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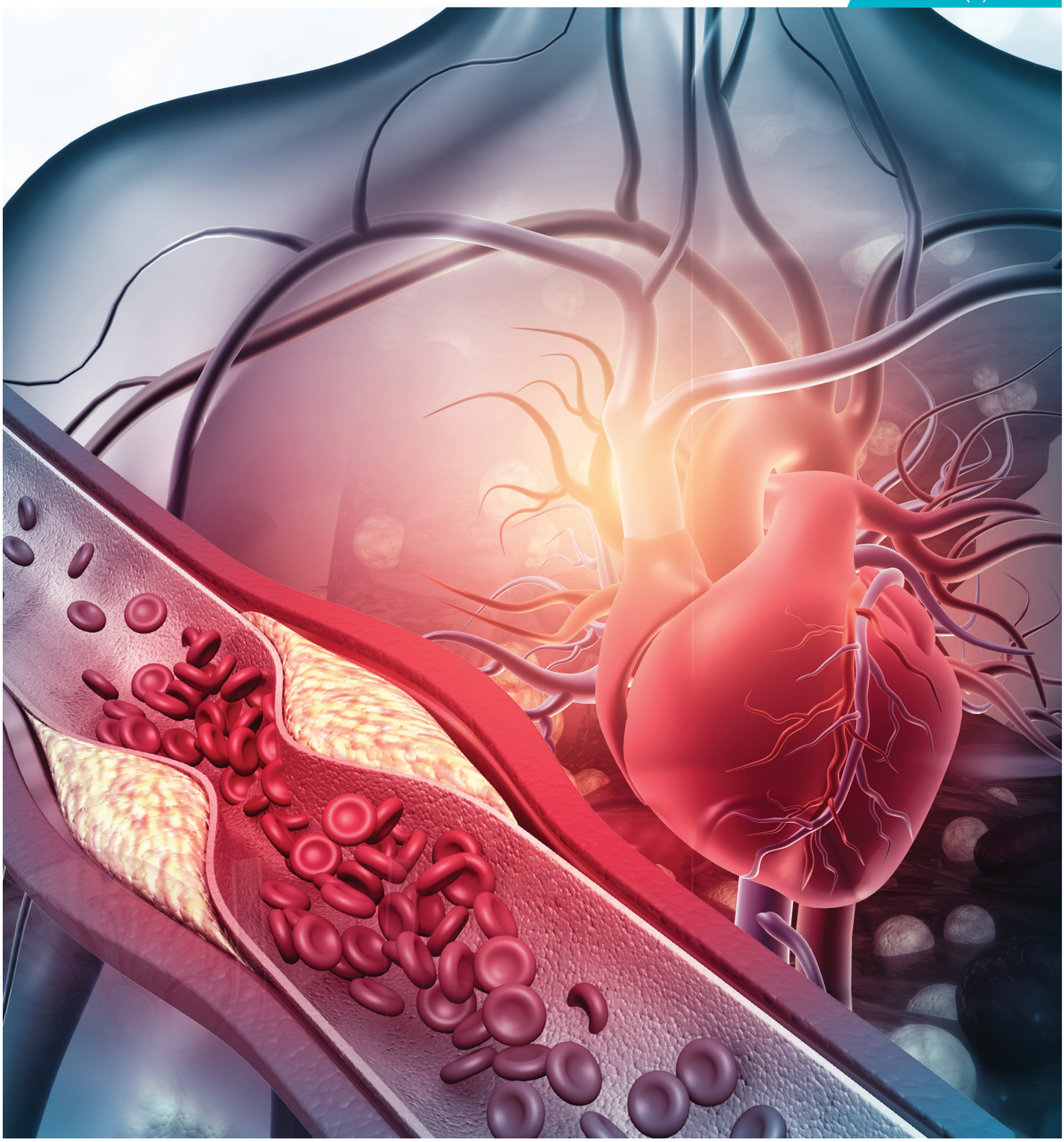

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EDITORIAL

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Eosinophilic Inflammation in COPD: Mechanisms, Diagnostic Markers, Clinical Features and Therapy Guidance

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Abstract

Chronic obstructive pulmonary disease (COPD) is one of the main causes of morbidity and mortality worldwide. Eosinophilia, which may have a significant role in the inflammation process and obstruction of airways, is present in about one third patients suffering from COPD. The significance of eosinophil counts in COPD is currently debated, but they can potentially serve as biomarkers for treatment selection. Blood eosinophil counts can assist in determining the patients who can benefit from inhaled corticosteroid therapy to prevent or eliminate exacerbations.

The aim of this review is to demonstrate the significance of eosinophilic inflammation COPD and the use of eosinophilic inflammation biomarkers to guide therapy decisions.

To reach this goal, a search of relevant literature on the subject was conducted. Articles were searched in electronic sources: PubMed and Google Scholar. The keywords "COPD and eosinophilia", "COPD exacerbations and eosinophilia", "inhaled corticosteroids, COPD and eosinophilia" were used. One hundred and twenty sources were found, of which 66 were selected.

Results of the review showed high frequency of eosinophilic phenotype detection in COPD. Blood eosinophilia becomes a potential universal marker of eosinophilic COPD. Blood eosinophils level can predict response to inhaled corticosteroids treatment patients with COPD, but increases the likelihood of pneumonia. Overall, the role of eosinophilia in COPD has not been obtained to date, which suggests it needs further study.

Keywords: chronic obstructive pulmonary disease; eosinophilia; inflammation; biomarkers; treatment outcome; corticosteroids; exacerbations; pneumonia; phenotype; personalized medicine.

Introduction

Chronic obstructive pulmonary disease (COPD) has a severe negative socio-economic impact on the healthcare system, causing significant mortality and disability worldwide [1-3]. Data shows that COPD causes 2.8 million deaths annually, which corresponds to about 5% of all deaths in the world [2]. Studies on the epidemiology of COPD in Kazakhstan are not numerous but indicate a high prevalence [4, 5]. According to the results of the Chronic Obstructive Respiratory disease study, carried out between 2013-2015 in Commonwealth of Independent States countries, the prevalence of COPD in Kazakhstan was found to be 114.1 per 1000 population [4]. In the near future, an increase in morbidity and mortality from COPD is expected due to the associated prevalence of tobacco smoking, environmental pollution, and increasing age of the population [6-9].

Chronic exposure to toxic particulates and gases leads to the development of COPD. Due to the influence of these factors, persistent inflammation with a complex

heterogeneous mechanism develops [10]. For a long time, the main cell responsible for the airway inflammation in COPD was considered to be the neutrophil, whereas eosinophilic airway inflammation was seen as typical for bronchial asthma [11-13]. However, there is increasing evidence of high eosinophil activity in a number of patients with COPD [14].

This review aims to describe the role of eosinophils in COPD, how they are related to clinical progression and prognosis, and how they can affect the choice of therapy.

A literature search was conducted on the topic of eosinophilia in COPD. Articles were searched in electronic sources: PubMed and Google Scholar. The keywords "COPD and eosinophilia", "COPD exacerbations and eosinophilia", "inhaled corticosteroids, COPD and eosinophilia" were used. One hundred and twenty sources were found, of which 66 were selected.

Prevalence of eosinophilic COPD

According to the results of the 3-year ECLIPSE study of COPD patients, which used a threshold level of 2% for blood eosinophilia: 37.4% of participants had persistent peripheral eosinophilia (>2%), 49% had variable eosinophil levels, and only 13.6% of patients had no peripheral eosinophilia at any visit [15]. Cross-sectional study by Bedolla-Barajas et al. demonstrated that out of 81 patients with COPD, 34 (42%) had a relative eosinophil concentration ≥2%; 21 (25.9%) ≥3%; 14 patients (17.3%) had ≥4%; and 10 patients (12.3%) had ≥5% [16]. A retrospective cohort study in China (2012-2015) by Yang et al., which selected 375 COPD patients, stratified them based on the blood eosinophil count into 3 groups, showed that: 125 patients had less than 100 eosinophils/μL, 195 patients had between 100-300 eosinophils/μL, and 55 patients had eosinophil count greater than 300 eosinophils/μL in the blood. The prevalence of eosinophilic COPD in that cohort demonstrated to be 66.7%, if the cut-off of 100 eosinophils/μL is used, and 14.7% if cut-off of 300 eosinophils/μL is used [17]. To date, COPD with eosinophilic inflammation is a distinct disease phenotype with its own characteristic clinical features [18, 19]. It has been shown that 25-40% of cases of COPD can present with eosinophilia [15]. Additionally eosinophilic inflammation was shown to be present in 28% of acute exacerbations of the disease [20].

Mechanism and markers of eosinophilic inflammation in COPD

The mechanism of eosinophilia development in COPD currently is not fully understood. However, the previous studies have indicated that eosinophilic inflammation in COPD patients correlates with an increase of cytokine Interleukin 5 (IL-5) in the respiratory tract [21]. This cytokine is produced by Th2 cells, ILC2 cells and it promotes differentiation, migration, activation, and effector functions of eosinophils [22, 23]. Another cytokine that promotes proliferation of hematopoietic cells into mature eosinophils is granulocyte-macrophage colony-stimulating factor [22]. As shown in Figure 1, the proinflammatory effect of eosinophils occurs through various mechanisms. The major basic protein of eosinophils disrupts epithelial permeability, promoting the penetration of antigens into the respiratory tract, eosinophil peroxidase causes tissue damage via oxidative stress, eosinophil cationic protein leads to the apoptosis of respiratory epithelium [24]. Cytokines and chemokines such as IL5, IL10, CCL5 also lead to development and persistence of eosinophilic inflammation. Sustained inflammation leads to oedema, basal membrane thickening, airway remodeling, and mucus hypersecretion [25, 26].

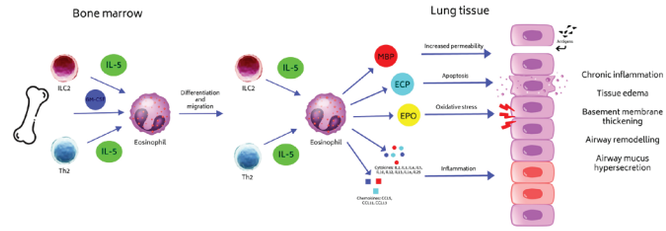

Figure 1 - Pathophysiology of eosinophilic inflammation in COPD: IL-5: interleukin 5, ILC2: type 2 innate lymphoid cell, Th2: T helper 2 cell, GM-CSF: granulocyte-macrophage colony-stimulating factor, MBP: major basic protein, ECP: eosinophil cationic protein, EPO: eosinophil peroxidase, IL2-25: interleukins 2-25, CCL5, 11, 13: chemokine ligand 5, 11, 13

There are several methods to estimate the severity of eosinophilic inflammation. The most common approaches are based on the estimation of eosinophil counts in various samples of biological material. For this purpose, biopsy material may be examined, sputum specimens obtained by conventional methods may be analyzed and induced sputum and bronchoalveolar lavage fluid may be analyzed.

A normal eosinophil count in induced sputum is <3% [27]. Elevation of eosinophils over 3% is a criterion for eosinophilic COPD and happens in 28% of all COPD exacerbations [20], as well as in 34-38% of COPD patients during the stable period [28]. It should be noted that cytological analysis of induced sputum is methodologically difficult and time consuming for most clinical laboratories and in many cases is not readily available in real clinical practice. Obtaining sufficient quantities of spontaneous sputum is technically challenging, as confirmed in the results of the SPIROMICS study [29]. Kolsum et al. revealed that blood eosinophilia is a marker of sputum eosinophilia. Patients with peripheral blood eosinophilia had higher levels of eosinophils in sputum: median percentage count 7.0%, compared to 2.5% in a group with no eosinophilia, $p = 0.002$, median absolute sputum eosinophil count 0.4 vs. 0.1, $p < 0.01$ [30]. Negewo et al. also demonstrated the relation of sputum eosinophilia to peripheral blood eosinophilia. Patients with sputum eosinophilia had a blood eosinophil count of 300 cells/μL, while the patients with normal sputum eosinophil levels had blood eosinophil count 150 cells/μL, $p<0.0001$) [31]. In a study by Singh et al., both percentage and absolute blood eosinophils positively correlated with the presence of eosinophils in sputum ($r=0.54$, $p<0.0001$) [32].

Recently, the blood eosinophilia threshold of 300 cells/μL is increasingly used as the most accurate marker of eosinophilic inflammation and with a subsequent positive response to inhaled corticosteroid (ICS) therapy [33, 34]. One of the limitations in using eosinophilia as a biomarker is its variability from day to day and throughout the day. According to Oshagbemi et al., in COPD patients, stable eosinophil counts (counts that are persistently above or below the threshold of 340 cells/μL) at 6 months of follow-up were observed in 85% of measurements, 62% at 2 years, then decreased afterwards [35]. Analysis of 2 prospective observational studies CHAIN and BODE conducted in Spain from 2016-2018 confirms the significant variability of eosinophilia in COPD patients over time: 40.5% of patients had an intermittently variable eosinophilia, defined as variation in eosinophil counts between ≥300 or <300 cells/μL in 3 assessments. 43.8% had a constantly normal eosinophil count, while only 15.7% had persistent eosinophilia [36].

Nitric oxide in exhaled air (FeNO) is a biomarker that has been shown to help distinguish eosinophilic from non-eosinophilic airway inflammation [37]. FeNO is positively correlated with eosinophil counts in induced sputum during COPD exacerbation [38]. Research by Tang et al. demonstrated a connection between FeNO and peripheral blood eosinophil count: blood eosinophilia positively correlated with FeNO (r=0.383, p=0.004) [39].

The above allows us to consider blood eosinophilia to be a surrogate for presence of eosinophilic inflammation in the airway. It should be noted that blood analysis, unlike the study of induced sputum, is widely available, easy to perform and can be performed in all patients, so the blood eosinophil count deserves the closest attention for the phenotypic characterization of inflammation in COPD.

Clinical features in eosinophilic COPD

Several studies have demonstrated that higher blood levels of eosinophils correlated with increased risk of re-hospitalization, prolonged hospitalizations, and worse lung function [40, 41]. Longitudinal prospective study ECLIPSE found that, COPD patients with blood eosinophilia ≥2% were characterized by higher values of the forced expiratory volume in 1 second (FEV1), lower body fat, less severe dyspnea measured by the modified Medical Research Council (mMRC) scale, higher scores on St. George's Respiratory Questionnaire which assessed life quality [15]. According to the study by Turato et al., patients were categorized into three distinct groups: those with a persistent elevatedeosinophil count (≥150 cells/μL), low eosinophil count (<150 cells/μL), and variable (fluctuating over/ below 150 cells/μL) eosinophil count. The study showed no notable distinctions among the groups regarding smoking history, results of spirometry, COPD severity, frequency of exacerbations, mMRC scale score, and exercise tolerance (according to the 6-minute walk test) [42]. As shown in the study by Chou et al., sputum eosinophil levels correlated weakly with the degree of reversibility of FEV1 (r=0.162, p=008.1) [43]. The differences between non-eosinophilic and eosinophilic COPD in terms of FEV1 and mMRC were found to be not-significant in a study of 7225 patients by Vedel-Krogh et al. [19].

At this point, the relation between sputum or blood eosinophilia, with severity of pulmonary emphysema in COPD patients is not clear. One study conducted in Greece, had shown that presence of emphysema was associated with significantly lower blood eosinophils: median absolute count 34.6 cells/μL (Interquartile range (IQR) 0-63) in emphysema group compared to 169.0 cells/μL (IQR 110-260) in non-emphysema group, p<0.001. Same was true for percent eosinophil count: median 0.6% (IQR 0-1%) vs. 3.0% (IQR 2-4%), p < 0.001 [44]. Same findings were confirmed in a study conducted in Korea by Oh et al. in 2018 [45]. Another study by Hastie et al. showed that increased eosinophils in sputum were related to higher exacerbation risk and more prominent emphysema, whilst no such relationship was observed for blood eosinophil levels [29].

Based on the above, convincing relation between clinical and functional parameters and eosinophilia in COPD has not been shown to date, which suggests it needs further study.

Exacerbations and prognosis in eosinophilic COPD

COPD exacerbations are considered extremely important in clinical practice as they are related to the disease prognosis, so risk of COPD exacerbations and their relationship with eosinophilia are actively investigated [46-48]. Cheng et al. observed that severe exacerbations were more common with baseline blood eosinophil levels ≥ 3%, compared to eosinophil levels <3% (27.1% vs. 7.4%; p<0.01) [49].

Vedel-Krogh et al. showed in 203 COPD patients that an increase in eosinophil count >340 cells/μL and a history of single exacerbation per year were linked to a 3.21-fold higher risk of severe exacerbations, additionally an increase in blood eosinophil count >2% corresponded with a 1.85 times higher risk of severe exacerbations [19]. Retrospective cohort study, which utilized the UK Clinical practice Research Datalink (CPRD) and US Optum Clinformatics Data Mart (Optum) databases, showed that among COPD patients with several (≥2) exacerbations per year, majority of patients: 76.8% in the CPRD database and 76.5% in the Optum database, had peripheral blood eosinophilia ≥300 cells/μL during one year of follow-up, while a significant minority: 17.0% in CPRD database, and 13.3% in Optum database had peripheral blood eosinophilia ≥400 cells/μL [50]. Yun and colleagues reported that higher blood eosinophil levels were linked to an increased risk of COPD exacerbations, with an eosinophil threshold of ≥300 cells/μL showing an adjusted incidence rate ratio for exacerbations of 1.32 (95% confidence interval (CI) 1.10-1.63) [51].

In a retrospective study by Müllerová et al., a subgroup of COPD patients receiving triple therapy with blood eosinophilia >150 cells/μL and a history of frequent exacerbations (n=2512) was analyzed. The RR of moderate and severe COPD exacerbations in this group was 2.32 (95% CI 2.22-2.43), the RR of non-COPD hospitalizations was 1.31 (95% CI 1.18-1.46) and the RR of all-cause mortality was 1.26 (95% CI 1.16-1.37). The researchers concluded that eosinophilic COPD is more resistant to triple therapy leading to a more severe disease and exacerbations [46]. Khamitov et al. in a retrospective study of 424 medical histories of hospitalized patients with COPD exacerbations over 4 calendar years (2015-2018), found that extremely severe obstructions were associated with higher eosinophil counts [52].

On the contrary, Acet-Öztürk and colleagues revealed that COPD patients with elevated eosinophil counts (≥2%) had fewer symptoms and reported higher quality of life in comparison to those with non-eosinophilic inflammation. Non-eosinophilic inflammation was more frequently linked with use of supplemental long-term oxygen therapy in comparison to eosinophilic inflammation (36.1% vs. 14.8%, p = 0.01) [53]. In a UK study where eosinophilia was defined as ≥200 cells/μL the length of hospitalization was shorter in the eosinophilic group compared to patients with non-eosinophilic exacerbations (5.0 days [range, 1-19 days] vs. 6.5 days [range, 1-33 days]; P = 0.015) [54].

Zysman et al. showed that at all cut-off values for eosinophilia (2; 3 or 4%) no significant differences in exacerbation frequency and 3-year survival, between patients with eosinophilic and non-eosinophilic COPD, were found [55]. Additionally retrospective study carried out in Tunisia, the aim of which was to show the relationship between the degree of eosinophilia and various parameters of severity of severe exacerbation of COPD, where the two groups were defined with eosinophils ≥ 200 cells/μL (103 cases, 20.4%), eosinophils < 200 cells/μL (403 patients: 79.6%), there was no notable variance in the course of severe exacerbation of COPD [56].

In the study by Turato et al., 867 patients with COPD died from various causes during the 5-year follow-up. Kaplan-Meier analysis revealed that patients with persistent blood eosinophilia had a higher survival rate (p<0.01) [42]. Another study had corresponding findings; with the median follow-up of 6 years, lower mortality rate was found in patients with COPD and blood eosinophilia ≥ 300 cells/μL (relative risk (RR) 0.29; 95% CI 0.09-0.97, p=0.045) [33].

Oh et al. confirm this in their study where patients with COPD were categorized into three groups based on their blood eosinophil count: high (≥5%), medium (2%-5%), and low (<2%). They found that participants in the group with high counts had longer survival time compared to those with medium or low counts (high = 9.52±0.23 years, medium = 8.47±1.94 years, low = 7.42±0.27 years; p<0.05) [45].

In summary, data on eosinophilic inflammation contributing to a negative prognostic outcome in COPD is still conflicting.

Efficacy of glucocorticosteroid therapy in eosinophilic COPD

Eosinophilic airway inflammation and blood eosinophil levels > 2% are correlated with the responsiveness of COPD patients to systemic glucocorticosteroid therapy for COPD exacerbations [57, 58]. In a study led by Ramarikshan et al., individuals who were current or former smokers with COPD diagnosis were allocated into two groups. The first group received oral prednisolone for 14 days once per day if their blood eosinophil level was higher than 2% or placebo if their eosinophil count was lower than 2%. The second group received standard care therapy irrespective of the blood eosinophil results. Both groups received antibiotic therapy. Practical implication of this non-inferiority trial showed that prednisolone therapy provided to patients according to their blood eosinophil count during the acute phase of COPD was non-inferior to the standardized care tactics and can be provided to patient to reduce potential risks related to the glucocorticoid therapy side effects [59]. There was a meta-analysis conducted by Bafadhel et al. of six clinical trials (300 cases of COPD exacerbations included), according to which similar findings were observed. In patients who did not receive prednisolone during the exacerbation and had blood eosinophil levels < 2%, therapy was ineffective in 20% of cases, with blood eosinophil levels ≥ 2% - in 66% of cases. In patients who received prednisolone and had blood eosinophil levels < 2%, therapy was ineffective in 26% of cases, and when the level was ≥ 2%, therapy was ineffective in 11% of cases [60].

In the prospective randomized study by Cheng et al., patients with COPD used a high dose of fluticasone 1 mcg/day or a medium dose of fluticasone 0.5 mcg/day combined with salmeterol (500 mcg/day). In this research, participants were separated into two groups based on their eosinophil counts: a group with high eosinophil levels (≥3%) and a group with low eosinophil levels (<3%). The group with high eosinophil counts who received higher doses of ICS showed a significant improvement in quality of life (determined by COPD assessment test) compared to the group with low eosinophil counts who received a medium dose of ICS (P=0.02). The incidence of exacerbations was decreased in patients in the first group compared to the second group (13.5% vs. 28.7%, P<0.01) [49]. A retrospective analysis of two 12-month randomized clinical trials comparing vilanterol and fluticasone furoate/vilanterol in COPD patients (n=3177) revealed that ICS reduced exacerbations in individuals with blood eosinophils ≥2%. Participants with eosinophil counts <2 % experienced a 10% reduction in exacerbations, while those with eosinophilia levels of 2-4%, 4-6%, and ≥6% saw reductions of 24%, 32%, and 42%, respectively [61].

