 hours. The patients were monitored with half-hourly pulse rate, oxygen saturation, and blood pressure.
The administration of the Magnesium sulphate solution started in addition to the Amiodarone infusion as the standard therapy at ED. However, there were no guidelines that amiodarone was recommended, but amiodarone is acceptable. All the patients were given efficient anticoagulant prior. Dose administered as per American college of cardiology protocol, and dose given to all patient presented to ED after counselling and consent.
Placebo: 0.9% sodium chloride+ standard therapy. In placebo, the administration of 0.9% sodium chloride solution as placebo was added to the standard therapy as Amiodarone infusion at the emergency department.
Assessment of Response: Ventricular response rate control (the pulse rate of <100 beats/ min), mean changes in the heart rate, the conversion to sinus rhythm, and major (hypotension and bradycardia) or minor adverse events.
ECG was taken before and after the infusion. Blood was drawn for a magnesium level estimation before the infusion. The patients were monitored and the rhythm was analyzed at the end of the infusion. The patients were followed up during their course of stay in the hospital, their ECG was repeated, and their heart rates were monitored continuously after the $MgSO_4$ infusion.
ECHO cardiograph was performed within ED and the patients were treated accordingly with a standard treatment. Transesophageal echocardiogram (TEE) was not done.
The patients discharged from the ED were instructed to continue their regular medications.
The statistical analysis was performed using Pearson's chi-square and Fischer's Tests. The effect of $MgSO_4$ on pulse rate measured at various points was analyzed by the analysis of variance-ANOVA (with a random intercept and slope for each subject), with contrasts to test the research hypotheses. The effect of treatment on the binary outcomes "any pulse rate of <100bpm," the conversion to sinus rhythm, and any adverse effects were analyzed using the chi-square test. The statistics package SPSS ver 18.0 was used for analyses (SPSS Inc, US).

RESULTS

Characteristics of patients

There were fifty-five subjects in the study and the control group, of which 75% of subjects were in the $MgSO_4$ treated group and 25% were in the placebo group. Most of the subjects presented to the emergency department were complaining of palpitations, only a few had chest pain and breathlessness [Tab 1].

Most of the subjects were in the fifth to seventh decade. The mean age in $MgSO_4$ treated group was 72.0 years, while it was 73.0 years in the placebo group. Female preponderance was observed in both groups, such as 41.46% of male, 58.53%of female was seen in the $MgSO_4$ treated group, whereas 14% of male and 86% of female were noted in the placebo. In the patients who were administered $MgSO_4$, 77% had a history of antiarrhythmic drug use in the past, and 23% of the Placebo had a history of antiarrhythmic drug use in the past.

Out of the total subjects who presented to the emergency department the least heart rate at the presentation was 127 bpm & maximum heart rate was 210 bpm, maximum subjects were having heart rates ranging from 150–170bpm [Tab 2][Figure 1].

Table 1 Comparison of Basic characteristics between the two groups

Characteristics	$MgSO_4$	Placebo
No randomized (n)	41	14
Sex		
Male (%)	41.46 %	0.71 %
Female	58.53 %	92 %
Age, yrs., Range	72.0 (50 – 70)	73.0 (51 – 71.0)
History of Alcohol Abuse (%)	51.95 %	72 %
History of Antiarrhythmic Use	77%	33%
History of RHD (%)	19.51 %	28.57 %
History of CAD (%)	17.07 %	21.42 %
History of Hypothyroid (%)	9.75 %	21.42 %
Currently receiving Antiarrhythmic drugs (%)	1.07 %	21.42 %
Duration of Symptoms:		
Within 24 hr (n)	41	14
After 24 hr (n)	1	14
ECG after 24 hrs – Rate (Mean)	84.51	112.2
Rhythm (%)	NSR (65%)	AF (85%)
ICU Stay (Mean)	2.25 days	4.25 days