Currently, ICS therapy is recommended for patients with blood eosinophil counts exceeding 300 cells/μL, as it is illustrated in Figure 2.

A study conducted over three years in Copenhagen, involving both healthy people and those with COPD, examined the link between blood eosinophils and the risk of COPD exacerbations. From a total population of 81,668 people, 7,225

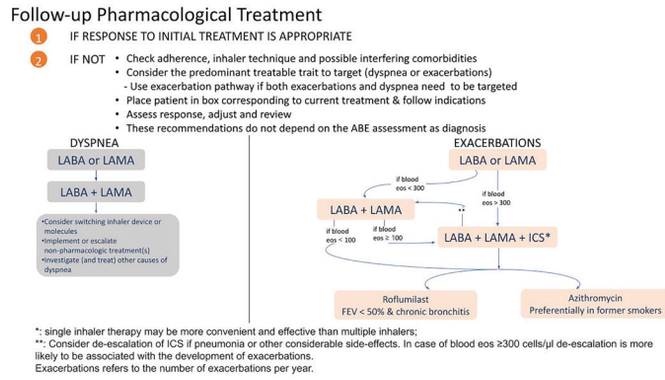

Figure 2 - Global Initiative for Chronic Obstructive Lung Disease 2023 recommendations for use of ICS in COPD [8]

patients with COPD were selected after spirometric respiratory function measurement. Among them, a subgroup of 203 patients who had experienced one or more exacerbations requiring systemic glucocorticoids or hospitalization in the year prior to the study was identified. In particular, the study found that the risk of severe exacerbations was higher in COPD patients with eosinophil counts exceeding 340 cells/μL in the blood, with adjusted incidence rate ratios of 1.76 (95% CI 1.56–1.99), which aligns with aforementioned blood eosinophil count of 300 cells/μL that requires ICS therapy as it is demonstrated in Figure 2 . Using a blood eosinophil count of 2% as a cut-off value, the risk of severe exacerbations necessitating hospitalization only increased in the subgroup of patients with a history of exacerbations [19].

It should be noted that the data on the benefits of ICS in eosinophilic COPD is not so clear-cut. For example, 52-week-long FLAME study analysis results did not show significant differences in reducing exacerbation frequency or time to first exacerbation between eosinophilic and non-eosinophilic COPD patients treated with combinations of ICS and long-acting beta agonist or combination of long-acting beta agonist and long-acting muscarinic antagonist. [62].

It is necessary to consider the level of peripheral blood eosinophilia when starting long-term and safe ICS therapy. A meta-analysis of 10 clinical trials involving 10,861 patients revealed that COPD patients with low eosinophil levels (<2%) had a 31% higher risk of pneumonia compared to those with high eosinophil levels (≥ 2%) (HR=1.31, 95% CI - 1.06-1.62). Moreover, severe pneumonia was more common in COPD patients with low blood eosinophils. The tendency to increase the incidence of pneumonia with decreasing eosinophil levels persisted after stratification of patients by ICS use. In the ICS therapy group, pneumonia episodes were reported in 40 (3.8%) of 1063 patients with eosinophil levels <2% and in 48 (2.4%) of 2002 patients with eosinophil levels ≥ 2% (HR=1.53, 95% CI 1.01-2.31) [63].

In the retrospective ISOLDE study, there were more cases of pneumonia in those receiving fluticasone propionate (15/263 (5.7%)) in the <2% eosinophil group as compared to the placebo group (3/242 (1.2%)). However, in patients who had higher eosinophil levels, the rate of pneumonia did not differ significantly between both treatment groups: 5/107 (4.7%) for fluticasone propionate and 6/126 (4.8%) for placebo. Comparable results were observed for severe pneumonia: 4.6% treated with fluticasone propionate and 0.8% treated with placebo experienced a severe pneumonia in the eosinophil <2% group, compared to 3.7% and 4.8% in the eosinophil ≥ 2% group [64].

According to the 4-year UPLIFT study, the use of ICS in COPD patients corresponded with an increased risk of pneumonia compared to no ICS use: the hazard ratio of 1.33 (95% CI 1.00-1.75; p = 0.046) [65].

In another meta-analysis, the risk of pneumonia-related events was shown to be significantly higher in patients with eosinophil counts ≥2% treated with ICS (RR, 1.969; 95% CI, 1.369-2.833; P<0.001). No significant differences were observed in those with eosinophil counts <2% (RR, 1.29; 95% CI, 0.888-1.879; P<0.181). The analysis also suggested that a threshold of 2% for blood eosinophils may help determine the response to ICS treatment in COPD patients but could also predispose the patient to develop pneumonia [66].

Conclusion

COPD with eosinophilic inflammation is a distinct disease phenotype with its own characteristic clinical features. Around 25% to 40% of COPD patients exhibit eosinophilic inflammation, and approximately 28% of COPD exacerbations are linked to airway eosinophilia. A number of cytokines acts in the development of eosinophilic inflammation in COPD, among which IL5 is a key one.

Determinations of blood eosinophil counts, sputum analysis and induced sputum are common methods, with blood eosinophilia becoming a potential universal marker. However, the role of other markers, including those in sputum, is not completely understood. The impact of eosinophils on exacerbations and prognosis is a topic of debate, with conflicting data available. Glucocorticosteroids are essential in the treatment of eosinophilic COPD, but concerns about the risk of pneumonia need careful consideration. Identifying patients who may respond to glucocorticosteroids remains a challenge. Overall, the identification of the eosinophilic phenotype of COPD is crucial because it details the clinical characteristics of the condition and justifies the use of ICS.

Author Contributions:

Conceptualization, A.A. and M.A.; methodology, N.L. and A.P.; investigation, A.A. and M.A.; resources, A.A.; data curation, S.S. and M.M.; writing – original draft preparation, A.A., S.S. and M.M.; writing – review and editing, N.Z.; visualization, S.S. and M.M.; supervision, N.L. and N.Z. All authors have read and agreed to the published version of the manuscript.

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References

1. Halpin DM, Criner GJ, Papi A, Singh D, Anzueto A, Martinez FJ, et al. Global initiative for the diagnosis, management, and prevention of chronic obstructive lung disease. The 2020 GOLD science committee report on COVID-19 and chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine.* 2021 Jan 1;203(1):24-36. https://doi.org/10.1164/rccm.202009-3533SO
2. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. *The lancet.* 1997 May 24;349(9064):1498-504. https://doi.org/10.1016/S0140-6736(96)07492-2
3. Carson-Chahhoud K, Noori M, Nejadghaderi SA, Sullman M, Ahmadian Heris J, Ansarin K, et al. The burden of chronic obstructive pulmonary disease and its attributable risk factors in 204 countries and territories, 1990-2019: Results from the Global Burden of Disease Study 2019.
4. Nugmanova D, Feshchenko Y, Iashyna L, Gyrina O, Malynovska K, Mammadbayov E, et al. The prevalence, burden and risk factors associated with chronic obstructive pulmonary disease in Commonwealth of Independent States (Ukraine, Kazakhstan and Azerbaijan): results of the CORE study. *BMC pulmonary medicine.* 2018 Dec;18(1):1-4. https://doi.org/10.1186/s12890-018-0589-5
5. Public health Republic of Kazakhstan and activities organizations health care in 2020 year. [Internet]. [cited 2023 Dec 28]. Available from: https://www.gov.kz/memleket/entities/dsm/documents/details/246287?lang=en
6. Viegi G, Maio S, Fasola S, Baldacci S. Global burden of chronic respiratory diseases. *Journal of aerosol medicine and pulmonary drug delivery*. 2020 Aug 1;33(4):171-7. https://doi.org/10.1089/jamp.2019.1576
7. McDaniel JT. Prevalence of chronic obstructive pulmonary disease: county-level risk factors based on the Social Ecological Model. *Perspectives in Public Health.* 2018 Jul;138(4):200-8. https://doi.org/10.1177/1757913918772598
8. Venkatesan P. GOLD report: 2022 update. The Lancet Respiratory Medicine. 2022 Feb 1;10(2):e20. https://doi.org/10.1016/S2213-2600(21)00561-0
9. Torén K, Olin AC, Lindberg A, Vikgren J, Schiöler L, Brandberg J, et al. Vital capacity and COPD: the Swedish cardiopulmonary bioImage study (SCAPIS). *International journal of chronic obstructive pulmonary disease.* 2016 May 2:927-33. https://doi.org/10.2147/COPD.S104644
10. Olloquequi J, Jaime S, Parra V, Cornejo-Córdova E, Valdivia G, Agustí À, Silva O R. Comparative analysis of COPD associated with tobacco smoking, biomass smoke exposure or both. *Respiratory research.* 2018 Dec;19(1):1-8. https://doi.org/10.1186/s12931-018-0718-y
11. Golpe R, Martín-Robles I, Sanjuán-López P, Pérez-de-Llano L, González-Juanatey C, López-Campos JL, et al. Differences in systemic inflammation between cigarette and biomass smoke-induced COPD. *International journal of chronic obstructive pulmonary disease.* 2017 Sep 1:2639-46. https://doi.org/10.2147/COPD.S141068
12. Lange P, Celli B, Agustí A, Boje Jensen G, Divo M, Faner R, et al. Lung-function trajectories leading to chronic obstructive pulmonary disease. *New England Journal of Medicine.* 2015 Jul 9;373(2):111-22. https://doi.org/10.1056/NEJMoa1411532
13. Górska K, Paplińska-Goryca M, Nejman-Gryz P, Goryca K, Krenke R. Eosinophilic and neutrophilic airway inflammation in the phenotyping of mild-to-moderate asthma and chronic obstructive pulmonary disease. COPD: *Journal of Chronic Obstructive Pulmonary Disease.* 2017 Mar 30;14(2):181-9. https://doi.org/10.1080/15412555.2016.1260539
14. Saha S, Brightling CE. Eosinophilic airway inflammation in COPD. *International journal of chronic obstructive pulmonary disease.* 2006 Jan 1;1(1):39-47. https://doi.org/10.2147/copd.2006.1.1.39
15. Singh D, Kolsum U, Brightling CE, Locantore N, Agusti A, Tal-Singer R. Eosinophilic inflammation in COPD: prevalence and clinical

characteristics. *European Respiratory Journal.* 2014 Dec 1;44(6):1697-700. https://doi.org/10.1183/09031936.00162414
16. Bedolla-Barajas M, Morales-Romero J, Bedolla-Pulido TI, Flores-Razo MM, Morales MA, Rosales G, et al. Prevalence of blood eosinophilia in adults with COPD according to the cut-off point. *Revista Alergia de Mexico.* 2021 Jul 1;68(3). https://doi.org/10.29262/ram.v67i3.893
17. Yang M, Yang T, Li X, Li D, Liao Z, Shen Y, et al. Clinical predictors of high blood eosinophils in chronic obstructive pulmonary disease. *International Journal of Chronic Obstructive Pulmonary Disease.* 2021 Aug 28:2467-74. https://doi.org/10.2147/COPD.S324511
18. Li A, Chan HP, Gan PX, Liew MF, Wong WF, Lim HF. Eosinophilic endotype of chronic obstructive pulmonary disease: similarities and differences from asthma. *The Korean Journal of Internal Medicine.* 2021 Nov;36(6):1305. https://doi.org/10.3904/kjim.2021.180
19. Vedel-Krogh S, Nielsen SF, Lange P, Vestbo J, Nordestgaard BG. Blood eosinophils and exacerbations in chronic obstructive pulmonary disease. The Copenhagen General Population Study. *American journal of respiratory and critical care medicine.* 2016 May 1;193(9):965-74. https://doi.org/10.1164/rccm.201509-1869OC
20. Bafadhel M, McKenna S, Terry S, Mistry V, Reid C, Haldar P, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. *American journal of respiratory and critical care medicine.* 2011 Sep 15;184(6):662-71. https://doi.org/10.1164/rccm.201104-0597OC
21. Bafadhel M, Saha S, Siva R, McCormick M, Monteiro W, Rugman P, et al. Sputum IL-5 concentration is associated with a sputum eosinophilia and attenuated by corticosteroid therapy in COPD. *Respiration.* 2009 Sep 1;78(3):256-62. https://doi.org/10.1159/000221902
22. Varricchi G, Bagnasco D, Borriello F, Heffler E, Canonica GW. Interleukin-5 pathway inhibition in the treatment of eosinophilic respiratory disorders: evidence and unmet needs. *Current opinion in allergy and clinical immunology*. 2016 Apr;16(2):186. https://doi.org/10.1097/ACI.0000000000000251
23. Molfino NA, Gossage D, Kolbeck R, Parker JM, Geba GP. Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor. *Clinical & Experimental Allergy.* 2012 May;42(5):712-37. https://doi.org/10.1111/j.1365-2222.2011.03854.x
24. Brusselle G, Pavord ID, Landis S, Pascoe S, Lettis S, Morjaria N, et al. Blood eosinophil levels as a biomarker in COPD. *Respiratory medicine.* 2018 May 1;138:21-31. https://doi.org/10.1016/j.rmed.2018.03.016
25. George L, Brightling CE. Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease. *Therapeutic advances in chronic disease.* 2016 Jan;7(1):34-51. https://doi.org/10.1177/2040622315609251
26. Davoine F, Lacy P. Eosinophil cytokines, chemokines, and growth factors: emerging roles in immunity. *Frontiers in immunology. 2*014 Nov 10;5:570. https://doi.org/10.3389/fimmu.2014.00570
27. McDonald VM, Higgins I, Wood LG, Gibson PG. Multidimensional assessment and tailored interventions for COPD: respiratory utopia or common sense?. *Thorax*. 2013 Mar 1:thoraxjnl-2012. https://doi.org/10.1136/thoraxjnl-2012-202646
28. Leigh R, Pizzichini MM, Morris MM, Maltais F, Hargreave FE, Pizzichini E. Stable COPD: predicting benefit from high-dose inhaled corticosteroid treatment. *European Respiratory Journal.* 2006 May 1;27(5):964-71. https://doi.org/10.1183/09031936.06.00072105
29. Hastie AT, Martinez FJ, Curtis JL, Doerschuk CM, Hansel NN, Christenson S, et al. Association of sputum and blood eosinophil concentrations with clinical measures of COPD severity: an analysis of the SPIROMICS cohort. *The Lancet Respiratory Medicine.* 2017 Dec 1;5(12):956-67. https://doi.org/10.1016/S2213-2600(17)30432-0
30. Kolsum U, Ravi A, Hitchen P, Maddi S, Southworth T, Singh D. Clinical characteristics of eosinophilic COPD versus COPD patients with a history of asthma. *Respiratory research.* 2017 Dec;18(1):1-7. https://doi.org/10.1186/s12931-017-0559-0
31. Negewo NA, McDonald VM, Baines KJ, Wark PA, Simpson JL, Jones PW, Gibson PG. Peripheral blood eosinophils: a surrogate marker for airway eosinophilia in stable COPD. *International journal of chronic obstructive pulmonary disease.* 2016 Jul 1:1495-504. https://doi.org/10.2147/COPD.S100338
32. Singh D, Watz H, Beeh KM, Kornmann O, Leaker B, Colgan B, et al. COPD sputum eosinophils: relationship to blood eosinophils and the effect of inhaled PDE4 inhibition. *European Respiratory Journal.* 2020 Aug 1;56(2). https://doi.org/10.1183/13993003.00237-2020
33. Shin SH, Park HY, Kang D, Cho J, Kwon SO, Park JH, et al. Serial blood eosinophils and clinical outcome in patients with chronic obstructive pulmonary disease. *Respiratory research.* 2018 Dec;19:1-9. https://doi.org/10.1186/s12931-018-0840-x
34. Yilmaz I, Turk M. What should be the cutoff value of blood eosinophilia as a predictor of inhaled corticosteroid responsiveness in patients with chronic obstructive pulmonary disease?. *American Journal of Respiratory and Critical Care Medicine. 2*017 Nov 1;196(9):1229-30. https://doi.org/10.1164/rccm.201705-0892LE
35. Oshagbemi OA, Burden AM, Braeken DC, Henskens Y, Wouters EF, Driessen JH, et al. Stability of blood eosinophils in patients with chronic obstructive pulmonary disease and in control subjects, and the impact of sex, age, smoking, and baseline counts. *American journal of respiratory and critical care medicine.* 2017 May 15;195(10):1402-4. https://doi.org/10.1164/rccm.201701-0009LE
36. Casanova C, Celli BR, de-Torres JP, Martínez-Gonzalez C, Cosio BG, Pinto-Plata V, et al. Prevalence of persistent blood eosinophilia: relation to outcomes in patients with COPD. *European Respiratory Journal.* 2017 Nov 1;50(5). https://doi.org/10.1183/13993003.01162-2017
37. Soter S, Barta I, Antus B. Predicting sputum eosinophilia in exacerbations of COPD using exhaled nitric oxide. *Inflammation*. 2013 Oct;36:1178-85. https://doi.org/10.1007/s10753-013-9653-8
38. Gao J, Zhang M, Zhou L, Yang X, Wu H, Zhang J, Wu F. Correlation between fractional exhaled nitric oxide and sputum eosinophilia in exacerbations of COPD. *International Journal of Chronic Obstructive Pulmonary Disease*. 2017 Apr 27:1287-93. https://doi.org/10.2147%2FCOPD.S134998
39. Tang B, Huang D, Wang J, Luo LL, Li QG. Relationship of blood eosinophils with fractional exhaled nitric oxide and pulmonary function parameters in chronic obstructive pulmonary disease (COPD) exacerbation. Medical Science Monitor: *International Medical Journal of Experimental and Clinical Research.* 2020;26:e921182-1. https://doi.org/10.12659%2FMSM.921182
40. Wu CW, Lan CC, Hsieh PC, Tzeng IS, Wu YK. Role of peripheral eosinophilia in acute exacerbation of chronic obstructive pulmonary disease. *World Journal of Clinical Cases.* 2020 Jul 7;8(13):2727. https://doi.org/10.12998/wjcc.v8.i13.2727
41. Xue J, Cui YN, Chen P, Cai S, Chen L, Dai ZS, Chen Y. Blood eosinophils: a biomarker of response to glucocorticoids and increased readmissions in severe hospitalized exacerbations of COPD. Zhonghua jie he he hu xi za zhi= Zhonghua jiehe he huxi zazhi= Chinese journal of tuberculosis and respiratory diseases. 2019 Jun 1;42(6):426-31. https://doi.org/10.3760/cma.j.issn.1001-0939.2019.06.005
42. Turato G, Semenzato U, Bazzan E, Biondini D, Tinè M, Torrecilla N, et al. Blood eosinophilia neither reflects tissue eosinophils nor

worsens clinical outcomes in chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*. 2018 May 1;197(9):1216-9. https://doi.org/10.1164/rccm.201708-1684LE
43. Chou KT, Su KC, Hsiao YH, Huang SF, Ko HK, Tseng CM, et al. Post-bronchodilator reversibility of FEV1 and eosinophilic airway inflammation in COPD. *Archivos de Bronconeumología (English Edition)*. 2017 Oct 1;53(10):547-53. https://doi.org/10.1016/j.arbr.2017.01.027
44. Papaioannou AI, Kostikas K, Papaporfyriou A, Angelakis L, Papathanasiou E, Hillas G, et al. Emphysematous phenotype is characterized by low blood eosinophils: a cross-sectional study. *COPD: Journal of Chronic Obstructive Pulmonary Disease.* 2017 Nov 2;14(6):635-40. https://doi.org/10.1080/15412555.2017.1386644
45. Oh YM, Lee KS, Hong Y, Hwang SC, Kim JY, Kim DK, et al. Blood eosinophil count as a prognostic biomarker in COPD. *International journal of chronic obstructive pulmonary disease.* 2018 Oct 31:3589-96. https://doi.org/10.2147/COPD.S179734
46. Müllerová H, Hahn B, Simard EP, Mu G, Hatipoğlu U. Exacerbations and health care resource use among patients with COPD in relation to blood eosinophil counts. *International Journal of Chronic Obstructive Pulmonary Disease.* 2019 Mar 22:683-92. https://doi.org/10.2147/COPD.S194367
47. Kerkhof M, Sonnappa S, Postma DS, Brusselle G, Agustí A, Anzueto A, et al. Blood eosinophil count and exacerbation risk in patients with COPD. *European Respiratory Journal.* 2017 Jul 1;50(1). https://doi.org/10.1183/13993003.00761-2017
48. Hegewald MJ, Horne BD, Trudo F, Kreindler JL, Chung Y, Rea S, Blagev DP. Blood eosinophil count and hospital readmission in patients with acute exacerbation of chronic obstructive pulmonary disease. *International Journal of Chronic Obstructive Pulmonary Disease.* 2020 Oct 23:2629-41. https://doi.org/10.2147/COPD.S251115
49. Cheng SL, Lin CH. Effectiveness using higher inhaled corticosteroid dosage in patients with COPD by different blood eosinophilic counts. International Journal of Chronic Obstructive Pulmonary Disease. 2016 Sep 21:2341-8. https://doi.org/10.2147/COPD.S115132
50. Vogelmeier CF, Kostikas K, Fang J, Tian H, Jones B, Morgan CL, et al. Evaluation of exacerbations and blood eosinophils in UK and US COPD populations. *Respiratory research.* 2019 Dec;20:1-0. https://doi.org/10.1186/s12931-019-1130-y
51. Yun JH, Lamb A, Chase R, Singh D, Parker MM, Saferali A, et al. Blood eosinophil count thresholds and exacerbations in patients with chronic obstructive pulmonary disease. *Journal of allergy and clinical immunology.* 2018 Jun 1;141(6):2037-47. https://doi.org/10.1016/j.jaci.2018.04.010
52. Hamitov RF, Zinnatullina AR, Mihoparova OJu. Eozinofilija krovi i obostrenija HOBL: vse li odnoznachno (Blood eosinophilia and exacerbations of COPD: whether everything is clear). [in Russian]. *Vestnik sovremennoj klinicheskoj mediciny.* 2022;15(4):60-4.
53. Acet-Öztürk NA, Dilektasli AG, Aydın-Güçlü Ö, Demirdöğen E, Coşkun F, Ursavaş A, et al. Long-term oxygen treatment need is less frequent in eosinophilic COPD patients. *The Clinical Respiratory Journal.* 2022 Jan;16(1):49-56. https://doi.org/10.1111/crj.13451
54. Bafadhel M, Greening NJ, Harvey-Dunstan TC, Williams JE, Morgan MD, Brightling CE, et al. Blood eosinophils and outcomes in severe hospitalized exacerbations of COPD. *Chest*. 2016 Aug 1;150(2):320-8. https://doi.org/10.1016/j.chest.2016.01.026
55. Zysman M, Deslée G, Caillaud D, Chanez P, Escamilla R, Court-Fortune I, et al. Relationship between blood eosinophils, clinical characteristics, and mortality in patients with COPD. *International journal of chronic obstructive pulmonary disease.* 2017 Jun 20:1819-24. https://doi.org/10.2147/COPD.S129787
56. Saad AB, Khemakhem R, Mhamed SC, Fahem N, Migaou A, Joobeur S, Rouatbi N. Etude de l'intérêt de l'éosinophilie sanguine au cours des exacerbations aigues sévères de bronchopneumopathie chronique obstructive (BPCO) dans un centre tunisien. *Pan African Medical Journal.* 2019 Nov 8;34(1). https://doi.org/10.11604/pamj.2019.34.138.17392
57. Pavord ID, Lettis S, Locantore N, Pascoe S, Jones PW, Wedzicha JA, Barnes NC. Blood eosinophils and inhaled corticosteroid/long-acting β-2 agonist efficacy in COPD. *Thorax.* 2015 Nov 19:thoraxjnl-2015. https://doi.org/10.1136%2Fthoraxjnl-2015-207021
58. Liu T, Xiang ZJ, Hou XM, Chai JJ, Yang YL, Zhang XT. Blood eosinophil count-guided corticosteroid therapy and as a prognostic biomarker of exacerbations of chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Therapeutic Advances in Chronic Disease.* 2021 Jul;12:20406223211028768. https://doi.org/10.1177/204062232110287
59. Ramakrishnan S, Jeffers H, Langford-Wiley B, Davies J, Thulborn SJ, Mahdi M, et al. Blood eosinophil-guided oral prednisolone for COPD exacerbations in primary care in the UK (STARR2): a non-inferiority, multicentre, double-blind, placebo-controlled, randomised controlled trial. *The Lancet Respiratory Medicine.* 2024 Jan 1;12(1):67-77. https://doi.org/10.1016/S2213-2600(23)00298-9
60. Bafadhel M, Davies L, Calverley PM, Aaron SD, Brightling CE, Pavord ID. Blood eosinophil guided prednisolone therapy for exacerbations of COPD: a further analysis. *European Respiratory Journal.* 2014 Sep 1;44(3):789-91. https://doi.org/10.1183/09031936.00062614
61. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. *The Lancet Respiratory medicine.* 2015 Jun 1;3(6):435-42. https://doi.org/10.1016/S2213-2600(15)00106-X
62. Roche N, Chapman KR, Vogelmeier CF, Herth FJ, Thach C, Fogel R, et al. Blood eosinophils and response to maintenance chronic obstructive pulmonary disease treatment. Data from the FLAME trial. *American journal of respiratory and critical care medicine.* 2017 May 1;195(9):1189-97. https://doi.org/10.1164/rccm.201701-0193OC
63. Pavord ID, Lettis S, Anzueto A, Barnes N. Blood eosinophil count and pneumonia risk in patients with chronic obstructive pulmonary disease: a patient-level meta-analysis. *The Lancet Respiratory medicine.* 2016 Sep 1;4(9):731-41. https://doi.org/10.1016/S2213-2600(16)30148-5
64. Barnes NC, Sharma R, Lettis S, Calverley PM. Blood eosinophils as a marker of response to inhaled corticosteroids in COPD. *European Respiratory Journal.* 2016 May 1;47(5):1374-82. https://doi.org/10.1183/13993003.01370-2015
65. Tashkin DP, Miravitlles M, Celli BR, Metzdorf N, Mueller A, Halpin DM, Anzueto A. Concomitant inhaled corticosteroid use and the risk of pneumonia in COPD: a matched-subgroup post hoc analysis of the UPLIFT® trial. *Respiratory research.* 2018 Dec;19:1-1. https://doi.org/10.1186/s12931-018-0874-0
66. Cheng SL. Blood eosinophils and inhaled corticosteroids in patients with COPD: systematic review and meta-analysis. *International journal of chronic obstructive pulmonary disease.* 2018 Sep 6:2775-84. https://doi.org/10.2147/COPD.S175017

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Genes, Antibodies, and Cytokines in Systemic Lupus Erythematosus: Update of Potential Biomarkers

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Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown aetiology, with the broad range of antibodies affecting various organs and tissues, leading to rapid disability and even to lethal outcome. Our objective was to make an analysis of the results of relevant global studies about genetic mutations, spectrum of antibodies and cytokines involved in the pathogenesis of SLE. The search was conducted in PubMed and Google Scholar platforms using keywords “systemic lupus erythematosus”, “genetic mutations”, “autoantibodies”, “cytokine production”, “sequencing”, “NGS" and "exome". Several genes were found to be involved in the pathogenesis of SLE, the majority of which were associated with B- and T-cell abnormal activation. The results of different studies revealed an association of active SLE with the increase in specific circulating autoantibodies. Based on the results of the current review, a preliminary list of autoantibodies, genes and cytokines associated with the development of SLE was prepared. Future directions will include the assessment of association of genes, antibodies and cytokines in SLE patients from the local Kazakh population, and the development of genetic-immunologic panel for the early diagnosis of this disease.

Keywords: systemic lupus erythematosus, genes, antibodies, cytokines.

Background

The urgency of the problem of autoimmune rheumatic diseases is determined by their high prevalence, the severity of the course with rapid disability, unfavourable life prognosis and the difficulty of early diagnosis. Autoimmune diseases represent a global burden on public health with an estimated incidence of 4.5%, disproportionately affecting women (6.4% of women compared to 2.7% of men) [1]. The prevalence of autoimmune diseases steadily rising, the incidence rate is about 3-9% per year according to the report of the British Society of Immunologists [2].

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with multiple organs impairment, a broad variety of clinical manifestations and life-threatening complications [3]. The mortality in SLE is 3-4 times higher than in general population with the young patients more preferably have SLE as the cause of death [4-6].

Ethnicity plays a vital role in the development of the disease. African Americans are 5-9 times more likely to develop SLE than Americans of European descent; in addition, they usually develop a more severe form of SLE [7]. Māori had worse prognosis in SLE mortality than others in New Zealand [6].

A few studies of SLE in Kazakhstan showed a 62.8% rise in the incidence of this disease from 2012 to 2017 year [8, 9]. Delayed verification of the diagnosis still exists, which highlighted the need of new tools for early diagnosis.

Little is known about the mechanisms underlying the observed differences between ethnic groups. Nevertheless, it is mentioned that differences in human leukocyte antigen (HLA) regions contribute to this fact. Tissue damage often leads to cell death and subsequent presentation of intracellular and nuclear components to adaptive immune cells, their activation and, as a result, to the production of autoantibodies and/or a self-directed lymphocytic response with the production of a large number of cytokines [10]. Finally, the gradual progression of LSE lead to serious damage to organs and tissues often developing even before the autoimmune disease can be diagnosed clinically.

The pathogenesis of SLE is compound; it is caused by the interaction of genetic and environmental factors leading to loss of immune tolerance and the occurrence of an autoimmune response. An analysis of the results of relevant global studies about genetic mutations, spectrum of antibodies and cytokines in patients with SLE due to identify commonalities, differences and gaps in existing knowledge could improve understanding of etiopathogenesis of this disease. This observation confirms the need for immunological and genetic studies aimed at improving the diagnosis of autoimmune diseases in the early stages, before irreversible damage of organs and tissues occurs. The importance of understanding the fundamental mechanisms of pathogenesis stimulated the search for new diagnostic methods.

The modern approach for the treatment of SLE is based on timely diagnosis, which determines the possibility of initiating treatment at an early stage of the disease ("window of therapeutic opportunities"). There is a therapeutic window in the pre-SLE phase, in which pathological processes can be stopped more quickly and effectively and the development of the disease prevented. Modern diagnostic technologies make it possible to detect autoimmune disease at an early stage in most patients, however, in some cases, the clinical picture, laboratory and instrumental methods do not provide complete data for the diagnosis. This requires the earliest and most complete diagnosis, which includes not only immunological tests, but also genetic analysis.

Objective

The aim of the study is to assess the antibodies, cytokines and genetic mutations involved in the pathogenesis of SLE by a systematic review and to develop a panel of genetic and immunological markers for early diagnosis of autoimmune diseases.

Methods

Possible genetic mutations, autoantibodies and cytokines produced during autoimmune aggression in the SLE were selected based on a systematic review of the available data from biomedical literature platforms, as well as comparison with available genetic panels of world manufacturers.

The literature search was made on the PubMed and Google Scholar platforms. A combination of keywords was used, including "systemic lupus erythematosus (SLE)", "genetic mutations", "autoantibodies", "cytokine production", "sequencing", "NGS" and "exome". Full-text publications in English devoted to these topics were included in the review. Retrieved articles were screened for relevance and included if they provided insight into the prevalence of genetic mutations, autoantibodies, or cytokine profiles from the research with targeted autoimmune disease. Exclusion criteria involved literature reviews, abstracts and other forms of small publications. Priority was given to peer-reviewed studies, systematic reviews and meta-analyses. In addition, Ion AmpliSeq™ Designer gene repository was used to allocate disease related genes. A schematic search strategy is shown in the Figure 1.

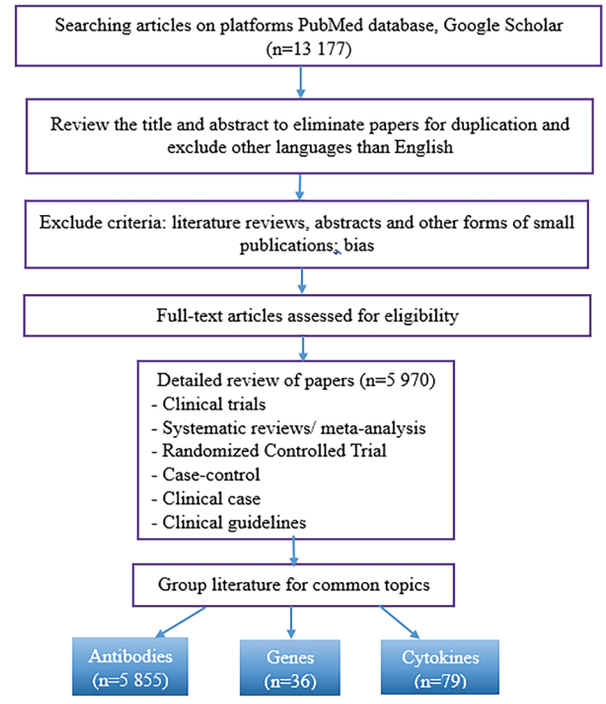

Figure 1 - Search strategy (n – number of articles).

Results

For the period from 1990-2023, the amount of 13 177 publications were found on the topic presented above, of which only 5 970 publications met the criteria of human research or systematic reviews and were included in this analysis.

The results of different studies revealed an association of active disease with increase in specific circulating autoantibodies and cytokines in SLE patients. The amount of 5 855 studies discovered the broad range of SLE autoantibodies. Autoantibody against cell nuclei (antinuclear antibodies, ANA) and other intranuclear antibodies production including double stranded DNA (dsDNA) were suggested as a hallmark of SLE [11]. The following antibodies were mentioned in the majority of the research: anti dsDNA first, then SS-A 60 kDA, SS-A 52 kDA, AFA, anti-Smith, anti U1RNP, anti Cq1 and La. The criteria for SLE recommended by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) included dsDNA and Sm nucleoprotein as relatively specific for SLE [12]. However, other antibodies assist to investigate the diagnosis of SLE as well. Some of them represented the link with special syndromes or symptoms. Thus, anti-SSA/Ro60 and anti-Ro52 were found to be positively associated with photosensitivity and xerophtalmia/xerostomia [13]. Additionally, hypocomplementemia was associated with anti-SSA/Ro60, while anti-Ro52 and anti-RNP antibodies were found to be correlated with Raynaud's syndrome [13, 14]. Anti-Sm antibodies were detectable in 5-30% of SLE patients (preferably in those who have the activity of renal involvement) and were included in the serological criteria for diagnosing of SLE [14]. Anti-C1q is known to be important in the inflammatory processes of SLE, but mainly associated with the hypocomplementemia and renal involvement [15, 16].

There is evidence of the association of different interleukins, both inflammatory and immunoregulatory, with SLE development. The excessive cytokine production was found in 79 studies reflecting both innate and adaptive immune system activation. Thus, over the past decade the type I interferon (IFN) was shown to play a vital role in SLE by promoting feedback loops, disturbing immune tolerance and enhance

disease activity. Analysis of the 79 research articles revealed cytokines commonly implicated in lupus, including IFN (IFN-α, IFN-β), TNF-α and IL-1β [17]. It was also showed an increase of cytokines IL-21, IL-17, IL-21, BlyS, IL-18, IL-2, IL-5 and IL-6 cytokines in serum obtained from patients with SLE [18]. Certain cytokines and autoantibodies contribute to more severe course of the disease. Thus, recent studies have shown that high levels of IL-5, IL-6, IL-4, IFN-γ were registered three years before clinical manifestation of SLE or even appearance of SLE-associated autoantibodies [19, 20].

Autoimmune diseases such as SLE, rheumatoid arthritis, and systemic sclerosis present complex pathogeneses with significant genetic predispositions. Current genetic screenings are insufficient in capturing the broad spectrum of genetic variations associated with these conditions. Several studies indicated the involvement of the following genes in the pathogenesis of SLE: BANK1, CD4, C4B, IRAK4, ORF2p, P2RX7, TREX1, BCR, LAG3, P40, PTPN22, TAM, TLR7, BLK, CR2, CXCL10, IL-1β, LTK, P450, RASGRP1, TLR5, CTLA4, FCGR2B, IL-18, MIF, PDCD1, RTK, TNFSF4, CCL19, C4A, NCF2, TNFAIP3, IFIT1, MX1, LY6E, ISG15, CXCL13, STAT1, STAT2, STAT3, PDGF, HAS2, SLC5A11 [21-23] (table 1). The majority of these genes were associated with B- and T-cell function and signaling pathways.

Currently the interferon-related immune pathway was detailly studied and showed the importance in the pathogenesis of SLE. IFIT1(interferon-induced with tetratricopeptide repeats 1) was the first gene described as a candidate gene for SLE. An overexpression of IFIT1 was confirmed in SLE patients included children SLE [24, 25]. IFIT1 interact with Rho/Rac guanine nucleotide exchange factor and activate Rho proteins which might be a potential target for novel tools in SLE therapy [24].

It is well-known that abnormal stimulation of innate immunity in SLE happens through Toll-like receptors. The toll-like receptor-7 (TLR-7) genetic variations (rs179019 and rs179010) are associated with an elevated risk of developing SLE according to meta-analysis of 15,472 SLE cases and 16,721 healthy controls [26]. Resent research reported mutations in TREX1 in SLE patients (especially SLE with neurological manifestations) that contribute to the accumulation of nucleic acid [27].

Human complement component 4 (C4) play the critical role in the processing of immune complexes in the pathogenesis of SLE. SLE patients are characterised by genetic deficiency for both C4A and C4B with low gene copy-number and decreased serum protein levels [28, 29]. The deletion of the C4A gene detected in different HLA haplotypes and might be suggested as a common genetic marker associated with SLE susceptibility [29]. Moreover, it was shown that low C4 gene copy number (GCN) in SLE were associated with the higher disease damage by Systemic Lupus International Collaborating Clinics – Damage Index (SLICC-DI) and serositis, while low C4B GCN was found in patients with arthritis [30]. The sodium-dependent glucose cotransporter (SLC5A11) was associated with low C4, anti-Sm, clinical serositis. It interacts with immune-related gene and might induce apoptosis through the TNF-alpha and PDCD1 pathway [31].

Proteomic quantification and genes sequencing of active SLE and inactive SLE were performed to determine biomarkers of active SLE [32]. Li et al. (2022) identified IFIT3, MX1, TOMM40, STAT1, STAT2, and OAS3 combination as biomarker for SLE diagnosis, while PHACTR2, GOT2, L-selectin, CMC4, MAP2K1, CMPK2, ECPAS, SRA1, and STAT2 were significantly increased in active SLE and may be uses for activity monitoring [32].

The genes HCY, HDL, GTF21, RIPK1, TYK2.34 were found to allocate SLE from Ion AmpliSeq™ Designer gene repository and double checked in GeneCards®: The Human Gene Database.

According to the data obtained, a comprehensive gene Next-Generation Sequencing panel was developed to identify germline mutations implicated in SLE, leveraging extensive PubMed and Google Scholar data and robust in-silico analyses. The finalized gene panel exhibited high diversity, covering key research areas in skin and connective tissue diseases, musculoskeletal disorders, and immune system pathologies. Preliminary in-silico assessments demonstrate a coverage of >99% and gene uniformity of >90% in wet-lab conditions, indicating a high potential to detect known and novel pathogenic variants.

Discussion

Autoimmune diseases are characterized by unregulated activation of immune cells, production of a broad range of autoantibodies and cytokines, which impair various organs and tissues. Nowadays, worldwide, chronic and steadily progressive autoimmune diseases affect approximately 5-10% of the population, while recently there has been an increase in the incidence, which promote a pronounced disability of able-bodied population [68, 69]. Early diagnosis of autoimmune diseases is extremely important, since timely therapy prevents the development of structural lesions and reduces the risk of disability of patients.

An increase in chronic and steadily progressing autoimmune diseases contributes to the growth of disability of the young, able-bodied population. SLE deserves great attention as the heterogeneous autoimmune disease with the life-threatening complications [3]. Over the past decade, the scope of therapeutic approaches has increased, however, there is still no cure for this disease.

The pathophysiology of autoimmune diseases is related to genetic mutations that cause the disease, or to a combination of genetic predisposition and epigenetic modifications resulting from environmental contact [70].

Autoimmune diseases such as SLE, rheumatoid arthritis, and systemic sclerosis present complex pathogeneses with significant genetic predispositions. Current genetic screenings are insufficient in capturing the broad spectrum of genetic variations associated with these conditions. The concordance rates of SLE consisted in 14.3–40% for monozygotic twins [71, 72]. These observations led to hypotheses that disease-causing mutations of a single gene in rare autoimmune diseases may result in the development of the disease, but do not predict outcomes [70].

Sequencing of the first human genome took place in 2003 [2]. Since then, advances in sequencing technologies allowed to reduce costs and enabled the generation of massive volumes of high-quality human sequence data used to create considerable catalogues of both population and disease-causing variations. The presence of such databases means that we can investigate all types of genetic variations of many diseases [2]. Genetic research identified different loci associated with autoimmune diseases. Several studies have proven the contribution of rare and suspected variants of pathogenic genes to the development of autoimmune diseases, for example, SLE. It was discovered that the missense variant rs35677470 of deoxyribonuclease I-like 3 (DNASE1L3) gene was associated with the development of SLE [73]. Mutations in C1QA, C1QC, C2, DNASE1L3 and IKZF1 were considered as congenital immune disorders and accounted for 7% of cases of SLE with onset in childhood, studied by a

Table 1 The genes included in the pathogenesis of SLE: currently available research data.

N	Gene abbreviation	Gene name	Summary (role, mechanism of working, findings)	Reference
Significant genes associated with susceptibility to the development of SLE				
1.	BANK1	B-cell scaffold protein with ankyrin repeats 1	Polymorphisms in BANK1 is associated with susceptibility to SLE Promote Lyn-mediated tyrosine phosphorylation of inositol 1,4,5-trisphosphate receptors	[33]
2.	BCR	Breakpoint cluster region	Patients with SLE showed increased BCR clonotypes in comparison with healthy people.	[34]
3.	BLK	BLK proto-oncogene, Src family tyrosine kinase	The risk variant rs922483 in the BLK gene is strongly associated with regulation of BLK mRNA and protein expression in B cells	[35]
4.	CR2	Complement component 3d receptor 2	CR2 connects with Complement component C3 and transit signals through CD19, so decrease the activation threshold of B-cells.	[36]
5.	CTLA4	Cytotoxic T-lymphocyte associated protein 4	CTLA-4 participates in regulation of signals by T-cells receptors	[37]
6.	CXCL10	C-X-C motif chemokine ligand 10	CXCL10 increases migration of proinflammatory cells through the activation of ERK	[38]
7.	IL1B	Interleukin 1 beta	Proinflammatory cytokine, play a key role in inflammatory and immune reactions	[39]
8.	ISG15	ISG15 ubiquitin-like modifier	SLE patients with lymphocytopenia have high ISG15 expression	[40]
9.	LTK	Leukocyte receptor tyrosine kinase	SLE is associated with LTK	[41]
10.	NCF2	Neutrophil cytosolic factor 2	NCF2 is a subunit of the NADPH enzyme that produces superoxide in the phagosomes of neutrophils and other phagocytic leukocytes	[42]
11.	PDCD1	Programmed cell death 1	An inhibitory receptor on antigen-activated T cells Plays a key role in the maintenance of immune self-tolerance	[43]
12.	PTPN22	Protein tyrosine phosphatase, non-receptor type 22	Participates in regulating CBL function in the T-cell receptor signaling pathway Mutations in PTPN22may be associated with SLE and rheumatoid arthritis	[44]
13.	RASGRP1	RAS guanyl releasing protein 1	Activates the Erk/MAP kinase cascade Regulates the development and differentiation of T- and B-cells Altered expression of various isoforms of this protein cause susceptibility to SLE	[45]
14.	SLC5A11	Solute carrier family 5 member 11	Induces apoptosis via the TNF-alpha, PDCD1 pathway associated with low C4, anti-Sm, clinical serositis	[31, 46]
15.	STAT1	Signal transducer and activator of transcription 1	SLE T- and B-cells characterised by the increased levels of STAT1 transcript enhance STAT1 signaling responses to IFN·	[32, 47]
16.	STAT2	Signal transducer and activator of transcription 2	In response to cytokines and cell growth elements, STATs phosphorylate receptors associated with kinases Than release homo- or heterodimers that translocate into the cell, where they act as transcriptional activators In response to interferon (IFN), this protein forms a complex with STAT1 and the IFN regulatory factor p48 protein group (ISGF3G), mediating innate antiviral activity	[32]
17.	STAT3	Signal transducer and activator of transcription 3	STAT3 plays a key role in the differentiation of Th17, T follicular helper and B-cells STAT3 inhibition may represent a possible future therapeutic target in SLE	[47]
18.	TLR5	Toll like receptor 5	This gene encodes a member of the toll-like receptor (TLR) family Play a key role in pathogen recognition and activation of innate immune responses Recognizes specific pathogen-associated molecular patterns that are expressed on infectious agents Activation of this receptor mobilizes the nuclear factor NF-kappa B, which activates a variety of target genes associated with inflammation	[48]
19.	TNFAIP3	Tumor necrosis factor alpha induced protein 3	The encoded protein: inhibit NF-kappa B activation and TNF-mediated apoptosis involved in cytokine-mediated immune responses	[49]