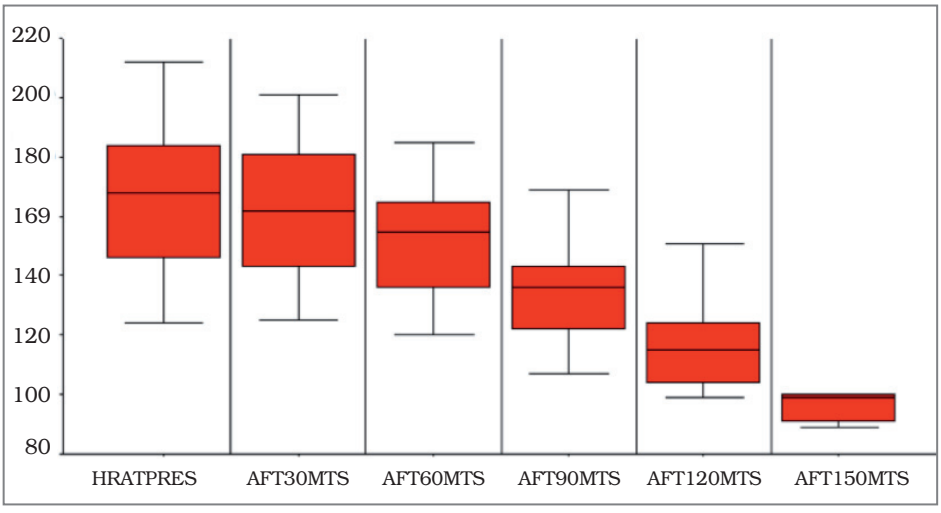

Fig. 1. Barograph plot shows achieving pulse rate <100 beats/min

Table 2 Measurement of effect of $MgSO_4$ using ECG and pulse rate

	Mean	S.D	S.E	95% CI	P value
Drugs Given					
Mgso4	1.8585	0.11175	0.01745	–	<0.0001
Placebo	1.8929	0.09169	0.02450		
ECG rate at presentation					
Mgso4	84.5122	8.54729	1.33486	–	<0.0001
Placebo	112.2857	7.89770	2.11075		
ECG Rhythm At 24hrs					
Mgso4	74.5	4.58	–		
Placebo	25.5	3.45	–		
Heart Rate					
At Presentation to ED				Mean Pulse rate difference, Beats/Min (95% CI)	
$MgSO_4$	162.7561	25.12845	3.92440	154.8246-170.6876	0.015
Placebo	177.2143	16.02008	4.28155	167.9646-186.464	
After 30 minutes					
$MgSO_4$	159.3902	23.75	3.70	-12.60 (-26.32 – 1.10)	
Placebo	172.0000	15.92	4.25		0.071
After 60 minutes					
$MgSO_4$	147.6829	19.63	3.066	-12.60 (-24.07 – -1.12)	0.032
Placebo	160.2857	14.35	3.836		
After 90 minutes					
$MgSO_4$	129.9756	14.218	2.22	-18.31 (-27.10 – -9.51)	0.001
Placebo	148.2857	13.980	3.736		
After 120 minutes					
$MgSO_4$	111.2195	8.802	1.374	-23.85 (-30.53 – -12.16)	0.001
Placebo	135.07	15.304	4.09		
After 150 minutes					
$MgSO_4$	96.1220	5.58	0.87	-27.94 (-33.29 – -13.60)	0.001
Placebo	24.07	14.34	3.83		
Paired Test of Heart Rate after 30 Mins & 150 Mins					
	Mean	Std dev	Std Err	95% CI	P
After 30 minutes	162.6	22.57	3.044	53.77-64.95	<0.0001
After 150 minutes	103.23	14.955	2.01		

At the end of the 150 minutes of Mgso4 infusion 31(70%) the subjects attained heart rate less than 100bpm and 2 subjects (14%) in the Placebo infusion attained the heart rate less than 100bpm [Fig 2].