20.	TLR-7	Toll like receptor 7	Enhanced TLR7 signaling leads to aberrant survival of B- cell receptor (BCR)-activated B-cells and cell-intrinsic accumulation of CD11c+ aged B cells Increases follicular and extrafollicular helper T-cells	[26, 50]
21.	TREX1	Three prime repair exonuclease 1	TREX1 deficiency result in: the accumulation of cytosolic DNA as well as activation of the cGAS-STING-IFN signaling pathway tissue inflammation and autoimmune diseases	[27, 51]
22.	C4B	Complement C4B null allele	Deficiency of the complement C4B null allele leads to the inflammatory, infectious or chronic autoimmune conditions included SLE	[28]
23.	C4A	*C4* complement components	Associates with the presence of SSA/SSB autoantibodies The copy number of *C4* correlates with C4 level in plasma	[29, 52]
24.	LAG3	Lymphocyte activation gene 3	High LAG3 expression associated with T cell dysfunction Decrease of LAG3 cause impaired immune tolerance and may lead to autoimmune conditions	[53]
25.	TAM	Tyro3, Axl, and Mer receptor tyrosine kinases	Participates in the apoptotic cell clearance and immune responses TAM deficiency leads to SLE	[54]
26.	RTK	TAM receptor tyrosine kinase	Recognises apoptotic cells Circulating TAM receptors have an immunoregulatory function May be a biomarker for SLE prognosis	[54]
27.	IFIT1	Interferon-induced with tetratricopeptide repeats 1	Highly expressed in SLE in children IFIT1 interact with Rho/Rac guanine nucleotide exchange factor May activate Rho proteins	[25]
Genes increased during active disease				
1.	CD4	CD4 molecule	Activation of CD4 (+) T-cells is a key factor for SSc and SLE Realise proinflammatory cytokines	[55]
2.	CXCL13	C-X-C motif chemokine ligand 13	CXCL13 attracts C-X-C chemokine receptor type 5 (CXCR5)-expressing B cells and T follicular helper cells to the follicle	[56]
3.	IL18	Interleukin 18	Proinflammatory cytokine; play a key role in immune reactions and activation of Th1 cells	[57]
4.	IRAK4	Interleukin 1 receptor associated kinase 4	Key regulator of native immune response	[58]
5.	MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	MIF works as a mediator of innate immunity by promoting host inflammatory responses through the induction of proinflammatory cytokines	[59]
6.	MX1	MX dynamin like GTPase 1	Patients with SLE and anti Smith (Sm), RNP, Ro/SSA или La/SSB have higher level of MX1	[60]
7.	PDGFB	Platelet derived growth factor subunit B	Participats in the stimulation of pronounced hyperplasia of fibroblast-like cells	[61]
8.	P2RX7	Purinergic receptor P2X 7	Receptor P2X7 induce release of proinflammatory molecules (IL1β, proteases)	[62]
9.	STAT2	Signal transducer and activator of transcription 2	In response to cytokines and cell growth elements, STATs phosphorylate receptors associated with kinases and then release homo- or heterodimers that translocate into the cell cell, where they act as transcriptional activators. In response to interferon (IFN), this protein forms a complex with STAT1 and the IFN regulatory factor p48 protein group (ISGF3G), mediating innate antiviral activity.	[32]
10.	C4A	*C4* Complement components	There is an association between low C4A gene copy number and SLE severity.	[30]
11.	LY6E	Lymphocyte antigen 6 complex, locus E	Increased level of LY6E was found in SLE patients with low C4 levels Active SLE patients were characterised by elevated LY6E in comparison with patients in remission. LY6E expression levels correlated with SLEDAI-2K scores.	[63, 64]
12.	P40	Protein subunit of interleukins 12	SLE antibodies reactive with p40 Higher in SLE patients with flare	[65]
13.	CCL19	Chemokine (C-C motif) ligand 19, MIP-3B	The level of CCL19 in serum is higher in patients with active SLE Potential marker for disease activity in SLE	[66]
14.	HAS2	Hyaluronan synthase 2	Plays a key role in fibrosis The level of HAS2 increases in the active phase of lupus nephritis	[67]

genetic analysis of British and French cohorts [74]. However, according to the growing number of known autoimmune conditions, the interindividual variability of phenotypes and outcomes, pathophysiology of SLE is not fully understood [75].

Now studies show that there are a relatively large number of risk loci associated with the development of SLE, but specific genes responsible for the development of pathogenetic mechanisms have not been found. According to the modern concept of understanding autoimmune diseases, SLE might be determined as a disease without single monogenic cause, but with genetic predisposition, which can be realised under the influence of environmental factors that determine the manifestation of the disease [10, 76]. Recently it was identified more than hundred gene loci associated with the development of SLE [77]. The gene loci associated with susceptibility to SLE may also contribute to the development of other autoimmune diseases [77]. And the same tendency may be seen in the other direction, having a family history of autoimmune disease increases the risk of SLE [78].

The study of genetic mutations in SLE can shed light on certain aspects of the aetiology of autoimmune diseases, and their connection with the activity of pathological process with the production of autoantibodies and cytokines.

Identification of gene mutations occurring in SLE and their associations with the severity of the disease, the production of autoantibodies and cytokines is necessary, given the importance of understanding the development of the disease at the genetic level. The modern strategy of therapy is based on early diagnosis, which determines the possibility of initiating treatment at a very early stage of the disease ("window of therapeutic possibilities") with appointment of active therapy. However, this requires the earliest and most complete diagnosis, which includes not only immunological tests, but also genetic analysis. Autoimmune diseases are variable in their clinical manifestations, organ and tissue injury, laboratory analysis, response to therapeutically agents; all of these differences might be associated with genetic variability [79]. The strategy of searching for pathogenic genetic mutations and their associations with the production of autoantibodies and cytokine secretion is crucial for the development of successful personalised programs for patients with severe autoimmune diseases such as SLE [2].

The applicability of the research results for development of healthcare consists in the improvement of the diagnostic tool for early diagnosis and initiation of therapy. The applicability of the results for science consists in the expanding the range of knowledge about genetic mutations and associations with the production of antibodies, cytokines and the severity of the disease. Due to include these cytokines analysis and genes sequencing into routine practice firstly it would be necessarily to assess them in SLE patients of Kazakh origin. Currently we collected material more over 100 patients and started the investigation. After that the list of autoantibodies, genes and cytokines will be propose for implementation to the Healthcare of Kazakhstan.

Conclusion

Based on the results of the current review, a preliminary list of autoantibodies, genes and cytokines associated with the development of SLE was prepared. The immunological-genetic panel made according to this review offers a targeted approach to uncover the genetic basis of SLE, facilitating the early diagnosis, personalized treatment and a deeper understanding of the molecular mechanisms underlying this autoimmune disease.

This is the first attempt of genetic examination of SLE patients in Kazakhstan during which we hope to investigate new panel.

Prospects for further research

Future directions will include the investigation of the unique genetic and immunological patterns on autoimmune diseases in the local Kazakh population. Ongoing work will validate gene panel in a clinical setting and assess its utility in patient stratification, prognostic evaluations, and as a tool for unveiling the genetic landscape of autoimmune diseases.

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References

1. Hayter SM, Cook MC. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. *Autoimmunity reviews*. 2012;11(10):754-765. https://doi.org/10.1016/j.autrev.2012.02.001.
2. Field MA. Detecting pathogenic variants in autoimmune diseases using high-throughput sequencing. *Immunology & Cell Biology*. 2021;99(2):146-156. https://doi.org/10.1111/imcb.12372.
3. Wallace B, Vummidi D, Khanna D. Management of connective tissue diseases associated interstitial lung disease: a review of the published literature. *Curr Opin Rheumatol*. 2016;28(3):236-245. https://doi.org/10.1097/bor.0000000000000270.
4. Rinaldi S, Doria A, Salaffi F, Ermani M, Iaccarino L, Ghirardello A, Zampieri S, Sarzi-Puttini P, Gambari PF, Perini G. Health-related quality of life in Italian patients with systemic lupus erythematosus. I. Relationship between physical and mental dimension and impact of age. *Rheumatology (Oxford, England)*. 2004;43(12):1574-1579. https://doi.org/10.1093/rheumatology/keh397.
5. Olesińska M, Saletra A. Quality of life in systemic lupus erythematosus and its measurement. *Reumatologia*. 2018;56(1):45-54. https://doi.org/10.5114/reum.2018.74750.
6. Lao C, White D, Rabindranath K, Dantzig PV, Foxall D, Lawrenson R. Mortality and causes of death in systemic lupus erythematosus in New Zealand: a population-based study. *Rheumatology*. 2023. https://doi.org/10.1093/rheumatology/kead427.
7. Lewis MJ, Jawad AS. The effect of ethnicity and genetic ancestry on the epidemiology, clinical features and outcome of systemic lupus erythematosus. *Rheumatology (Oxford, England)*. 2017;56(suppl_1):i67-i77. https://doi.org/10.1093/rheumatology/kew399.

8. Issayeva B, Aseeva E, Saparbayeva M, Issayeva S, Kulshymanova M, Kaiyrgali SM, Amanzholov A, Bizhanova M, Kalykova M, Solovyev S, et al. Features of clinical manifestations, course, outcomes and health related quality of life in patients with systemic lupus erythematosus in the Republic of Kazakhstan. *Rheumatology Science and Practice.* 2022;60:602-611. https://doi.org/10.47360/1995-4484-2022-602-611.
9. Isayeva B, Saparbayeva M, Isayeva S, Kulshymanova M, Kaiyrgali SM, Bizhanova M, Nurgaliyev K, Kosherbayeva L, Kulymbetova B, Rysbekova K, et al. The current status of the problem with systemic lupus erythematosus in Kazakhstan. *Modern Rheumatology Journal.* 2020;14:32-39. https://doi.org/10.14412/1996-7012-2020-1-32-39.
10. Hedrich CM. Shaping the spectrum - From autoinflammation to autoimmunity. *Clinical immunology (Orlando, Fla)*. 2016;165:21-28. https://doi.org/10.1016/j.clim.2016.03.002.
11. Kubota T. An Emerging Role for Anti-DNA Antibodies in Systemic Lupus Erythematosus. *Int J Mol Sci.* 2023;24(22). https://doi.org/10.3390/ijms242216499.
12. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, Smolen JS, Wofsy D, Boumpas DT, Kamen DL. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Arthritis & rheumatology.* 2019;71(9):1400-1412.
13. Menéndez A, Gómez J, Caminal-Montero L, Díaz-López JB, Cabezas-Rodríguez I, Mozo L. Common and specific associations of anti-SSA/Ro60 and anti-Ro52/TRIM21 antibodies in systemic lupus erythematosus. *TheScientificWorldJournal.* 2013;2013:832789. https://doi.org/10.1155/2013/832789.
14. Migliorini P, Baldini C, Rocchi V, Bombardieri S. Anti-Sm and anti-RNP antibodies. *Autoimmunity*. 2005;38(1):47-54. https://doi.org/10.1080/08916930400022715.
15. Trendelenburg M. Autoantibodies against complement component C1q in systemic lupus erythematosus. *Clinical & translational immunology*. 2021;10(4):e1279. https://doi.org/10.1002/cti2.1279.
16. Stojan G, Petri M. Anti-C1q in systemic lupus erythematosus. *Lupus*. 2016;25(8):873-877. https://doi.org/10.1177/0961203316645205.
17. Inoue K, Ishizawa M, Kubota T. Monoclonal anti-dsDNA antibody 2C10 escorts DNA to intracellular DNA sensors in normal mononuclear cells and stimulates secretion of multiple cytokines implicated in lupus pathogenesis. *Clinical and experimental immunology*. 2020;199(2):150-162. https://doi.org/10.1111/cei.13382.
18. Ohl K, Tenbrock K. Inflammatory cytokines in systemic lupus erythematosus. *Journal of biomedicine & biotechnology.* 2011;2011:432595. https://doi.org/10.1155/2011/432595.
19. Lu R, Munroe ME, Guthridge JM, Bean KM, Fife DA, Chen H, Slight-Webb SR, Keith MP, Harley JB, James JA. Dysregulation of innate and adaptive serum mediators precedes systemic lupus erythematosus classification and improves prognostic accuracy of autoantibodies. *Journal of autoimmunity.* 2016;74:182-193. https://doi.org/10.1016/j.jaut.2016.06.001.
20. Nepal D, Gazeley D. Role of IL-6 and IL-6 targeted therapy in systemic lupus erythematosus. *Rheumatology (Oxford, England)*. 2023;62(12):3804-3810. https://doi.org/10.1093/rheumatology/kead416.
21. Cunninghame Graham DS, Graham RR, Manku H, Wong AK, Whittaker JC, Gaffney PM, Moser KL, Rioux JD, Altshuler D, Behrens TW, et al. Polymorphism at the TNF superfamily gene TNFSF4 confers susceptibility to systemic lupus erythematosus. *Nature genetics.* 2008;40(1):83-89. https://doi.org/10.1038/ng.2007.47.
22. Wang YF, Zhang Y, Zhu Z, Wang TY, Morris DL, Shen JJ, Zhang H, Pan HF, Yang J, Yang S, et al. Identification of ST3AGL4, MFHAS1, CSNK2A2 and CD226 as loci associated with systemic lupus erythematosus (SLE) and evaluation of SLE genetics in drug repositioning. *Ann Rheum Dis.* 2018;77(7):1078-1084. https://doi.org/10.1136/annrheumdis-2018-213093.
23. Díaz-Barreiro A, Bernal-Quirós M, Georg I, Marañón C, Alarcón-Riquelme ME, Castillejo-López C. The SLE variant Ala71Thr of BLK severely decreases protein abundance and binding to BANK1 through impairment of the SH3 domain function. *Genes and immunity.* 2016;17(2):128-138. https://doi.org/10.1038/gene.2016.1.
24. Ye S, Pang H, Gu YY, Hua J, Chen XG, Bao CD, Wang Y, Zhang W, Qian J, Tsao BP, et al. Protein interaction for an interferon-inducible systemic lupus associated gene, IFIT1. *Rheumatology (Oxford, England).* 2003;42(10):1155-1163. https://doi.org/10.1093/rheumatology/keg315.
25. Li H, Wang T, Li B, Huang T, Hai Y, Huang C, Xiang W. Bioinformatic analysis of immune-related transcriptome affected by IFIT1 gene in childhood systemic lupus erythematosus. *Translational pediatrics.* 2023;12(8):1517-1526. 10.21037/tp-23-365.
26. Ranjan S, Panda AK. Association of Toll-Like Receptor 7 (TLR7) Polymorphisms with Predisposition to Systemic Lupus Erythematosus (SLE): A Meta and Trial Sequential Analysis. *Biochemical genetics.* 2023. https://doi.org/10.1007/s10528-023-10600-9.
27. Fredi M, Bianchi M, Andreoli L, Greco G, Olivieri I, Orcesi S, Fazzi E, Cereda C, Tincani A. Typing TREX1 gene in patients with systemic lupus erythematosus. *Reumatismo*. 2015;67(1):1-7. https://doi.org/10.4081/reumatismo.2015.782.
28. Zhou D, Rudnicki M, Chua GT, Lawrance SK, Zhou B, Drew JL, Barbar-Smiley F, Armstrong TK, Hilt ME, Birmingham DJ, et al. Human Complement C4B Allotypes and Deficiencies in Selected Cases With Autoimmune Diseases. *Front Immunol.* 2021;12:739430. https://doi.org/10.3389/fimmu.2021.739430.
29. Kemp ME, Atkinson JP, Skanes VM, Levine RP, Chaplin DD. Deletion of C4A genes in patients with systemic lupus erythematosus. *Arthritis and rheumatism*. 1987;30(9):1015-1022. https://doi.org/10.1002/art.1780300908.
30. Pereira KMC, Perazzio S, Faria AGA, Moreira ES, Santos VC, Grecco M, da Silva NP, Andrade LEC. Impact of C4, C4A and C4B gene copy number variation in the susceptibility, phenotype and progression of systemic lupus erythematosus. *Advances in Rheumatology.* 2019;59(1):36. https://doi.org/10.1186/s42358-019-0076-6.
31. Tsai LJ, Hsiao SH, Tsai LM, Lin CY, Tsai JJ, Liou DM, Lan JL. The sodium-dependent glucose cotransporter SLC5A11 as an autoimmune modifier gene in SLE. *Tissue antigens.* 2008;71(2):114-126. https://doi.org/10.1111/j.1399-0039.2007.00975.x.
32. Li Y, Ma C, Liao S, Qi S, Meng S, Cai W, Dai W, Cao R, Dong X, Krämer BK, et al. Combined proteomics and single cell RNA-sequencing analysis to identify biomarkers of disease diagnosis and disease exacerbation for systemic lupus erythematosus. *Front Immunol.* 2022;13:969509. https://doi.org/10.3389/fimmu.2022.969509.
33. Jiang SH, Athanasopoulos V, Ellyard JI, Chuah A, Cappello J, Cook A, Prabhu SB, Cardenas J, Gu J, Stanley M, et al. Functional rare and low frequency variants in BLK and BANK1 contribute to human lupus. *Nature communications.* 2019;10(1):2201. https://doi.org/10.1038/s41467-019-10242-9.

34. Zheng F, Xu H, Zhang C, Hong X, Liu D, Tang D, Xiong Z, Dai Y. Immune cell and TCR/BCR repertoire profiling in systemic lupus erythematosus patients by single-cell sequencing. *Aging*. 2021;13(21):24432-24448. https://doi.org/10.18632/aging.203695.
35. Simpfendorfer KR, Olsson LM, Manjarrez Orduño N, Khalili H, Simeone AM, Katz MS, Lee AT, Diamond B, Gregersen PK. The autoimmunity-associated BLK haplotype exhibits cis-regulatory effects on mRNA and protein expression that are prominently observed in B cells early in development. *Human molecular genetics.* 2012;21(17):3918-3925. https://doi.org/10.1093/hmg/dds220.
36. Soto L, Ferrier A, Aravena O, Fonseca E, Berendsen J, Biere A, Bueno D, Ramos V, Aguillón JC, Catalán D. Systemic Sclerosis Patients Present Alterations in the Expression of Molecules Involved in B-Cell Regulation. *Front Immunol.* 2015;6:496. https://doi.org/10.3389/fimmu.2015.00496.
37. Flores-Borja F, Jury EC, Mauri C, Ehrenstein MR. Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis. *Proceedings of the National Academy of Sciences of the United States of America.* 2008;105(49):19396-19401. https://doi.org/10.1073/pnas.0806855105.
38. Lee JH, Kim B, Jin WJ, Kim HH, Ha H, Lee ZH. Pathogenic roles of CXCL10 signaling through CXCR3 and TLR4 in macrophages and T cells: relevance for arthritis. *Arthritis Res Ther.* 2017;19(1):163. https://doi.org/10.1186/s13075-017-1353-6.
39. 39. Mohammadoo-Khorasani M, Salimi S, Tabatabai E, Sandoughi M, Zakeri Z, Farajian-Mashhadi F. Interleukin-1β (IL-1β) & IL-4 gene polymorphisms in patients with systemic lupus erythematosus (SLE) & their association with susceptibility to SLE. *The Indian journal of medical research.* 2016;143(5):591-596. https://doi.org/10.4103/0971-5916.187107.
40. Shen M, Duan C, Xie C, Wang H, Li Z, Li B, Wang T. Identification of key interferon-stimulated genes for indicating the condition of patients with systemic lupus erythematosus. *Front Immunol.* 2022;13:962393. https://doi.org/10.3389/fimmu.2022.962393.
41. Shao WH, Cohen PL. The role of tyrosine kinases in systemic lupus erythematosus and their potential as therapeutic targets. *Expert Rev Clin Immunol.* 2014;10(5):573-582. https://doi.org/10.1586/1744666x.2014.893827.
42. Bakutenko IY, Haurylchyk ID, Nikitchenko NV, Sechko EV, Kozyro IA, Tchitchko AM, Batyan GM, Sukalo AV, Ryabokon NI. Neutrophil cytosolic factor 2 (NCF2) gene polymorphism is associated with juvenile-onset systemic lupus erythematosus, but probably not with other autoimmune rheumatic diseases in children. *Molecular genetics & genomic medicine.* 2022;10(1):e1859. https://doi.org/10.1002/mgg3.1859.
43. Chua KH, Lian LH, Sim XJ, Cheah TE, Lau TP. Association between PDCD1 Gene Polymorphisms and Risk of Systemic Lupus Erythematosus in Three Main Ethnic Groups of the Malaysian Population. *Int J Mol Sci.* 2015;16(5):9794-9803. https://doi.org/10.3390/ijms16059794.
44. Román-Fernández IV, Machado-Contreras JR, Muñoz-Valle JF, Cruz A, Salazar-Camarena DC, Palafox-Sánchez CA. Altered PTPN22 and IL10 mRNA Expression Is Associated with Disease Activity and Renal Involvement in Systemic Lupus Erythematosus. *Diagnostics (Basel, Switzerland)*. 2022;12(11). https://doi.org/10.3390/diagnostics12112859.
45. Molineros JE, Singh B, Terao C, Okada Y, Kaplan J, McDaniel B, Akizuki S, Sun C, Webb CF, Looger LL, et al. Mechanistic Characterization of RASGRP1 Variants Identifies an hnRNP-K-Regulated Transcriptional Enhancer Contributing to SLE Susceptibility. *Front Immunol.* 2019;10:1066. https://doi.org/10.3389/fimmu.2019.01066.
46. Lanata CM, Paranjpe I, Nititham J, Taylor KE, Gianfrancesco M, Paranjpe M, Andrews S, Chung SA, Rhead B, Barcellos LF, et al. A phenotypic and genomics approach in a multi-ethnic cohort to subtype systemic lupus erythematosus. *Nature communications.* 2019;10(1):3902. https://doi.org/10.1038/s41467-019-11845-y.
47. Goropevšek A, Holcar M, Avčin T. The Role of STAT Signaling Pathways in the Pathogenesis of Systemic Lupus Erythematosus. *Clinical reviews in allergy & immunology.* 2017;52(2):164-181. https://doi.org/10.1007/s12016-016-8550-y.
48. Petrackova A, Horak P, Radvansky M, Skacelova M, Fillerova R, Kudelka M, Smrzova A, Mrazek F, Kriegova E. Cross-Disease Innate Gene Signature: Emerging Diversity and Abundance in RA Comparing to SLE and SSc. *Journal of immunology research*. 2019;2019:3575803. https://doi.org/10.1155/2019/3575803.
49. Kim T, Bae SC, Kang C. Synergistic activation of NF-κB by TNFAIP3 (A20) reduction and UBE2L3 (UBCH7) augment that synergistically elevate lupus risk. *Arthritis Res Ther.* 2020;22(1):93. https://doi.org/10.1186/s13075-020-02181-4.
50. Brown GJ, Cañete PF, Wang H, Medhavy A, Bones J, Roco JA, He Y, Qin Y, Cappello J, Ellyard JI, et al. TLR7 gain-of-function genetic variation causes human lupus. Nature. 2022;605(7909):349-356. https://doi.org/10.1038/s41586-022-04642-z.
51. Fang L, Ying S, Xu X, Wu D. TREX1 cytosolic DNA degradation correlates with autoimmune disease and cancer immunity. *Clinical and experimental immunology.* 2023;211(3):193-207. https://doi.org/10.1093/cei/uxad017.
52. Lundtoft C, Pucholt P, Martin M, Bianchi M, Lundström E, Eloranta ML, Sandling JK, Sjöwall C, Jönsen A, Gunnarsson I, et al. Complement C4 Copy Number Variation is Linked to SSA/Ro and SSB/La Autoantibodies in Systemic Inflammatory Autoimmune Diseases. *Arthritis & rheumatology (Hoboken, NJ)*. 2022;74(8):1440-1450. https://doi.org/10.1002/art.42122.
53. Chen K, Li X, Shang Y, Chen D, Qu S, Shu J, Zhang M, Wang Z, Huang J, Wu M, et al. FGL1-LAG3 axis impairs IL-10-Producing regulatory T cells associated with Systemic lupus erythematosus disease activity. *Heliyon*. 2023;9(10):e20806. https://doi.org/10.1016/j.heliyon.2023.e20806.
54. Cohen PL, Shao WH. Gas6/TAM Receptors in Systemic Lupus Erythematosus. *Disease markers.* 2019;2019:7838195. 10.1155/2019/7838195.
55. Liu M, Wu W, Sun X, Yang J, Xu J, Fu W, Li M. New insights into CD4(+) T cell abnormalities in systemic sclerosis. *Cytokine Growth Factor Rev*. 2016;28:31-36. https://doi.org/10.1016/j.cytogfr.2015.12.002.
56. Greisen SR, Schelde KK, Rasmussen TK, Kragstrup TW, Stengaard-Pedersen K, Hetland ML, Hørslev-Petersen K, Junker P, Østergaard M, Deleuran B, et al. CXCL13 predicts disease activity in early rheumatoid arthritis and could be an indicator of the therapeutic 'window of opportunity'. *Arthritis Res Ther.* 2014;16(5):434. https://doi.org/10.1186/s13075-014-0434-z.
57. Favilli F, Anzilotti C, Martinelli L, Quattroni P, De Martino S, Pratesi F, Neumann D, Beermann S, Novick D, Dinarello CA, et al. IL-18 activity in systemic lupus erythematosus. *Annals of the New York Academy of Sciences.* 2009;1173:301-309. https://doi.org/10.1111/j.1749-6632.2009.04742.x.
58. Winkler A, Sun W, De S, Jiao A, Sharif MN, Symanowicz PT, Athale S, Shin JH, Wang J, Jacobson BA, et al. The Interleukin-1 Receptor-Associated Kinase 4 Inhibitor PF-06650833 Blocks Inflammation in Preclinical Models of Rheumatic Disease and in Humans Enrolled in a Randomized Clinical Trial. *Arthritis & rheumatology (Hoboken, NJ).* 2021;73(12):2206-2218. https://doi.org/10.1002/art.41953.

59. Lang T, Foote A, Lee JP, Morand EF, Harris J. MIF: Implications in the Pathoetiology of Systemic Lupus Erythematosus. *Front Immunol.* 2015;6:577. https://doi.org/10.3389/fimmu.2015.00577.
60. Huijser E, van Helden-Meeuwsen CG, Groot N, Bodewes ILA, Wahadat MJ, Schreurs MWJ, van Daele PLA, Dalm V, van Laar JAM, van Hagen PM, et al. MxA is a clinically applicable biomarker for type I interferon activation in systemic lupus erythematosus and systemic sclerosis. *Rheumatology (Oxford, England)*. 2019;58(7):1302-1303. https://doi.org/10.1093/rheumatology/kez078.
61. Remmers EF, Sano H, Lafyatis R, Case JP, Kumkumian GK, Hla T, Maciag T, Wilder RL. Production of platelet derived growth factor B chain (PDGF-B/c-sis) mRNA and immunoreactive PDGF B-like polypeptide by rheumatoid synovium: coexpression with heparin binding acidic fibroblast growth factor-1. *The Journal of rheumatology*. 1991;18(1):7-13.
62. Baroja-Mazo A, Pelegrín P. Modulating P2X7 Receptor Signaling during Rheumatoid Arthritis: New Therapeutic Approaches for Bisphosphonates. *Journal of osteoporosis.* 2012;2012:408242. https://doi.org/10.1155/2012/408242.
63. Juárez-Vicuña Y, Pérez-Ramos J, Adalid-Peralta L, Sánchez F, Martínez-Martínez LA, Ortiz-Segura MDC, Pichardo-Ontiveros E, Hernández-Díazcouder A, Amezcua-Guerra LM, Ramírez-Bello J, et al. Interferon Lambda 3/4 (IFNλ3/4) rs12979860 Polymorphisms Is Not Associated With Susceptibility to Systemic Lupus Erythematosus, Although It Regulates OASL Expression in Patients With SLE. *Frontiers in genetics.* 2021;12:647487. https://doi.org/10.3389/fgene.2021.647487.
64. Tang J, Gu Y, Zhang M, Ye S, Chen X, Guo Q, Qian J, Bao C, Chen S, Shen N. Increased expression of the type I interferon-inducible gene, lymphocyte antigen 6 complex locus E, in peripheral blood cells is predictive of lupus activity in a large cohort of Chinese lupus patients. *Lupus.* 2008;17(9):805-813. https://doi.org/10.1177/0961203308089694.
65. Carter V, LaCava J, Taylor MS, Liang SY, Mustelin C, Ukadike KC, Bengtsson A, Lood C, Mustelin T. High Prevalence and Disease Correlation of Autoantibodies Against p40 Encoded by Long Interspersed Nuclear Elements in Systemic Lupus Erythematosus. *Arthritis & rheumatology (Hoboken, NJ).* 2020;72(1):89-99. https://doi.org/10.1002/art.41054.
66. Geneva-Popova MG, Popova-Belova SD, Gardzheva PN, Kraev KI. A Study of IFN-α-Induced Chemokines CCL2, CXCL10 and CCL19 in Patients with Systemic Lupus Erythematosu. *Life (Basel, Switzerland)*. 2022;12(2). https://doi.org/10.3390/life12020251.
67. Iwamoto T, Niewold TB. Genetics of human lupus nephritis. *Clinical immunology (Orlando, Fla).* 2017;185:32-39. https://doi.org/10.1016/j.clim.2016.09.012.
68. Conrad N, Misra S, Verbakel JY, Verbeke G, Molenberghs G, Taylor PN, Mason J, Sattar N, McMurray JJV, McInnes IB, et al. Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort study of 22 million individuals in the UK. *Lancet (London, England).* 2023;401(10391):1878-1890. https://doi.org/10.1016/s0140-6736(23)00457-9.
69. Ahmed SS, Lambert P-H. Chapter 20 - Autoimmune Diseases: The Role for Vaccines. In: Rose NR, Mackay IR, editors. The Autoimmune Diseases (Fifth Edition). Boston: *Academic Press;* 2014. p. 275-282.
70. Surace AEA, Hedrich CM. The Role of Epigenetics in Autoimmune/Inflammatory Disease. *Front Immunol.* 2019;10:1525. https://doi.org/10.3389/fimmu.2019.01525.
71. Ulff-Møller CJ, Asmar F, Liu Y, Svendsen AJ, Busato F, Grønbaek K, Tost J, Jacobsen S. Twin DNA Methylation Profiling Reveals Flare-Dependent Interferon Signature and B Cell Promoter Hypermethylation in Systemic Lupus Erythematosus. *Arthritis & rheumatology (Hoboken, NJ).* 2018;70(6):878-890. https://doi.org/10.1002/art.40422.
72. Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P, Walker A, Mack TM. A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis and rheumatism.* 1992;35(3):311-318. https://doi.org/10.1002/art.1780350310.
73. Zervou MI, Andreou A, Matalliotakis M, Spandidos DA, Goulielmos GN, Eliopoulos EE. Association of the DNASE1L3 rs35677470 polymorphism with systemic lupus erythematosus, rheumatoid arthritis and systemic sclerosis: Structural biological insights. *Molecular Medicine Reports.* 2020;22(6):4492-4498.
74. Belot A, Rice G, Omarjee S, Rouchon Q, Smith E, Moreews M, Tusseau M, Frachette C, Bournhonesque R, Thielens N, et al. Contribution of rare and predicted pathogenic gene variants to childhood-onset lupus: a large, genetic panel analysis of British and French cohorts. *The Lancet Rheumatology.* 2020;2. https://doi.org/10.1016/S2665-9913(19)30142-0.
75. Balow JE, Jr., Ryan JG, Chae JJ, Booty MG, Bulua A, Stone D, Sun HW, Greene J, Barham B, Goldbach-Mansky R, et al. Microarray-based gene expression profiling in patients with cryopyrin-associated periodic syndromes defines a disease-related signature and IL-1-responsive transcripts. *Ann Rheum Dis.* 2013;72(6):1064-1070. https://doi.org/10.1136/annrheumdis-2012-202082.
76. Hedrich CM, Tsokos GC. Epigenetic mechanisms in systemic lupus erythematosus and other autoimmune diseases. Trends in molecular medicine. 2011;17(12):714-724. https://doi.org/10.1016/j.molmed.2011.07.005.
77. Goulielmos GN, Zervou MI, Vazgiourakis VM, Ghodke-Puranik Y, Garyfallos A, Niewold TB. The genetics and molecular pathogenesis of systemic lupus erythematosus (SLE) in populations of different ancestry. *Gene*. 2018;668:59-72. https://doi.org/10.1016/j.gene.2018.05.041.
78. Sestan M, Kifer N, Arsov T, Cook M, Ellyard J, Vinuesa CG, Jelusic M. The Role of Genetic Risk Factors in Pathogenesis of Childhood-Onset Systemic Lupus Erythematosus. *Current issues in molecular biology.* 2023;45(7):5981-6002.
79. Cho JH, Feldman M. Heterogeneity of autoimmune diseases: pathophysiologic insights from genetics and implications for new therapies. *Nature medicine.* 2015;21(7):730-738. https://doi.org/10.1038/nm.3897.

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New Mechanisms of Barrett's Esophagus Development

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Abstract

Barrett's esophagus (BE) is a pathological condition that develops as a result of metaplastic transformation of the stratified squamous non-keratinized epithelium of the mucous membrane of the distal part of esophagus into columnar epithelium of the intestinal type. The purpose of this review was to investigate novel hypotheses and mechanisms related to the development of BE, aiming to identify emerging trends and enhance understanding of the disease's pathogenesis for the purpose of preventing esophageal adenocarcinoma. A thorough investigation of recent scholarly publications was carried out to examine the mechanisms contributing to the development of BE. In the process of scrutinizing an extensive array of literature, novel pathways involving cell transdifferentiation and transcommitment were elucidated, supplementing the conventional theory of esophageal cell replacement.

Many aspects remain unclear, especially concerning the cell population from which BE originates and the molecular processes or phases involved in its progression to esophageal adenocarcinoma. These questions hold immense importance for researchers, as the answers will profoundly influence efforts in disease prevention and treatment. Although there are presently few experimental model systems accessible for the study of BE and esophageal adenocarcinoma, advancements in tissue engineering and organotypic cell culture systems utilizing human cells present promising avenues for future research into the pathogenesis and advancement of these conditions.

Key words: Barrett's esophagus, gastroesophageal reflux disease, Barrett's metaplasia, esophageal cell differentiation, esophageal dysplasia.

Introduction

BE stands as one of the foremost concerns in modern gastroenterology. This condition emerges through the metaplastic alteration of the multilayered non-keratinizing squamous epithelium of the lower esophageal mucosa into a columnar epithelium resembling that of the intestines [1]. BE typically develops in the distal part of esophagus against the background of chronic gastroesophageal reflux disease (GERD) and characterized histopathologically by the the replacement of normal squamous epithelium with intestinal-type columnar epithelium [2]. The clinical importance of BE lies in its role as a significant risk factor for the development of esophageal adenocarcinoma. Furthermore, it stands as the sole recognized precursor to esophageal adenocarcinoma, an exceptionally lethal form of cancer whose incidence has shown a concerning rise over the last fifty years [3]. In patients afflicted with BE, the metaplastic transformation occurs wherein columnar mucosa, comprising epithelial cells exhibiting characteristics of both gastric and intestinal types, replaces the esophageal squamous mucosa that has been damaged by gastroesophageal reflux disease (Figure 1) [4]. According to US guidelines, the diagnosis of Barrett's disease requires its endoscopic confirmation with the presence of columnar mucosa extending at least 1 cm proximal to the esophago-gastric junction, and histological evidence of intestinal-type metaplasia [4].

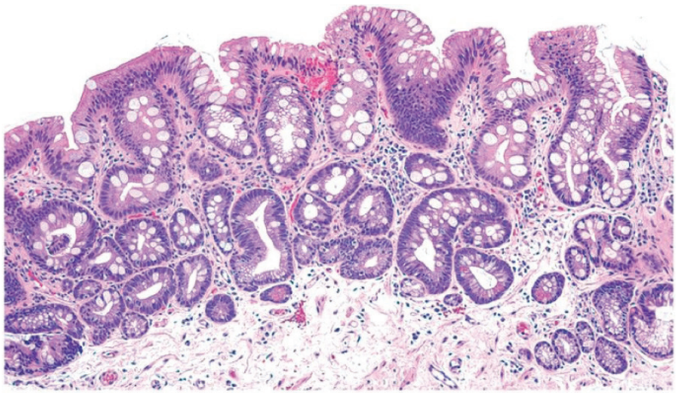

Figure 1 - Barrett's intestinal metaplasia with mucin-secreting gastric foveolar-type cells and prominent intestinal-type goblet cells.

(Photomicrograph provided by Robert Genta).

Materials and methods

We undertook a systematic search of pertinent medical databases spanning the last 15 years to gather relevant literature for our study. In accordance with the Cochrane collaboration recommendations, we conducted searches in Medline, Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL). Additionally, we explored relevant publications in databases such as Web of Science and Scopus, as well as in the "Russian Medicine" database based on e-library.ru. The search terms used for literature search included "Barrett's esophagus", "gastroesophageal reflux disease", "Barrett's metaplasia", "esophageal cell differentiation", and "esophageal dysplasia".

We established specific inclusion and exclusion criteria for publications to ensure the thoroughness and accuracy of our review process. Thus, primary attention was directed towards multicenter randomized clinical trials, cohort studies with large sample sizes, and with proper statistical analysis. The review analysis did not include descriptions of individual clinical cases or case series. The authors analyzed each article, paying attention to the quality of each publication, study design, sample size, quality of statistical analysis of results, and completeness of reference citations. An essential criterion for the inclusion of a publication in the review was the presence in the study protocol of mandatory histological examination of patients with BE.

Discussion

The established mechanism previously known for the development of BE is associated with an increase in the intensity and extent of esophageal damage caused by acid, bile, and pancreatic enzyme reflux [2, 4]. Activation of cyclooxygenase-2 (COX-2) occurs under the influence of bile salts, and experiments with laboratory rats have shown that inhibiting its activity results in a decreased risk of cancer development. Patients diagnosed with dysplasia and cancer frequently exhibit increased levels of COX-2 suppression. In vitro research has revealed that intermittent (pulse-like) acid exposure to the esophageal mucosa exerts a more pronounced effect on epithelial proliferation compared to continuous exposure.

Nevertheless, the pathogenetic mechanism underlying the development of metaplasia in BE remains unclear. It is proposed that the appearance of metaplasia arises from continual exposure to aggressive substances that damage mature cells, simultaneously stimulating the distorted differentiation of immature proliferating cells (gastric acid, bile acids, and pancreatic enzymes), which damage mature esophageal epithelial cells [1]. Indeed, at a certain stage, intestinal metaplasia appears to be an adaptive response that promotes the formation of columnar epithelium, which is more resistant to damage from various pathological factors. The reflux of bile acids and pancreatic enzymes causing damage to the esophageal mucosa results in the onset of "chemical" esophagitis in the terminal part of the esophagus. This condition is distinguished by dystrophic and inflammatory alterations in the mucous membrane, which may include the appearance of intestinal metaplasia [2, 5].

The article authored by researchers from North Carolina examine two histological variants of esophageal cancer: squamous cell carcinoma and adenocarcinoma, and explore the contribution of BE to their pathogenesis. They also underscore the importance of investigating the pathogenesis of BE and adenocarcinoma to enable effective risk stratification and facilitate the development of treatments [2].

There is a suggestion that BE may originate from either differentiated cells or stem cells. Within this framework, in the article investigates four potential cellular sources that may contribute to the development of BE. At the molecular level, metaplasia is presumably caused by activation or inactivation of transcription factors. The article conducts an analysis of microchip and SAGE data to identify potential "drivers" and "passengers" involved in the development of BE [6].

Additionally, potential cellular origins for BE are contemplated, encompassing basal cells of the squamous epithelium, submucosal gland cells of the esophagus, cells originating from the upper part of the stomach, and specialized cells located at the esophagogastric junction (Figure2) [1].

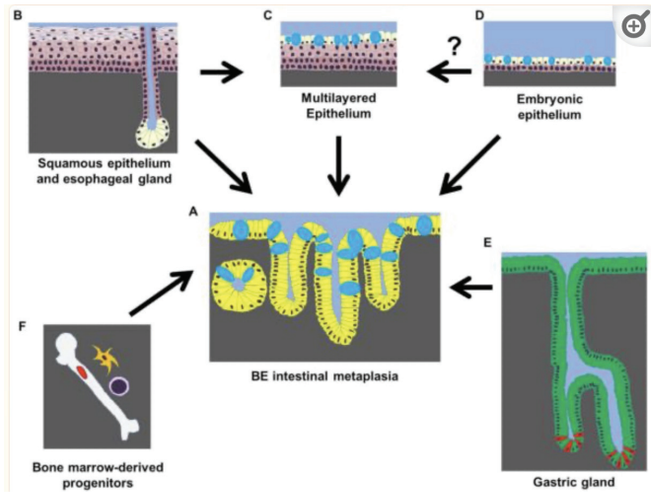

Figure 2 - Potential Cellular Substrates for Barrett's Esophagus (BE).

Columnar epithelial cells with glandular cells (mucin depicted as blue oval shapes) in (A). Direct sources of BE may be flat epithelial cells of the esophageal epithelium (keratinocytes) or ductal epithelial cells of the esophageal submucosal glands (B). Mucosal eosinophilic change (MLE) is suspected to be a precursor of BE (C). MLE is associated with esophageal glands in humans. BE may be caused by migration of residual embryonic esophageal cells or reactivation of developmental pathways (e.g., BMP4, Hh) (D). Possible variants include migration of gastric cells (e.g., Lgr5- positive stem cells in red at the base of glands) (E) and circulating bone marrow precursors (F).

1. Transcription factors: P63, Sox2, and Pax9

P63 acts as a critical regulator of epithelial stratification and progenitor cell survival in esophageal squamous epithelium. Sox2 is identified as an oncogene in lung cancer and esophageal squamous cell cancer [7, 8]. Pax9 is involved in the regular process of differentiating esophageal squamous epithelium [9].

2. Intestinal transcription factors: Cdx1 and Cdx2, HNFs, GATA4, and GATA6

Cdx1 and Cdx2 are pivotal regulators of intestinal development. HNFs are involved in gene regulation within the liver, pancreas, and intestine. GATA4 and GATA6 contribute to the differentiation of mesodermal and endodermal tissues [10, 11, 12].

3. Signaling pathways: TGFβ/BMP, WNT, NFkB, Hedgehog, Notch

The significance of BMP4 in inducing metaplasia in GERD is highlighted, alongside NFkB activation in gastroesophageal reflux [13, 14]. The influence of WNT on Cdx1 and Cdx2, as well as its regulation of Sox9, is acknowledged [15]. Hedgehog activation and Notch inhibition are also considered potential factors in BE development [16].

4. Stromal factors

The interaction between epithelial-mesenchymal cells plays a pivotal role in epithelial cell differentiation. The association between inflammation and BE, driven by proinflammatory cytokines, is well-studied. Inflammatory alterations occur before cell damage in GERD, with esophageal squamous epithelial cells secreting chemokines such as IL8 and IL1β, initiating spontaneous inflammation and metaplasia. Metaplasia, characterized by TFF2 and Cdx2 expression, is stimulated by bile acid exposure [17-19].

5. MicroRNAs

Several studies have demonstrated alterations in microRNA profiles in individuals with BE and adenocarcinoma. Some microRNAs (e.g., miR-203) express key genes associated with BE (e.g., p63), while others modulate the expression of such genes. Transcription factors like p63 are known to modulate microRNA processing, such as miR-21. The role of microRNAs as drivers or passive participants in BE development is yet to be determined [20-22].

6. Other factors

The level of retinoic acid increases in BE tissues compared to normal esophagus and decreases with adenocarcinoma. Changes in retinoic acid receptors are observed, and treatment with choleretic drugs enhances the activity of retinoic acid receptors, further promoting the differentiation of esophageal epithelial cells into columnar cells.

The RUNX3 gene, part of the transcription factor family with a regulatory RUNT domain, is crucial for esophageal cellular differentiation. Loss of RUNX3 results in gastric epithelium differentiation into intestinal-type cells, which may contribute to BE development [23-26].

7. Transcommitment

Transcommitment, a process akin to transdifferentiation, shares similarities with paligenosis in that it involves the initial dedifferentiation of mature cells into progenitor-like cells, followed by abnormal redifferentiation [27]. However, unlike transdifferentiation, transcommitment begins with immature progenitor cells that undergo abnormal differentiation, potentially triggered by factors such as GERD. This process could elucidate why different cell types persist even when GERD is managed [16, 27].

The exact progenitor cells responsible for Barrett's metaplasia are not fully elucidated, but four categories of candidates are proposed (Figure 3) [28]: progenitor cells native to the esophagus, those from the proximal stomach (gastric cardia), specialized populations at the esophago-gastric junction (EGJ), and bone marrow progenitor cells transported to the esophagus.