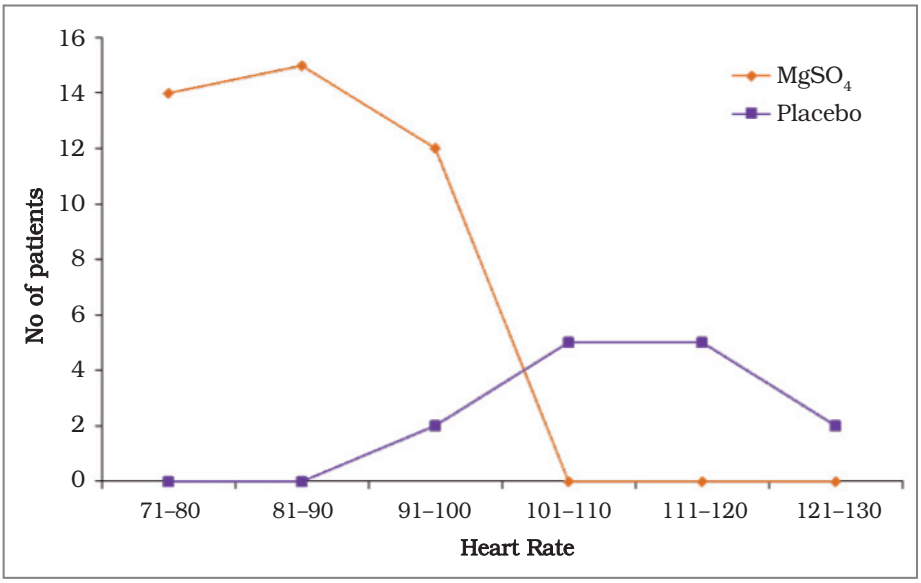

Fig. 2 Pulse Rate monitoring up to at 24 hours after MgSO4 infusion

Achieving the heart rate of <100beats/min in the $MgSO_4$ treated group, 84.5% achieved a heart rate <100bpm, and 65% of the subjects attained normal sinus rhythm, and in the placebo group 85% were in Atrial fibrillation and only 12.28% attained a heart rate of <100 bpm at the end of 24 hours. ECG reverted to normal in 74.5% within 24 hours in the $MgSO_4$ treated group and in 25.5 % within 24 hours in the Placebo group [**Fig. 3**].

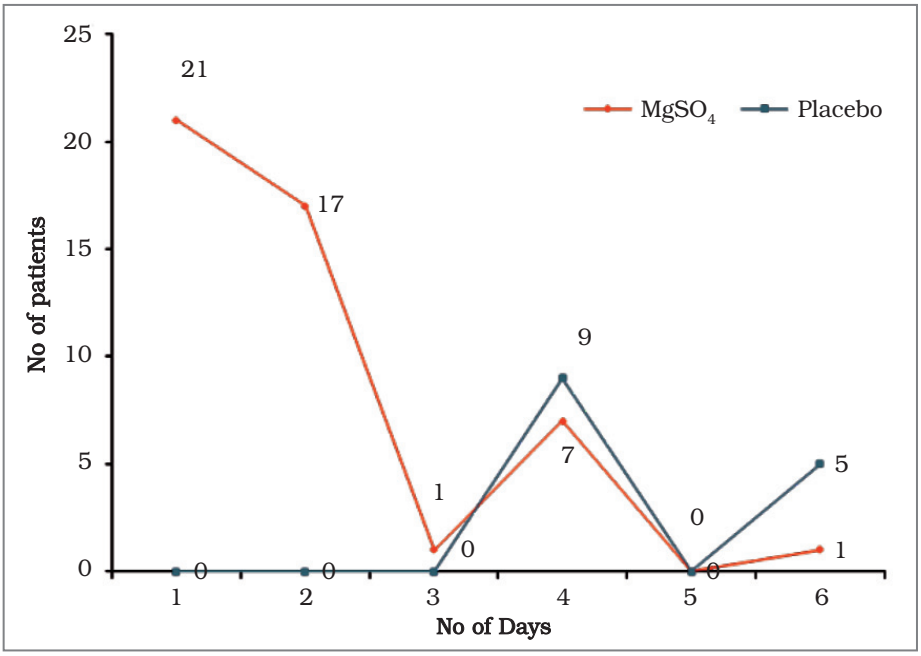

Fig. 3 ICU Stay during the treatment period

Seven patients in the magnesium sulphate treated group had minor side effects like flushing, headache, and nausea, P-value =0.839, Chi-square test = 0.041, Fischer's test = 1.00 [Tab 3] [Tab 4].