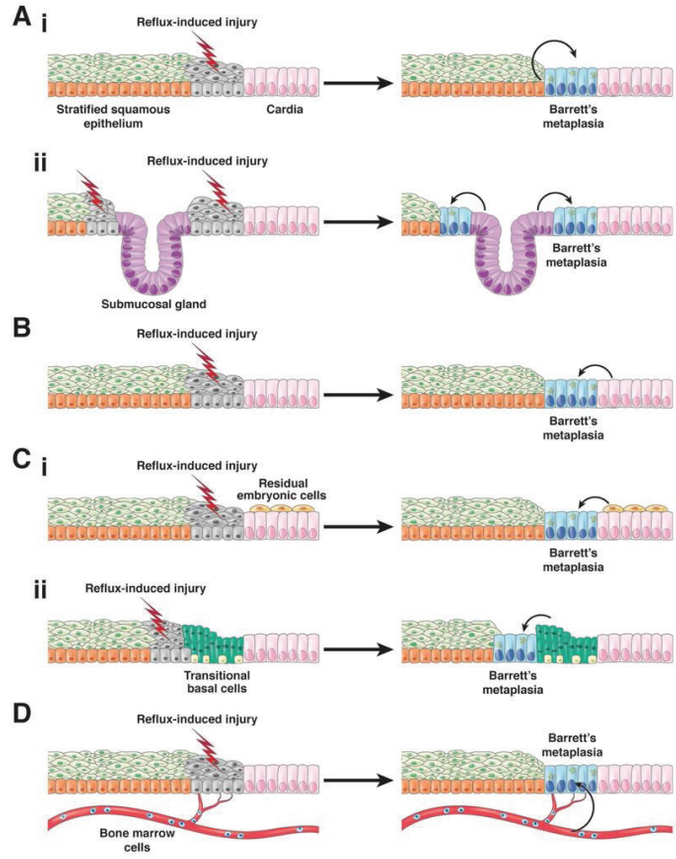

Figure 3 - Proposed cells of origin for BE.

1) Cells native to the esophagus including (1a) squamous epithelial cells that undergo reflux-induced transdifferentiation or transcommitment to produce the columnar cells of Barrett's metaplasia and (1b) Progenitor cells in esophageal submucosal glands and/or their ducts. 2) Progenitor cells in the gastric cardia. 3) Specialized populations of cells at the esophago-gastric junction migrate into the reflux-damaged esophagus including (3a) residual embryonic cells (RECs) or (3b) transitional basal cells (TBCs). 4) Circulating bone marrow cells. For all of these proposed progenitor cells, reflux-induced injury to the esophageal squamous mucosa is assumed to initiate the metaplastic process, perhaps by stimulating progenitor cell migration into the damaged esophagus via a wound-healing process. In addition, reflux is assumed to induce the transcommitment of the progenitor cells to produce the multiple columnar cell types of Barrett's metaplasia. (Figure modified from Jiang et al [14]).

Furthermore, research by Jiang et al. suggests that transitional epithelium is inherent and instigates metaplasia. They observed distinctive expression patterns of three protein markers—cytokeratins KRT5 and KRT7, and the transcription regulator p63—in cell types at the murine gastroesophageal junction [1]. KRT7 expression in BE cells in humans is particularly characteristic of transitional epithelium [29].

Jiang and colleagues discovered a group of cells, different from RECs, located at the transitional zone of the SCJ in mice and humans. These cells are believed to potentially lead to BE [30]. Their research revealed that this transitional epithelium is sustained by a group of precursor cells referred to as transitional basal cells (TBCs). The TBCs expressed squamous markers such as KRT5, KRT14, and p63, along with a columnar cytokeratin characteristic of Barrett's metaplasia (KRT7+), which differed from nearby squamous basal cells and cardia mucosal progenitor cells (Figure 4) [30]. Following the establishment of an esophago-jejunostomy to stimulate bile reflux, Jiang and team observed an increase in proliferation and differentiation of TBCs into columnar epithelium expressing genes typical of intestinal cells, such as Cdx2. Additionally, the overexpression of Cdx2 in TBCs facilitated their differentiation into intestinal-type epithelial cells [1].

Additionally, a mechanism contributing to the onset of BE is chronic reflux esophagitis, triggered by gastroesophageal reflux and other harmful substances, including exposure to high concentrations of nitric oxide (NO) derived from dietary nitrates found in green leafy vegetables. The majority of ingested nitrate is absorbed in the small intestine and excreted the body unchanged through urine.

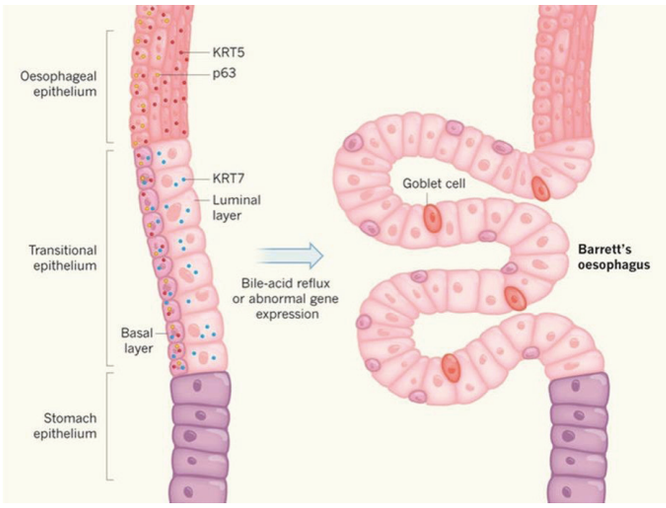

Figure 4 - Cross-section of epithelial cell types in the esophagus.

The gastroesophageal junction in mammals consists of various epithelial cells. Jiang et al. describe the transitional epithelial zone between the esophagus and the stomach, showing differential expression of three marker proteins (KRT5, KRT7, and p63) in the basal and luminal layers, and surrounding epithelium. They provide evidence in mice and humans that bile reflux or abnormal gene expression can cause abnormal expansion of this transitional epithelium, forming precancerous tissue containing goblet cells.

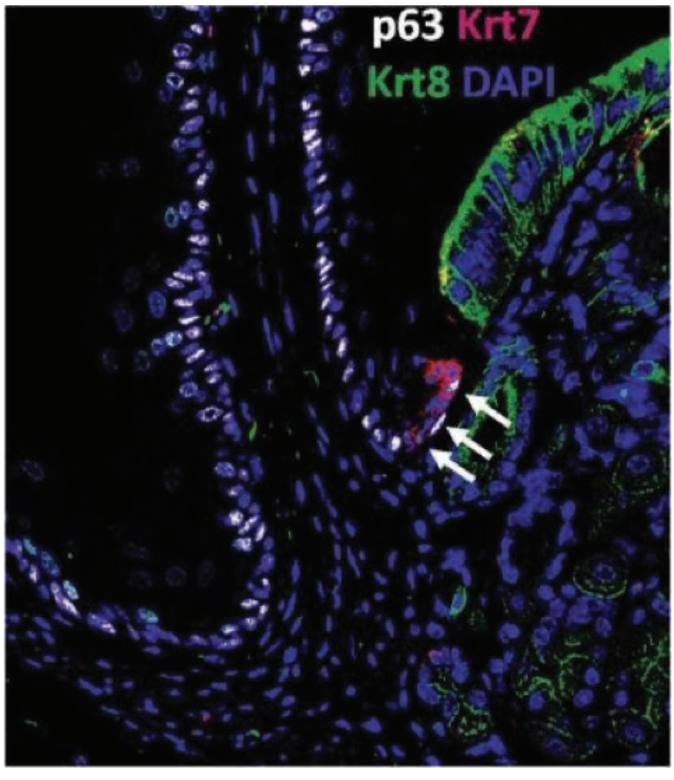

Figure 5 - Transitional basal cells at the mouse squamo-columnar junction.

Ttransitional basal cells (p63+ Krt7+ Krt8-negative) located at the squamo-columnar junction. The neighboring squamous cells (p63+, Krt7- negative, Krt8-negative) on the left and the columnar gastric cells (p63-negative, Krt7-negative, Krt8+) on the right (see details in Jiang et al[14]).

However, around 25% of it is concentrated by salivary glands and secreted into the mouth, where bacteria on the tongue convert recycled nitrate into nitrite. After being swallowed, nitrite comes into contact with acidic gastric juice and quickly transforms into nitric oxide (NO). Increased levels of NO have been observed at the gastroesophageal junction following the consumption of nitrates [31, 32].

Scientists from Tohoku University Graduate School of Medicine (Japan) investigated the proposition that elevated NO levels affect the Rho/ROCK signaling pathway in esophageal fibroblasts, potentially leading to aberrant wound healing characterized by delayed wound contraction. This phenomenon, they hypothesized, could contribute to the onset of BE [31, 33].

The study provides an overview of the molecular, immunological, and genetic mechanisms involved in BE development.

In 1950, British surgeon Norman Barrett coined the term "Barrett's esophagus" and described the classical mechanism of its development, characterized by changes in the epithelium of the lower esophagus, which can occur as a result of chronic acid reflux from the stomach. Initially, there is incompetence of the gastroesophageal barrier function, leading to the development of reflux containing acid, bile, and pancreatic enzymes. Under the influence of aggressive factors from gastric juice, the stratified squamous epithelium of the esophagus is destroyed, and it is replaced by columnar and intestinal epithelium [2].

In 2009, Spechler SJ and Rhonda F. Souza proposed an alternative concept of reflux esophagitis development, suggesting that the pathology begins with cytokine-mediated injury rather than acidic chemical exposure [1, 34].

Jiang M. and colleagues from the UK provided evidence supporting the idea that transitional epithelium is innate and initiates metaplasia. The authors identified differences in the expression of cytokeratins KRT5 and KRT7, as well as the transcription regulator p63, delineating cell types in the murine gastroesophageal junction. It was confirmed that KRT7, expressed in BE cells in humans, is specific to transitional epithelium [30].

Researchers from North Carolina identify potential cellular sources such as differentiated and stem cells. Additionally, the role of transcription factors, including P63, Sox2, Pax9, as well as intestinal factors and signaling pathways (TGFβ/BMP, WNT, NFκB, Hedgehog, Notch), is discussed in the context of BE development [7-9].

Stromal factors, epithelial-mesenchymal cell interactions, and inflammation, particularly the association with proinflammatory cytokines, are considered key in BE development. MicroRNAs, such as miR-203, are also extensively analyzed in light of their role in regulating genes associated with BE [17-19].

Factors such as retinoic acid levels, RUNX3, KLF4, and KLF5 genes are highlighted in the context of esophageal epithelial cell differentiation [26]. The study provides important data for understanding the molecular mechanisms of esophageal pathologies development, which may contribute to effective risk stratification and treatment methods development [23- 25].

Results

After analyzing the latest data on this topic, we have reached the following conclusions:

The research carried out by scientists from North Carolina emphasized the importance of recognizing two histological types of esophageal cancer – squamous cell carcinoma and

adenocarcinoma – and explored the role of precision medicine in their classification and therapy.

Transcription factors such as P63, Sox2, and Pax9 play a key role in the development of esophageal squamous epithelium. Interference in their activity provides promising targets for therapeutic interventions. This also emphasizes the need for detailed molecular-level analysis to understand metaplasia and the activation/inactivation of transcription factors.

Signaling pathways such as TGFβ/BMP, WNT, NFκB, Hedgehog, and Notch represent a complex network of interconnections that influence the development of BE. Identifying BMP4 as a key player in the induction of metaplasia in gastroesophageal reflux reveals potential points of intervention for preventing this process.

Stromal factors and their interaction with epithelial-mesenchymal cells emphasize the role of inflammation in the development of BE. This is associated with pro-inflammatory cytokines such as IL8 and IL1β, highlighting the importance of comprehensive study not only of epithelial but also of mesenchymal aspects.

MicroRNA profiles are an additional aspect of the molecular heterogeneity of BE and adenocarcinoma. Studying the impact of microRNAs such as miR-203 on key genes related to BE provides new opportunities for understanding and therapeutically affecting the developmental processes of these dangerous conditions.

Changes in the levels of retinoic acid in esophageal tissues under various conditions raise questions about its impact on cellular differentiation. Discussions of molecular mechanisms associated with retinoic acid receptors can complement the understanding of the connection between these changes and the development of pathology.

The role of the RUNX3 gene in esophageal cellular differentiation and its loss in the context of Barrett's development are subject to discussion. Analyzing the mechanisms by which the loss of RUNX3 affects cell types may reveal pathogenetic processes.

The consideration of KLF4 and KLF5 involvement in the differentiation of squamous epithelium in the esophagus highlights their possible role in the formation of BE.

The use of immortalized cell lines was discussed, emphasizing the importance of model systems for studying the molecular mechanisms of Barrett's development. The limitations of current methods highlight the need for new approaches for more accurate modeling of the precancerous state.

The possible link between chronic reflux and high levels of nitric oxide was also discussed. This discussion may shed light on the impact of environmental factors on the development of the disease".

Conclusion

Much remains unknown, particularly: from which cellular population the cells originate and what are the molecular events or stages through which BE progresses to esophageal adenocarcinoma. These are crucial questions for researchers, the answers to which will significantly impact disease prevention and treatment. Despite the current lack of extensive experimental model systems for studying BE and esophageal adenocarcinoma, advancements in tissue engineering and organotypic cell-based culture systems provide promising avenues for future research into the pathogenesis and progression of these conditions.

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References

1. Que J, Garman KS, Souza RF, Spechler SJ.Pathogenesis and Cells of Origin of Barrett's Esophagus. *Gastroenterology*. 2019; 157(2): 349–364. https://doi.org/10.1053/j.gastro.2019.03.072.
2. Chen H, Fang Y, Tevebaugh W, Orlando RC, Shaheen NJ and Chen X. Mechanism of Barrett's Esophagus. *Dig Dis Sci.* 2011; 56(12): 3405–3420. https://doi.org/10.1007/s10620-011-1885-6.
3. Wijnhoven BP, Hussey DJ, Watson DI, Tsykin A, Smith CM, Michael MZ. MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. *Br J Surg.* 2010; 97: 853–61. https://doi.org/10.1002/bjs.7000.
4. Spehler SY, Sousa RF. Esophagus Barretta. N Engl J Med. 2014; 371: 836-45. https://doi.org/10.1056/NEJMra1314704.
5. Shaheen NJ, Falk GW, Iyer PG, Gerson LB; American College of Gastroenterology. ACG Clinical Guideline: Diagnosis and Management of Barrett's Esophagus. Am J Gastroenterol 2016; 111:30–50. https://doi.org/10.1038/ajg.2015.322.
6. Wang J, Qin R, Ma Y, et al. Differential gene expression in normal esophagus and Barrett's esophagus. *J Gastroenterol.* 2009; 44: 897–911. https://doi.org/10.1007/s00535-009-0082-2.
7. Bass AJ, Watanabe H, Mermel CH, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. *Nat Genet.* 2009; 41: 1238–42. https://doi.org/10.1038/ng.465.
8. Mutoh H, Sashikawa M, Sugano K. Sox2 expression is maintained while gastric phenotype is completely lost in Cdx2-induced intestinal metaplastic mucosa. *Differentiation*. 2010; 81: 92–8. https://doi.org/10.1016/j.diff.2010.10.002.
9. Sherwood RI, Chen TY, Melton DA. Transcriptional dynamics of endodermal organ forquanmation. *Dev Dyn*. 2009; 238: 29–42. https://doi.org/10.1002/dvdy.21810.
10. Benoit YD, Pare F, Francoeur C, et al. Cooperation between HNF-1alpha, Cdx2, and GATA-4 in initiating an enterocytic differentiation program in a normal human intestinal epithelial progenitor cell line. *Am J Physiol Gastrointest Liver Physiol.* 2010; 298: G504–17. https://doi.org/10.1152/ajpgi.00265.2009.

11. Kazumori H, Ishihara S, Kinoshita Y. Roles of caudal-related homeobox gene Cdx1 in oesophageal epithelial cells in Barrett's epithelium development. *Gut*. 2009; 58: 620–8. https://doi.org/10.1136/gut.2008.152975.
12. Verzi MP, Shin H, Ho LL, Liu XS, Shivdasani RA. Essential and redundant functions of Caudal family proteins in activating adult intestinal genes. *Mol Cell Biol.* 2011: 31(10): 2026–2039. https://doi.org/10.1128/MCB.01250-10.
13. Domyan ET, Ferretti E, Throckmorton K, Mishina Y, Nicolis SK, Sun X. Signaling through BMP receptors promotes respiratory identity in the foregut via repression of Sox2. *Development.* 2011; 138: 971–81. https://doi.org/10.1242/dev.053694.
14. Rodriguez P, Da Silva S, Oxburgh L, Wang F, Hogan BL, Que J. BMP signaling in the development of the mouse esophagus and forestomach. *Development*. 2010; 137: 4171–6. https://doi.org/10.1242/dev.056077.
15. Yamanaka Y, Shiotani A, Fujimura Y, et al. Expression of Sonic hedgehog (SHH) and CDX2 in the columnar epithelium of the lower oesophagus. *Dig Liver Dis.* 2011; 43 :54–9. https://doi.org/10.1016/j.dld.2010.04.014.
16. Wang DH, Clemons NJ, Miyashita T, et al. Aberrant epithelial-mesenchymal Hedgehog signaling characterizes Barrett's metaplasia. *Gastroenterology*. 2010; 138: 1810–22. https://doi.org/10.1053/j.gastro.2010.01.048.
17. Lao-Sirieix P, Fitzgerald RC. Role of the micro-environment in Barrett's carcinogenesis. *Biochem Soc Trans.* 2010; 38: 327–30. https://doi.org/10.1371/journal.pone.0000177.
18. Menke V, van Es JH, de Lau W, et al. Conversion of metaplastic Barrett's epithelium into post-mitotic goblet cells by gamma-secretase inhibition. *Dis Model Mech.* 2010; 3: 104–10. https://doi.org/10.1242/dmm.003012.
19. Quante M, Abrams JA, Marrache F, Wang TC. Barrett's esophagus correlates with increased putative gastrointestinal stem cell markers DCLK1 and CCK2R in a IL1b mouse model and in humans. *Gastroenterology*. 2010; 138 (5): S-128-S-128. https://doi.org/10.1016/S0016-5085(10)60586-0.
20. Bansal A, Lee IH, Hong X, et al. Feasibility of MicroRNAs as Biomarkers for Barrett's Esophagus Progression: A Pilot Cross-Sectional, Phase 2 Biomarker Study. *Am J Gastroenterol.* 2011; 106(6): 1055-1063. https://doi.org/10.1038/ajg.2011.37.
21. Fassan M, Volinia S, Palatini J, et al. MicroRNA expression profiling in human Barrett's carcinogenesis. *Int J Cancer.* 2010. https://doi.org/10.1002/ijc.25823.
22. Wijnhoven BP, Hussey DJ, Watson DI, Tsykin A, Smith CM, Michael MZ. MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. *Br J Surg.* 2010; 97: 853–61. https://doi.org/10.1002/bjs.7000.
23. Gudas LJ, Wagner JA. Retinoids regulate stem cell differentiation. J Cell Physiol. 2011; 226: 322–30. https://doi.org/10.1002/jcp.22417.
24. Hu D, Wan Y. Regulation of Kruppel-like factor 4 by the anaphase promoting complex pathway is involved in TGF-beta signaling. *J Biol Chem.* 2011; 286: 6890–901. https://doi.org/10.1074/jbc.M110.179952.
25. Kazumori H, Ishihara S, Takahashi Y, Amano Y, Kinoshita Y. Roles of Kruppel-like factor 4 in oesophageal epithelial cells in Barrett's epithelium development. *Gut*. 2011; 60: 608–17. https://doi.org/10.1136/gut.2008.152975.
26. Zheng H, Pritchard DM, Yang X et al. KLF4 gene expression is inhibited by the notch signaling pathway that controls goblet cell differentiation in mouse gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol.* 2009; 296: G490–8. https://doi.org/10.1152/ajpgi.90393.2008.
27. Van DH, Sousa RF. Transcription: the way to Barrett's metaplasia. *Adv Exp Med Biol*. 2016; 908: 183-212. https://doi.org/10.1007/978-3-319-41388-4_10.
28. Jin RU, Mills JC. Are gastric and esophageal metaplasia relatives,the case for Barrett's stemming from SPEM. *Dig Dis Sci.* 2018; 63: 2028–2041. https://doi.org/10.1007/s10620-018-5150-0.
29. Lizhe Zhuang & Rebecca C. Fitzgerald Origins in the oesophagus. Nature. 2017; 550: 463–464. https://doi.org/10.1038/nature24150.
30. Jiang M, Li H, Zhang Y et al. Transitional basal cells at the squamous-columnar junction generate Barrett's oesophagus. *Nature*. 2017; 550: 529–533. https://doi.org/10.1038/nature24269.
31. Asanuma K, Huo X, Agoston A, Zhang X, Yu C, Cheng E, Zhang Q, Dunbar KB, Pham TH, Wang DH, Iijima K, Shimosegawa T, Odze RD, Spechler SJ, Souza RF: In oesophageal squamous cells, nitric oxide causes s-nitrosylation of akt and blocks Sox2 (sex determining region y-box 2) expression. *Gut* 2015. https://doi.org/10.1136/gutjnl-2015-309272.
32. Kusaka G, Uno K, Iijima K, Shimosegawa T. Role of nitric oxide in the pathogenesis of Barrett's-associated carcinogenesis. *World J Gastrointest Pathophysiol.* 2016 Feb 15; 7(1): 131–137. https://doi.org/10.4291/wjgp.v7.i1.131.
33. Fujiya T, Asanuma K, Koike T, Okata T, Saito M, Asano N, Imatani A, Masamune A. Nitric oxide could promote development of Barrett's esophagus by S-nitrosylation-induced inhibition of Rho-ROCK signaling in esophageal fibroblasts. *Am J Physiol Gastrointest Liver Physiol.* 2022; 322 (1): G107-G116. https://doi.org/10.1152/ajpgi.00124.2021.
34. Wang DH. Souza RF. Transcommitment: paving the way to Barrett's metaplasia. *Adv Exp Med Biol.* 2016; 908: 183-21. https://doi.org/10.1007/s10620-018-5069-5.

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Effects of Distance Education Models on Senior Nursing Students' Readiness for E-learning, Self-Directed Learning, and Clinical Practice

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Abstract

Aim: The present study aimed to examine the effects of distance education models on the readiness of senior nursing students for e-learning, self-directed learning, and clinical practice.

Material and methods: This study was conducted with 58 senior nursing students in the 2020-2021 fall semester. Descriptive Characteristics Form, University Students' E-learning Readiness Scale, Self-Directed Learning Readiness Scale, Clinical Practice Readiness Assessment Form, and focus group interviews were used as data collection tools.

Results: The mean age of the students was 21.50 ± 0.71. The majority (77.2%) were female students, 22.8% of which were male students. The findings showed that students' readiness for e-learning increased statistically significantly over time. The e-learning mean score, 156.77 at the beginning of the term, increased to 187.72 in the 11th week ($F = 6779.370$, $p < 0.05$). The mean clinical readiness score of the students was 2.63 ± 0.40. It was seen that the clinical readiness of the students was higher than the average. In the analysis performed to evaluate the relationship between clinical readiness and e-learning and self-directed learning, the findings showed that the clinical readiness of the students whose e-learning readiness and self-directed learning increased in the last week of the term increased statistically ($p < 0.05$).

Conclusions: In distance education, as well as in face-to-face nursing education, the importance of using interactive education methods in extraordinary situations such as the COVID-19 pandemic and earthquakes has emerged.

Keywords: COVID-19; distance education; nursing students; e-learning.

Introduction

The COVID-19 pandemic has had a detrimental impact on every facet of daily life, necessitating measures such as social isolation [1]. Education, a crucial component of daily life and a societal necessity, is among the affected domains. In our country, the distance education process has continued with digital opportunities in all universities with distance education capacity since March 23, 2020 [2]. Students considered the transition to distance education as a positive change because it offers the opportunity to use technology, access to information whenever they want, easy access to course materials and homework, the opportunity to watch the recording of the lessons again, and the absence of transportation to the campus [3,4].

Nevertheless, applied departments like nursing education faced challenges in providing essential practical training, a pivotal component of the curriculum, and were unable to leverage the benefits of distance education in this regard [5]. Nursing profession education requires a meaningful combination and synthesis of theoretical knowledge and practical skills [6]. However, with the

introduction of theoretical courses through distance education, some students did not have technological opportunities or were not inclined to use technology, causing challenges in the education process [7]. In the literature, students' negative views on distance education include a low level of computer skills, anxiety about accessing technological platforms, low motivation, not being able to work independently, and feeling lonely because they have to look at the computer screen [7,8]. At the same time, they are faced with the risk of decreasing teacher-student interaction and not being able to meet their socialization needs with their peers [7]. Therefore, due to the isolation specific to the COVID-19 pandemic process, the students who received training in applied fields and who are in their final year have had concerns about their competencies in the field and their readiness for clinical practice due to the distance education and the inability to deliver one-to-one hands-on training [9].

Despite the loosening of the pandemic rules and the start of face-to-face training in many countries worldwide, this seemingly disadvantageous process has been transformed into a kind of advantage by integrating technology into education and developing in this sense. Therefore, this study aimed to examine the effects of distance education models on the readiness for e-learning, self-directed learning, and clinical practice of senior nursing students educated in obstetrics and gynecology nursing.

Materials and methods

Design

The effectiveness of distance education can provide better evidence for nursing students, who are educated with the distance education model and practice for self-improvement, with questionnaires and also with scales and focus group interviews. Therefore, a mixed-method method was used. The study was conducted in a nursing faculty through an online distance education platform.

Participants

In the present study, 58 senior nursing students who participated within the scope of obstetrics and gynecology nursing courses agreed to participate in this study. Being a last-year student and taking online education related to obstetric and gynecologic cases were studied, including criteria. However, students who did not attend three or more of the online courses were excluded from this study.

Procedures

In this study, case discussions, concept maps, debate discussions, skill video demonstrations, and Patient–Intervention– Comparison– Outcome" (PICO) games were used as distance education methods/models.

The PICO game was played in online courses conducted over the online distance education platform. For all these activities, students were divided into groups of five or six over the online distance education platform, and group work was performed. This program, which lasted for four weeks, is explained with an example in Figure 1 below.

The students participated in the case discussion sessions for five hours every week. The trainers selected the cases that were most frequently seen in the clinic.

Data Collection Method

Data were collected both quantitatively and qualitatively.

Quantitative process

Theoretical courses were carried out using the distance education method in the fall semester of the 2020-2021 academic year. "Descriptive Characteristics Form," "University Students' Readiness for E-learning Scale," and "Self-Directed

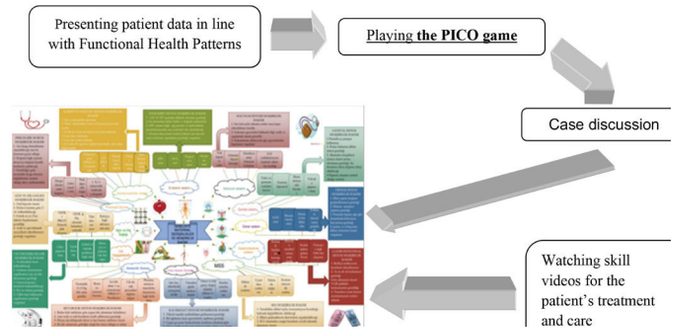

Figure 1 - Session Program Process

Learning Readiness Scale" were applied just before starting the education. During the semester, both scales, "University Students' Readiness for E-learning Scale" and "Self-Directed Learning Readiness Scale," were applied five times in total (2nd, 5th, 8th, 11th, and 14th weeks of the education period). At the end of the semester, the Clinical Practice Readiness Assessment Form" was applied to the students. All forms were applied online using Google Forms.

Data Collection Tools

Descriptive Characteristics Form: This is an eight-question diagnostic form prepared by researchers based on the literature on the subject [3,4,10]. The form included questions about gender, age, easy learning path, preferred education system, duration of distance education courses, and experience with active teaching methods.

University Students' Readiness for E-learning Scale: It was developed by Yurdagül and Demir (2017) to measure students' e-learning readiness structure for the effective implementation of e-learning. The options for the items on the scale were designed in a 7-point Likert type, ranging from Not suitable for me at all (1")" to Completely suitable for me (7")." The scale consisted of 33 items and six factors (Computer Self-efficacy, Internet Self-efficacy, Online Communication Self-efficacy, Self-learning, Learner Control, Motivation for e-learning). A maximum of 231 and a minimum of 33 points can be obtained from the scale. A high score on the scale means that one is more ready for e-learning. The total 'Cronbach's alpha coefficient of the scale is 0.93 [11]. In this study, the 'Cronbach's alpha coefficient of the scale was calculated as 0.96.

Self-Directed Learning Readiness Scale: The Self-Directed Learning Readiness Scale (SDLRS), developed by Fisher et al. (2001) [12] and adapted to Turkish culture by Sahin and Erden (2009), was used in this study to determine the students' readiness for self-directed learning. The options for the items of the scale were designed in a five-point Likert type, ranging from "strongly disagree (1)" to "strongly agree (5)." The scale consists of three sub-dimensions: “Self-Management”, “Willingness to Learn” and “Self-control Skills” and 40 items. A maximum of 200 and a minimum of 40 points can be obtained from the scale. High scores obtained from the scale indicate that readiness for self-directed learning is high. The total Cronbach's alpha coefficient of the scale is 0.93 [13]. In this study, the Cronbach's alpha coefficient of the scale was calculated as 0.97.

Clinical Practice Readiness Assessment Form: It was a nine-question form prepared by researchers based on the literature on the subject, assessing students' readiness for clinical practice [3,4,6,9-11]. In this form, closed-ended questions were created to consider the clinical practice skills that nursing students should have (e.g., preparation of treatment, caregiving, patient registration, communicating with the patient, and

exhibiting teamwork). In creating the questionnaire, the opinions of eleven specialists who were teaching staff in nursing and who had clinical training experience were taken.

Qualitative Process

At the end of the semester, the effectiveness of distance education methods was evaluated by conducting focus group interviews with the students. The focus group interviews were held over the online distance education platform by forming two separate meeting groups. Thirty students who agreed to participate in the qualitative part of this study were divided into two groups of 15 regarding the interview quality. Both focus group interviews were conducted by the same researcher. The average duration of the interviews was 45.09 minutes.

Evaluation of data

Quantitative data evaluation process: Students' changes in e-learning and self-directed learning over time were tested using analysis of variance (ANOVA) with repeated measures. Readiness for clinical practice was expressed with mean and standard deviation values. Pearson correlation analysis was applied to examine whether there was a significant difference between clinical practice readiness and e-learning and self-directed learning. The statistical significance level was accepted as 0.05.

Qualitative data evaluation process: The data obtained using the interview form were evaluated using the thematic analysis developed by Braun and Clarke (2006) [14]: (1) The researcher's familiarity with the data, (2) The creation of the initial codes, (3) The collection of the codes under potential themes, (4) The revision of the themes, (5) Identification and naming of themes, (6) Preparation of the report. In this study, the texts were read several times by three researchers during the thematic analysis to ensure a detailed understanding of the data. Researchers read each one separately. While reading the texts, a code was added to each section that was thought to be related to the subject investigated by the researchers. Researchers then discussed these independently developed codes together and tried to create common codes. This process continued until a consensus was reached. Themes were created by combining the sub-themes from the codes. While evaluating the data, the researchers paid attention that their beliefs and values did not affect the interpretations taken from the data. To ensure the reliability of the data, we focused on the strategies of validity, transferability, reliability, and acceptability [15].

Ethics

Institutional permission from the nursing faculty and approval from the Research Ethics Board were obtained for the study (with the decision dated 18.01.2021 and numbered 2021/02-14). The students were informed verbally and in writing about the research purpose. Data collection was continued after the students' written consent was obtained. Verbal consent was obtained from the students who attended the online meeting, which was recorded before the qualitative data of the present study were collected. Each student was given a number when mentioning their comments to ensure their privacy.

Results

The mean age of the students was 21.50 ± 0.71. The majority (77.2%) were female students, 22.8% of which were male students. They stated that the average duration of the courses they attended through distance education was 149.10 ± 51.29 minutes. The experiences and preferences of the students regarding the learning methods are given in Table 1.

Table 1 Students' experiences and preferences regarding learning methods

	n	%
Best way to learn		
Visual	13	22.8
Visual, kinesthetic	11	17.5
Visual, auditory, kinesthetic	10	19.3
Kinesthetic	23	40.4
Preferred education system		
Distance education	8	13.79
Face to face education	50	86.21
Active participation in the course		
Participating	49	84.49
Non-participating	9	15.51
Education method that facilitates learning the most		
Drawing a concept map	10	17.24
Case discussion	12	20.68
Video display	15	25.86
PICO game	21	36.22

Quantitative research findings

Readiness of students for e-learning

The findings showed that students' readiness for e-learning increased statistically significantly over time. The e-learning mean score, 156.77 at the beginning of the term, increased to 187.72 in the 11th week ($F = 6779.370$, $p = 0.00$). The difference was mainly due to the e-learning readiness measurement between the 11th week and the 15th week (Table 2).

Readiness for Self-directed Learning

When the mean scores of readiness for self-directed learning were examined, a statistically significant increase was found over time. Although the self-directed learning score, which was 4.09 in the second week, increased to 4.49 in the last week, this difference was not statistically significant. There was a significant increase in students' readiness only from the 11th week to the 15th week of the term ($F = 5624.968$, $p = 0.00$) (Table 3).

Clinical readiness

Students evaluated how ready they felt to go to the clinic in the next semester. At the end of the semester, they took theoretical courses. Scoring was no (1), sometimes (2), yes (3). The mean clinical readiness score of the students was 2.63 ± 0.40. It was seen that the clinical readiness of the students was higher than the average.

The relationship between clinical readiness and e-learning and self-directed learning

In the analysis performed to evaluate the relationship between clinical readiness and e-learning and self-directed learning, the findings showed that the clinical readiness of the students whose e-learning readiness and self-directed learning increased in the last week of the term increased statistically ($p = 0.00$) (Table 4).

Qualitative research findings

Three themes and three sub-themes emerged in the reporting made as a result of the researchers' evaluations of the interviews. Three themes emerged as outlined below: (1) high readiness, (2) ambivalence, and (3) low readiness (Table 5).

Theme 1. High readiness

Sub-theme 1. Orientation

Some of the students stated that the interactive methods of distance education models applied regarding readiness for clinical practice are a positive support for clinical orientation.

Table 2 Readiness of students for e-learning

Readiness of students for e-learning	X ± SS**	5th week***		8th week***		11th week***		15th week***	
2nd week	156.77 ± 35.29	0.144	0.70						
5th week	160.06 ± 29.29			1.702	0.20				
8th week	174.75 ± 30.66					1.611	0.21		
11th week	187.72 ± 23.78							8.594	0.00*
15th week	182.96 ± 28.20								

*Statistically significant, **X: Mean, SS: Standard deviation, ***Analysis of variance in repeated measurements and p-value were given, respectively.

Table 3 Readiness for Self-directed Learning

Readiness of students for e-learning	X ± SS	5th week***		8th week***		11th week***		15th week***	
2nd week	4.09 ± 0.76	0.051	0.82						
5th week	4.05 ± 0.63			0.028	0.86				
8th week	4.08 ± 0.59					1.847	0.18		
11th week	4.30 ± 0.52							10.933	0.00*
15th week	4.49 ± 0.43								

**Analysis of variance in repeated measurements and p-value were given, respectively.

Table 4 The relationship between students' clinical readiness and their readiness for e-learning and self-directed learning

		Clinical readiness**	
2nd week	e-learning	-0.166	0.40
	Self-directed learning	-0.082	0.67
5th week	e-learning	-0.114	0.56
	Self-directed learning	0.011	0.95
8th week	e-learning	-0.007	0.97
	Self-directed learning	0.009	0.96
11th week	e-learning	-0.097	0.62
	Self-directed learning	-0.242	0.21
15th week	e-learning	0.789	0.00*
	Self-directed learning	0.709	0.00*

*Statistically significant **Pearson correlation test and p-value are given, respectively.

Table 5 Distance Education Models and Themes and Sub-themes

Themes	Sub-themes	Codes
High Readiness	Orientation support	Research for clinical practice Facilitate practical thinking Gaining self-confidence Saving time Creating a learning environment Professor support Active method use Developing computer skills
Ambivalence	Pros and Cons	Thinking that they will have difficulties in medication and care practices Thinking that face-to-face education will be encouraging in clinical practice. Thinking that it will facilitate patient care Thinking that they will have difficulty communicating with the patient Feeling unable to fit in with the team at the clinic
Low Readiness	Insufficiency	Inability to practice invasive procedures Fear of harming the patient Fear of contagious COVID-19 Feeling uncertainty Having difficulty learning Perspective of the immediate surroundings/society on the distance education process Failure to provide a hospital atmosphere Not memorable Internet access restriction

Theme 2. Ambivalence

Sub-theme 1. Pros and Cons

Most of the students evaluated the distance education models and the readiness for clinical practice as both pros and cons.

Theme 3. Low readiness

Sub-theme 1. Insufficiency

Some of the students evaluated the readiness for clinical practice with distance education models as insufficient.

Discussion

In this study, the findings obtained in the present study showed that distance education models (case discussions, concept maps, debate discussions, professional skills video demonstrations, and PICO) increased the readiness for e-learning and self-directed learning of senior nursing students studying obstetrics and gynecology nursing. In the e-learning feasibility study conducted by Alhassan (2020) in Ghana with 233 nursing and midwifery students, students have reported that they are ready to receive education in e-learning circumstances [16]. In a systematic review evaluating the results of 22 different systematic reviews conducted in Canada, positive attitudes and behaviors toward e-learning were reported at a rate of 79.41% in the results of 11 different comparison studies on e-learning with nurses and nursing students [17]. Most of the studies conducted with nurses and nursing students report that the participants have positive opinions about e-learning. In their study with 312 nursing students, Sener et al. (2022) reported that students with high online learning attitudes had positive views on online learning in nursing education [18]. In this study, consistent with other studies, students' e-learning readiness levels were 156.77 at the beginning of the term, at a medium-high level, but increased to 187.72 at the 11th week. As a result of the study we conducted, the e-learning readiness levels of nursing students increased statistically significantly. The findings suggest that this significant increase in students' e-learning and self-directed learning readiness levels is due to the educational methods that facilitate learning in the distance education process. Students found it useful to draw the PICO game with a rate of 36.22% at the most and draw a concept map at a rate of at least 17.24% among the education methods applied in the distance education process and facilitating learning. This result can be interpreted as the significance of gamification, which is the today's reality, combined with technology and its use in education.

In our study, students stated that distance education methods increased their clinical readiness. The opinions of the students are that nursing education is supported by distance education methods that facilitate learning by influencing clinical and academic success. Thus, distance education methods should become a part of nursing education. The students expressed that the education models utilized in distance learning, which facilitate learning, positively contribute to their readiness for clinical practice, particularly during clinical orientation. At the same time, in the results of the present study, the clinical readiness of the students whose e-learning readiness and self-directed learning increased in the last week of the term increased statistically. In this direction, the readiness for clinical practice of students whose e-learning readiness level increases will also increase. On the other hand, in the interviews, most of the students evaluated the distance education models and the readiness for clinical practice as both pros and cons.

Nursing education is primarily practice-based [6]. While distance education aids students in becoming familiar with the clinical environment, it might not have the same impact on their readiness for actual clinical applications. McDonald et al. (2018) have reported that e-learning programs provide a flexible teaching method, according to the results of their integrative review, in which they present the available evidence for the effectiveness of e-based learning in improving clinical knowledge and skills in nursing students. However, the review results show existing evidence that e-learning alone does not provide the learning that face-to-face patient care provides [19]. In a study by Thapa et al. (2021) involving 470 nursing students, 58.9% of participants had a favorable attitude toward e-learning. However, only 34% perceived e-learning to be as effective as traditional face-to-face learning [20]. On the other hand, in the results of the study conducted by Kabir et al. (2022), nursing students' e-learning readiness was low due to various factors, such as less technology availability and less self-confidence in technology use. The prevalence of preferring e-learning in nursing education among students was reported as 43.46% [21]. This result may be because nursing education also includes clinical education. Students may think that e-learning environments are useful but insufficient to provide clinical learning opportunities.

In our study, the mean clinical readiness score of the students was 2.63±0.40. Some of the students evaluated the readiness for clinical practice with distance education models as insufficient. The students reported the best learning path as kinesthetic learning" with 40.4%, and they defined harms, such as "thinking that it will have difficulties in medicine and care applications" “thinking that it will be difficult in communication with the patient” and “feeling they you cannot adapt to the team in the clinic” in preparing for clinical practice with distance education. In the study's findings, as kinesthetic learning, which is best for hands-on experience, is not attainable during distance education, students might perceive their readiness levels as inadequate in clinical skills necessitating experiential learning. These skills include patient communication, personalized nursing care, administering drug therapy, and collaborating with clinical teams. These challenges experienced by students in their readiness for clinical practice may also affect their stress levels. For example, in a study by Oducado and Estoque (2021) with 108 nursing students, nursing students who received online education during the COVID-19 pandemic period evaluated online education as stressful by 44.4% and very stressful by 47.2% [22]. The COVID-19 pandemic process has already caused negative (academic stress, etc.) effects on students' own psychology [23]. Therefore, it can be thought that the stress levels of students whose e-learning, self-directed learning, and clinical readiness levels increase with web-based education supported by distance education methods will also decrease. Thus, along with the decrease in the stress levels of students supported by distance education methods, their academic success may also be affected.

Distance education methods have been included in today's nursing education, together with the COVID-19 pandemic conditions. Today, with the widespread use of technology, distance education methods have become a part of nursing education [24]. It is seen that e-learning, self-directed learning, and clinical readiness of nursing students are positively affected by an education supported by distance education methods. Thus, it is seen that nursing education has been enriched with techniques that facilitate learning and create positive views and attitudes in students.

Conclusion

The study results indicate that despite the integration of interactive methods, students perceive face-to-face education as more effective than distance education. This is due to the inability of nursing students to practice their nursing skills in the clinic/field. Hence, both in distance education and traditional face-to-face nursing education, the significance of employing interactive teaching methods during extraordinary circumstances such as the COVID-19 pandemic and earthquakes has become evident.

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References

1. Evans D. How will COVID-19 affect women and girls in low- and middle-income countries? 2020. https://www.cgdev.org/blog/how-will-covid-19-affect-women-and-girls-low-and-middle-income-countries
2. The Higher Education Council Presidency. *Press briefing.* 2020. https://covid19.yok.gov.tr/alinan-kararlar
3. Cabı E, Kalelioglu F. A fully online course experience from students’ perspective: readiness, attitudes and thoughts. *Turkish Online Journal of Distance Education,* 2019;20(3):165-180. doi:10.17718/tojde.601934
4. Platt CA, Amber ANW, Yu N. Virtually the same?: Student perceptions of the equivalence of online classes to face-to-face classes. *MERLOT Journal of Online Learning and Teaching,* 2014;10(3):489-503.

5. Turkish Council of Higher Education. Analysis- The impact of online education during COVID-19 pandemic in Turkish higher education. 2020. https://www.aa.com.tr/en/analysis/analysis-the-impact-of-online-education-during-covid-19-pandemic-in-turkish-higher-education/2163525
6. Boztepe H, Terzioğlu F. Skill Assessment ın nursing education. *Journal of Anatolia Nursing and Health Sciences,* 2013;16(1):57-63. [Turkish].
7. Emin MN. Coronavirus Outbreak and Education in Emergency. 2020. https://setav.org/assets/uploads/2020/04/P268.pdf [Turkish].
8. Ullah O, Khan W, Khan A. Students' attitude towards online learning at tertiary level. *PUTAJ–Humanities and Social Sciences*, 2017;25(1-2):63-82.
9. Ulenaers D, Grosemans J, Schrooten W, Bergs J. Clinical placement experience of nursing students during the COVID-19 pandemic: A cross-sectional study. *Nurse Education Today*, 2021;99:104746. doi: 10.1016/j.nedt.2021.104746.
10. Gómez-Urquizaa JL, Gómez-Salgadob J, Albendín-Garcíaa L, Correa-Rodrígueza M, González-Jiméneza E, Cañadas-De la Fuentea GA. The impact on nursing students' opinions and motivation of using a "Nursing Escape Room" as a teaching game: A descriptive study. *Nurse Education Today,* 2019;72:73–76. doi: 10.1016/j.nedt.2018.10.018.
11. Yurdugül H, Demir Ö. An investigation of pre-service teachers' readiness for e-learning at undergraduate level teacher training programs: the case of Hacettepe University. *Hacettepe Unıversity the Journal of Education,* 2017;32(4):896-915 doi: 10.16986/HUJE.2016022763 [Turkish]
12. Fisher M, King J, Tague G. Development of a self-directed learning readiness scale for nurse education. *Nurse Education Today,* 2001;21(7):516-525. doi: 10.1054/nedt.2001.0589.
13. Sahin E, Erden M. Reliability and Validity of Self-Directed Learning Readiness Scale (SDLRS). e-Journal of New World Sciences Academy, 2009;4(3):695-706. [Turkish]
14. Braun V, Clarke V. Using thematic analysis in psychology. Qualitative Research in Psychology, 2006;3(2):77-101. doi:10.1191/1478088706qp063oa
15. Yildirim A, Simsek H. Qualitative research method. 2016, Ankara: Seckin Publication.
16. Alhassan RK. Assessing the preparedness and feasibility of an e-learning pilot project for university level health trainees in Ghana: a cross-sectional descriptive survey. *BMC Medical Education,* 2020;20:465 doi:10.1186/s12909-020-02380-2
17. Rouleau G, Gagnon MP, Côté J, Payne-Gagnon J, Hudson E, Dubois CA, et al. Effects of e-learning in a continuing education context on nursing care: systematic review of systematic qualitative, quantitative, and mixed-studies reviews. *Journal of Medical Internet Research*, 2019;21(10):e15118. doi: 10.2196/15118.
18. Sener Y, Sener Taplak A, Hobek Akarsu R. Views and attitudes of nursing students towards online learning during COVID-19 pandemic. *Value in Health Sciences,* 2022;12(1):137-146. doi:10.33631/sabd.1055486 [Turkish]
19. McDonald EW, Boulton JL, Davis JL. E-learning and nursing assessment skills and knowledge – An integrative review. *Nurse Education Today,* 2018;66:166-174. doi: 10.1016/j.nedt.2018.03.011.
20. Thapa P, Bhandari SL, Pathak S. Nursing students' attitude on the practice of e-learning: A cross-sectional survey amid COVID-19 in Nepal. *PLoS ONE*, 2021;16(6):e0253651. doi:10.1371/journal.pone.0253651
21. Kabir H, Tonmon TT, Hasan M, Biswas L, Chowdhury M, Hasnat A, et al. Association between preference and e-learning readiness among the Bangladeshi female nursing students in the COVID-19 pandemic: a cross-sectional study. *Bulletin of the National Research Centre,* 2022;46(1):1-10. doi:10.1186/s42269-022-00697-0
22. Oducado RM, Estoque H. Online learning in nursing education during the COVID-19 pandemic: Stress, satisfaction, and academic performance. *International Journal of Nursing Practice,* 2021;4(2):143-153. doi:10.30994/jnp.v4i2.128
23. Lee SY, Jang SJ. High school students' social jetlag, lifelong competency, and academic stress during the COVID-19 pandemic: a cross-sectional study. *The Journal of School Nursing,* 2022;10598405221094847.
24. Satır G, Beji Kızılkaya N. Electronic teaching methods in the field of healthcare. *Turkish Journal of Health Sciences and Research,* 2021;4(2):14-24. doi:10.51536/tusbad.870675 [Turkish]

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The Effects Of Surgical Arthroscopy And Intraarticular Medication On The Antioxidant System And Lipid Peroxidation In Knee Osteoarthritis

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Abstract

Aim. This study aims to evaluate the effects of joint surgery through arthroscopy, intraarticular medication, and antioxidant therapy on the antioxidant system and lipid peroxidation in patients with knee osteoarthritis (KOA). The study examines the ability of high-molecular weight hyaluronan, sodium hyaluronate, and oral Vitamin E to modulate oxidative stress markers in the knee joint.