Table 3 Minimum adverse effects after drug administration

		Frequency	Percent	Valid Percent	Cumulative Percent	P value
Valid	MgSO4	5	12.2	12.2	12.2	–
	Placebo	2	14.3	14.3	14.3	–
CROSS TABULATION						
Drug Administration/ Adverse effects			Adverse effects		Total	
			1.00	2.00		
DRUG Administration	MgSO4	Count	5	36	41	0.839
		% Within DRUG Administration	12.2%	87.8%	100.0%	
		% Within DRUG adverse effects	71.4%	75%	74.5%	
		% Of total	9.1%	65.5%	74.5%	
	Placebo	Count	2	12	14	
		% Within DRUG Administration	14.3	85.7%	100%	
		% Within DRUG adverse effects	28.6%	25.0%	25.5%	
		% Of total	3.6%	21.8%	25.5%	
	Total	Count	7	48	55	
		% Within DRUG Administration	12.7%	87.3%	100%	
		% Within DRUG adverse effects	100%	100%	100%	
		% Of total	12.7%	87.3%	100%	

Table 4 Descriptive statistics of the patient characteristics

	N	Minimum	Maximum	Sum	Mean		std
	Statistic	Statistic	Statistic	Statistic	Statistic	Std error	Statistic
Age	55	32	80	2981	54.2	1.62	12.05
Heart rate at presentation	55	124	212	9154	166.43	3.219	23.87
MgSO4	55	1.7	2.1	102.7	1.867	0.014	0.107
After 30 minutes	55	125	201	8943	162.6	3.044	22.57
After 60 minutes	55	120	185	8299	150.9	2.57	19.12
After 90 minutes	55	107	169	7405	134.6	2.18	16.17
After 120 minutes	55	99	151	6451	117.29	2.016	14.95
After 150 minutes	55	89	147	5678	103.23	2.016	14.95
ECG Rate 24Hr	55	72	127	5037	91.58	1.99	14.77
ICU stay	55	1.0	6.0	124	2.25	0.17	1.322

DISCUSSION

Magnesium is an important electrolyte in cardiovascular physiology. Magnesium sulphate is capable of increasing the conduction time and refractoriness at the atrium and the atrioventricular nodal level while suppressing atrial automaticity. It has been found to be useful for treating various atrial tachyarrhythmias[15-18] but there are few if any data on its use in paroxysmal atrial fibrillation. Our study showed that magnesium sulphate used in conjunction with other agents results in 86.4% of the patients achieving a heart rate of less than 100 beats/min compared with the placebo. The current study demonstrated that magnesium sulphate increases the rate of conversion from atrial fibrillation to sinus rhythm when added to standard therapies.

Thirteen of the 41 patients in $MgSO_4$ treated group were on antiarrthymic drugs at the time of the admission [77%], two of the fourteen patients in the Placebo group were on antiarrthymic drugs at the time of the admission [23%]. This indicates most of the patients presented to ED were new onset atrial fibrillation patients who were not on any medication.

Out of total patients presenting to the emergency department, most of the patients were having heart rates ranging from 150-170bpm, of which the least heart rate at the presentation was 127 bpm & the maximum heart rate was 210 bpm. At the end of 150 minutes $MgSO_4$ infusion 31 [70%] patients [of a total of 41] attained a heart rate of < 100bpm and in the Placebo infusion, two [14%] patients [of the total of 14] attained the heart rate of < 100bpm.

These patients were followed for the next 24hrs and their heart rate and rhythm were recorded, which was not done in previous studies. Analyses at 30 min, 60 min, 90 min, and 150 min revealed mean pulse rate differences of -12.60, -12.60, -23.85, and 27.94 beats/min respectively. This shows the heart rate started to decrease once the MgSO4 infusion was started and at the end of 150 min about 31 subjects attained a heart rate of <100bpm, which proves its efficacy in atrial fibrillation patients.

In the $MgSO_4$ treated group, 35 out of 41 patients (84.5%) achieved a heart rate less than 100beats /min and 25 of 35 patients [65%] attained normal sinus rhythm. In the placebo group, 2 of 14 patients [12.28%] achieved a heart rate less than 100beats /min and no patient attained normal sinus rhythm, 87.72% were continued to be in atrial fibrillation at the end of 24 hours. This proves the effectiveness of $MgSO_4$ in reducing heart rate and attaining rhythm control at the end of 24hrs in conjunction with antiarrhythmic drugs when compared to the placebo.

The subjects in the $MgSO_4$ treated group had [2.25] days of ICU stay compared to the placebo group [4.25] days. Hence, MgSO4 also reduces the duration of ICU stay, thereby reducing the cost factors and burden on the patients.

Seven patients in the magnesium sulphate treated group had minor side effects like flushing, headache, and nausea, which shows it is safe and effective in rate and rhythm control when used in conjunction with other antiarrhythmic agents in atrial fibrillation with fast ventricular rate.

In our study, the Antiarrhythmic drug used was Amiodarone and $MgSO_4$. ECG was taken after 24 hrs to detect the Rate Rhythm and ICU Stay has been studied.

Whereas a study by David Teubner, Michael John Davey used Antiarrhythmic Drug as Digoxin, Verapamil, & Beta-blocker. They measured ECG after 24 hrs., Rate Rhythm, but ICU stay has not been taken into their study.

Our study demonstrated that $MgSO_4$ produced a statistically significant reduction in ventricular response rates at all duration intervals up to 150 minutes. We showed that magnesium sulphate used in conjunction with other agents results in 56.4% of the patients achieving a pulse rate of less than 100 beats/min compared with the placebo. In the magnesium sulphate treated group, the majority of adverse effects were minor. Only 7 patients in the magnesium sulphate treatment group had minor side effects confirming its safety.

Numerous studies conducted on to use of intravenous magnesium injection to treat acute onset atrial fibrillation.

An event is successful cardioversion to sinus rhythm or the absence of atrial fibrillation on follow-up.

Ho KM et al., 2007, used intravenous magnesium injection and observed cardioversion or prevention; 42 events in 166 cases in Mgso4, 31 events in 161 cases in the placebo group, respectively [13].Chu et al., 2009, identified events of 2/24 in Mgso4, and 6/24 in placebo groups, respectively [19].

Cook et al., 2012, identified events of 465/595 in the Mgso4 and 457/595 in the placebo groups, respectively [20]. Sultan et al., 2012, identified events of 81/84 in the Mgso4 treated and 74/86 in the placebo groups, respectively [21]. Klinger et al., 2015, identified events of 107/186 in the $MgSO_4$ treated and 110/177 in the placebo groups, respectively [22].

Rajagopalan et al., add to the growing evidence base on this issue by performing a randomized, double-blind, placebo-controlled trial of intravenous magnesium before electric cardioversion of AF. A total of 261 patients were enrolled with normal magnesium levels at baseline (2.1±0.2 mg/dL; 0.86±0.08 mmol/L). Their key finding was that 1-hour conversion to sinus rhythm was similar in both the magnesium-treated patients and the placebo group (86.4% magnesium versus 86.0% placebo). They also found no difference in biphasic energy requirement, or the number of shocks needed in a ramping energy protocol. Rajagopalan et al., identified events of 114/132 in the $MgSO_4$ treated, and 111/129 in the placebo groups, respectively [23].

Literature related to the management of atrial fibrillation has analysed the evidence of the pharmacologic agents [1,2,3]. The literature recommends that calcium-channel blockers and b-blockers were considered as the first-line agents for rate control in patients with preserved left ventricular function.

The current study also believes that physicians can use digoxin even if it is a slow onset of effect due to uncertainty of the patients' underlying cardiac status. The current study demonstrated that an infusion of magnesium sulphate marginally improved the early ventricular response rate control and increases the rates of conversion to sinus rhythm in rapid atrial fibrillation when added to a usual conventional pharmacological treatment.

The limitations include sample size is not adequate, the majority of patients who present to the ED with atrial fibrillation are elderly, and it is often difficult to rapidly confirm the reliability of medical history in this setting.

CONCLUSION

Magnesium sulphate showed a statistically significant reduction of ventricular response rates at all-time intervals up to 150 minutes. In summary, 2.5 g of IV magnesium sulfate administered along with a standard therapy in patients presenting to ED with atrial fibrillation causes improvement in the heart rate and conversion to sinus rhythm. Larger double-blind studies with this design should be done to find the potential benefits of IV $MgSO_4$ as additional treatment along with standard drugs.

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