Methods and Materials. There were 60 patients diagnosed with KOA that were divided into four groups according to the type of treatment for this prospective study at the Department of Orthopaedics and Traumatology. Blood and synovial fluid samples collected before and after treatment were evaluated for superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), lipid peroxidation (malondialdehyde, MDA) catalase (CAT). SPSS software was used to perform statistical analysis where the significance level was set at $p<0.05$.

Results. Synovial fluid malondialdehyde levels that showed a decreased tendency among treated groups indicated a reduced state of antioxidant activity. However, no significant changes were observed in systemic oxidative stress markers. These findings show that localized antioxidant therapy within the knee can be effective in reducing oxidative stress, therefore, may have implications for nonsurgical treatment of KOA.

Conclusion. The results emphasize possible gains made from combining surgical arthroscopy together with antioxidant treatment in managing KOA. By reducing the level of oxidative stress within the knee joint, this combined method can provide a viable solution to improve symptoms and quality of life among KOA patients.

Keywords: Knee osteoarthritis (KOA), Oxidative stress, antioxidant therapy, lipid peroxidation

Introduction

Knee osteoarthritis (KOA) can often cause pain, impairments, and disability. It has far-reaching implications for the daily lives of many people around the world [1]. This condition is caused by the degeneration of cartilage that is supposed to protect the ends of bones from damage. Evidence suggests that knee deterioration has high prevalence rates among elderly individuals and women [2].

The aging process and several other factors have escalated knee osteoarthritis (KOA) into a global public health concern. Osteoarthritis (OA) poses a significant challenge for public health, as it is increasing at an alarming rate with substantial economic consequences and deteriorates quality of life [3]. Considering its significance and priority, preventive measures should be prioritized. However, effective management and control will rely on early detection indicators, precise diagnosis, and the appropriate treatment options [4].

Research typically focuses on methods of drug administration, mechanical means, and sometimes surgical procedures. Therapeutic interventions are designed to reduce pain symptoms, enhance functional abilities, and slow the disease's progression [5]. Physical exercises often complement most therapies since they help maintain the essential body mass for joint protection while taking medications. Otherwise, surgery is required when these remedies are ineffective [6].

Pharmacological approaches include the administration of analgesics along with anti-inflammatory agents used for both inflammation reduction and pain alleviation. Antioxidants may benefit elderly individuals who are prone to poor physical performance, thus putting them at risk of developing osteoarthritis (OA) [7]. Recent studies have targeted the use of antioxidants to prevent damage by OA within cartilage. The effects of antioxidants on mortality or morbidity in diseases remain contradictory, requiring further investigation. For example, antioxidant supplements should be considered therapeutic products and undergo rigorous evaluation before being marketed [8]. These studies were conducted through dietary modification in which the ratio of antioxidant-rich diets was increased while subjects received antioxidant pharmaceutical formulations in addition to a supplement [9]. There have also been studies showing increased or improved muscle strength, facilitation of physical capacities, and reduced likelihood of advancing the disease. All this is derived from the free radical theory of aging, which proposes that oxygen-based free radicals are responsible for age-related cell and tissue damage [10]. Because this issue is crucial, it is necessary to obtain more information from policymakers and physicians so that they can develop effective remedies. The current study has been designed as a comprehensive systematic review that focuses only on articles relevant enough to investigate the effects of antioxidants on KOA and done with utmost care to provide reliable evidence.

Methods

This study was conducted in the Department of Orthopedics and Traumatology of SDO Faculty of Medicine between January 2005 and May 2005. The diagnosis included knee complaints about pain, clinical and radiographic evidence that the patients had knee osteoarthritis, while those who fulfilled the American College of Rheumatology (ACR) criteria for knee osteoarthritis were eligible.

The research involved a total of 60 knee OA patients where women and men were 24. Inclusion criteria for this group included ACR criterion as well as no pregnancy or desire to become pregnant, no breastfeeding, no drug allergies or hypersensitivities, no severe systemic diseases, absence of conditions that could affect an assessment of the treated knee joint, absence of patients who received intraarticular therapy in the study knee within a three month period prior to entry in that case series, and finally exclusion due to arthroscopic surgery performed in last three years (figure 1).

The patients were randomly placed into four groups. Anamnesis has been specified in detail along with systemic physical examination and examination of the motion system. Haematological tests including routine blood biochemistry erythrocyte sedimentation rate c-reactive protein rheumatoid factor levels blood type coagulation time complete urine analysis have been carried out for each patient before surgery. All participants underwent a bilateral X-ray examination of their knees; posteroanterior chest radiograph (CXR) and electrocardiography were added for age over 40.

After arthroscopy treatment first group received high molecular weight hyaluronan (Hylan G-F 20) at weekly intervals after arthroscopy with three doses given per person. Furthermore, Hylan G-F-20 used oral vitamin E (Grandpherol®), taken once daily at a dose of 400 IU for three weeks. This control group was not treated with any type of intraarticular therapy after arthroscopy. Sodium hyaluronate was injected as a dose of 2 ml into the joint cavity after arthroscopy weekly basis for five consecutive times in patients in the fourth group.

Before and one week after the last injection, blood samples and synovial fluid were collected to determine the SOD, CAT, GSH-Px, and MDA content.

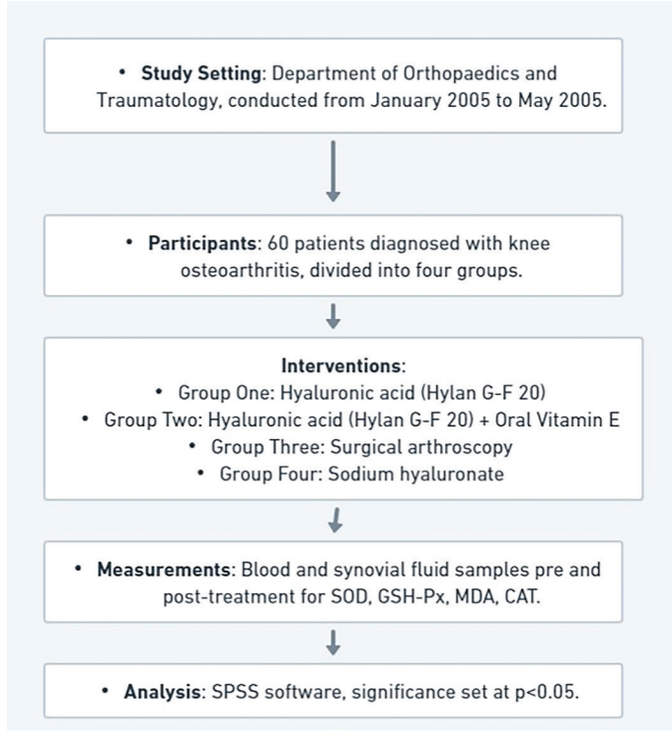

Figure 1 - Study Design

The fasting period started at midnight before the operation. Immediately prior to surgery, the leg to be operated on was shaved from the knee to the thigh. The arthroscopic intervention was performed under general or spinal anaesthesia in an antiseptic operating room. All patients had debridement arthroscopically, Kirchner wire used while microfracture K wires during entry into knee joint through the anterolateral approach described by the Watanabe anatomical approach described by the Watanabe versatile access route described by the Watanabe anterolateral approach as told by Watanabe through which cartilage lesions seen during arthroscopy were classified according to the Outerbridge classification system.

At our institution, this study was approved by the local institutional review board (IRB) meeting the ethical standards described in the Declaration of Helsinki. Furthermore, written informed consent has been obtained from all individuals participating in this experiment. The reference number and name will be provided on request.

Data analysis using SPSS software version 25 windows was performed for descriptive statistics of demographic, clinical biochemical data, including Wilcoxon test and other tests within each treatment group were done; p-value <0.05 is considered significant statistically.

Results

This is an intricate study that examines in detail various treatments for knee osteoarthritis among 60 patients divided into four groups. The study focusses mainly on the classifications of cartilage lesions and assessing biochemical markers before and after treatment.

A total of 60 people participated in this investigation who were further divided into four groups: Group One (Hylan G-F 20), Group Two (Hylan G-F 20 plus oral vitamin E), Group Three (surgical arthroscopy) and Group Four (Na-Hyaluronate). In group one, Hylan G-F20 was used to provide fifteen cases (10 women and five males) with average age determined to be fifty three point two six.

Table 1 Patient Demographics and Distribution Across Treatment Groups

Treatment Group	Therapy Applied	Male Patients	Female Patients	Average Age	Total Patients
Group 1	Hyalan G-F 20	5	10	53.26	15
Group 2	Hyalan G-F 20 + Oral Vitamin E	6	9	52.93	15
Group 3	Surgical Arthroscopy	7	8	52.13	15
Group 4	Na-Hyaluronate	7	8	51.8	15
Total		25	35	52.53	60

Note: The patient population comprised 60% females and 40% males, with the youngest participant being 40 years old and the oldest 68 years old. There was no statistically significant difference in the average age across treatment groups (p>0.05).

Table 2 Grading and Distribution of Cartilage Lesions by Treatment Group and Joint Structure

Joint Structure	Grade-1 Lesions	Grade-2 Lesions	Grade-3 Lesions	Grade-4 Lesions	Total Lesions
Patella	4	28	35	11	78
Femur Medial Condyle	3	41	44	14	102
Femur Lateral Condyle	2	11	10	3	26
Femur Trochlea	4	38	38	4	84
Tibia Medial Condyle	5	25	28	3	61
Tibia Lateral Condyle	2	10	11	2	25
Total	20	153	166	37	376

Similarly, the second group consisted of 15 subjects (9 women and six men) with a mean age of fifty two point nine three receiving Hylan G-F 20 plus oral vitamin E. The third group had fifteen patients [8 women + 7 men] whose average age was fifty two point one three. Finally, Na-hyaluronate was administered at its recommended dose to 15 cases [8 females +7 males] aged about fifty and one point eight as illustrated in Table I below. This distribution demonstrates an equitable representation of gender within the sample population across all groups with middle-aged being primarily affected by knee arthritis of the knee (Table 1).

Therefore, it is important for us also to test for severity as well as distribution so that we can get insights into how therapeutic interventions affect those suffering from osteoarthritis of the knees during our investigation. These grades were used in diagnosing about three hundred seventy-six cartilage injuries present in 60 patients according to Outerbridge's grading system. This discovery reveals that there are more osteoarthritic changes that occur in the medial femoral condyle compared to any other site within the knee joint.

Table 3 Pre- and Post-Treatment Biochemical Values in Blood and Synovial Fluid

Treatment Group	Parameter	Pre-Treatment (Blood)	Post-Treatment (Blood)	Pre-Treatment (Synovial Fluid)	Post-Treatment (Synovial Fluid)
Group 1 (Hyalan G-F 20)	CAT (U/g)	9.144	9.128	-	-
	MDA (µmol/L)	35.95	35.96	0.3108	0.1066*
	SOD (U/g)	1761.9	1761.92	-	-
	GPX (U/g)	74.85	74.82	-	-
Group 2 (Hyalan G-F 20 + Vitamin E)	CAT (U/g)	10.854	10.861	-	-
	MDA (µmol/L)	33.48	33.43	0.4928	0.334*
	SOD (U/g)	2174.33	2174.33	-	-
	GPX (U/g)	87.43	87.44	-	-
Group 3 (Surgical Arthroscopy)	CAT (U/g)	8.436	8.397	-	-
	MDA (µmol/L)	32.48	32.85	0.9302	0.928
	SOD (U/g)	1691.8	1691.85	-	-
	GPX (U/g)	85.03	85.04	-	-
Group 4 (Na-Hyaluronate)	CAT (U/g)	12.92	12.11	-	-
	MDA (µmol/L)	34.42	34.52	1.9308	1.6813*
	SOD (U/g)	2147.45	2146.8	-	-
	GPX (U/g)	112.91	112.25	-	-

Table 4 Statistical Analysis of Biochemical Marker Changes Pre- and Post-Treatment

Treatment Group	Biochemical Marker	Pre-Treatment Average	Post-Treatment Average	p-Value	Statistical Significance
Group 1 (Hyalan G-F 20)	Catalase (CAT) U/g	9.144	9.128	>0.005	No
	Malondialdehyde (MDA) µmol/L	35.95	35.96	>0.005	No
	Superoxide Dismutase (SOD) U/g	1761.9	1761.92	>0.005	No
	Glutathione Peroxidase (GPX) U/g	74.85	74.82	>0.005	No
	MDA in Synovial Fluid µmol/L	0.3108	0.1066	<0.005	**Yes**
Group 2 (Hyalan G-F 20 + Vitamin E)	MDA in Synovial Fluid µmol/L	0.4928	0.334	<0.005	**Yes**
Group 3 (Surgical Arthroscopy)	MDA in Synovial Fluid µmol/L	0.9302	0.928	>0.005	No
Group 4 (Na-Hyaluronate)	MDA in Synovial Fluid µmol/L	1.9308	1.6813	<0.005	Yes

The area around the lunate surface, patella and lateral aspect femoral trochlea are some important areas that easily wear out through common biomechanical stress points in the knee joint. Table 2 shows such classifications and therefore helps to guide which treatment is best for different degrees of cartilage damage.

As a result, our extensive biochemistry study aimed to determine systemic as well as local biochemical responses associated with the therapies employed herein. Additionally, no significant differences were observed in catalase, malondialdehyde, superoxide dismutase, and glutathione peroxidase levels before and after blood samples from all subjects in all groups. Therefore, this shows that any treatment does not have much impact on general oxidative stress or the antioxidant capacity of an organism. In contrast, Groups 1, 2 and 4 had reduced malondialdehyde in their synovial fluids during the post-treatment period indicating increased localised oxidative stresses within their knee joints. These findings presented in Table 3 provide a clear target for local therapy rather than systemic biochemical changes that result in redox injury within the affected joint.

A thorough statistical analysis was performed to assess how the treatments had an impact. It is possible that intraarticular knee injections treatment may help reduce lipid peroxidation, which could be as a result of suppressing cellular damage and thus nitric oxide (NO) production through increased enzymatic activities. The findings imply that such therapeutic strategies can directly influence markers of oxidative stress within the knee joint, but not the systemic biochemical parameters (Table 4). These discoveries highlight the localised nature of the therapy and its potential as a non-intrusive method for treating osteoarthritis of the knee. This method targets oxidative stress in order to prevent cartilage degradation, it is a direct way to slow down arthritis progression from occurring in knees.

In conclusion, these detailed observations have demonstrated how different treatments affect knee osteoarthritis at various levels. However, there is a significant reduction in synovial fluid malondialdehyde after therapy within the chosen groups, indicating another way to attenuate local oxidative stresses from inside the knee joint itself, apart from some notable findings about systemic biochemical markers, among others.

Discussion

According to this study, the use of antioxidants in a specific area is effective in treating oxidative stress in the knee joint, which is one of the most important causes of knee osteoarthritis (OA). Although there were no significant systemic changes in oxidative markers; this shows that treating joints with antioxidants can reduce the levels of malondialdehyde in synovial fluid - a biomarker of local tissue damage caused by free radicals - and thus relieve pain locally. In other words, it means that this treatment can focus on particular symptoms or sites only where they occur without affecting general health conditions. This implies that although very little happens elsewhere apart from around where an operation has been done, still much relief could come about through dealing with oxidation changes near an affected area. Therefore, further research is needed on how best surgery and nonsurgical methods can be combined to improve quality of life among these patients.

Furthermore, it is important to note that there were no significant changes in systemic oxidative stress markers. Therefore, the conclusion is made that antioxidant and anti-inflammatory properties are more specific for local organs such as the knees than the whole body [13]. This suggests that there may be a treatment modality which provides relief and therapeutic benefits at the source of pain and dysfunction associated with OA without affecting any systemic biomarkers of oxidative stress. Consequently, addressing localised pathways to reduce oxidative stress within the knee joint could lead to the development of therapies targeting symptoms caused by KOA specifically. In addition, it opens up possibilities for therapeutic strategies aimed at reducing the systemic side effects that often accompany many pharmacological options [14]. Consequently, all of these point to the need for effective treatment modalities not only against localised pathologies linked to osteoarthritis but also safe without unwanted systemic consequences.

Comparing these results with other studies helps to unravel how antioxidants help to manage OA. Although some researchers have questioned whether antioxidants such as vitamin E can effectively treat osteoarthritic signs, others claim that their combination with hyaluronic acid treatments may cause a significant decrease in levels of oxidative stress within knee joint. Thus, combined therapy may improve its efficacy [15]. In this sense, these data brings out issues on the complexity of osteoarthritis and show what can be improved through combining several forms of therapy. Therefore, differences between the results of the trials have arisen because OA has proven quite complicated as a disease; therefore, one needs to consider a wide range of treatments directed towards different substrates [7].

Our analysis revealed that hyaluronic and vitamin E have intricate curative actions within the KOA oxidative stress mechanisms of KOA [16]. This means that the pathogenesis of KOA is based on oxidative stress, resulting in cartilage degradation and facilitating inflammation; therefore, antioxidants can be considered as potential modulatory agents for disease progression [17]. Moreover, HA is viscoelastic in addition to being a knee joint lubricant that may decrease mechanical loading in order to generate a suitable milieu. It aims to decrease stress levels by reducing ROS production. Moreover, this could make it more secure when combined with a lipid-soluble antioxidant such as Vitamin E. In addition, in this case, Vitamin E acts as an antioxidant molecule that neutralises free radicals, and inhibits chain propagation reactions that lead to lipid peroxidation hence preserving cell integrity in joint tissues against oxidative challenges that would otherwise affect articular cartilage due to oxidation [18].

However, certain restrictions must be kept in mind in order to fully comprehend the scope and relevance of our findings. Additionally, we note that despite having enough weakness size to make some tentative conclusion based on our data on this condition we realize it was not representative of all patients with KOA and could not incorporate all possible cases throughout its spectrum. Different individuals manifest various grades of severity of this ailment, as well as different responses to treatment modalities applied for its control. Furthermore, since it was a short study, we did not know if these therapies were effective or influenced the progression of KOA after this period. Therefore, longer follow-up studies are required than what was used here, noting changes in local and body-wide indicators of oxidative stress over time to establish whether such treatments are sustainable for improving health outcomes improvement or improving quality enhancement respectively [19].

Additionally, one should consider carefulness about generalisations among dissimilar affected populations by osteoarthritis, various stages of diseases, and other joints except knee also. This is because OA is a complex condition that arises from various aetiology factors such as genetic background,

lifestyle components that include everyday movement patterns and types of mechanical stresses people undergo among many others which can interact differently with the findings of this research [20]. More patients suffering from osteoarthritis at all stages from common sites including hands and hips, should be enrolled for future analysis to establish these observations. Furthermore, in addition to confirming the present results, they would help to understand how hyaluronic acid and Vitamin E function more fully by providing a clearer idea of the conservative treatment approaches discussed against osteoarthritis [7]. In another recent study, these authors reported on the comparative effects of different treatments for osteoporosis bone loss on joint pain and showed ways through which bone health indices involving the vertebrae or hip could be improved [16].

Treatment for osteoarthritis of the knee can be further established by this study. Furthermore, it discloses that antioxidants can decrease oxidative stress within the knee joint. These findings imply that medical interventions are possible and therefore more research looking into treatment approaches, combinatorial therapies, and quality of life improvement in KOA patients. Consequently, this can lead to change in clinical practice to more targeted symptom relief and improved joint function while minimising systemic side effects which are currently prevalent with a wider spectrum of adverse effects. As such, these results support the inclusion of antioxidant therapy in KOA guidelines pending future studies.

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References

1. Litwic A, Edwards MH, Dennison, EM, Cooper C. Epidemiology and burden of osteoarthritis. *British Medical Bulletin*. 2013; 105: 185-199. https://doi.org/10.1093/bmb/lds038
2. Canter PH, Wider B, Ernst E. The antioxidant vitamins A, C, E and selenium in the treatment of arthritis: A systematic review of randomized clinical trials. *Rheumatology (Oxford)*. 2007; 46(8): 1223-1233. https://doi.org/10.1093/rheumatology/kem116
3. Hsiao AF, Lien YC, Tzeng IS, Liu CT, Chou SH, Horng YS. The efficacy of high- and low-dose curcumin in knee osteoarthritis: A systematic review and meta-analysis. *Complementary Therapies in Medicine.* 2021; 63: 102775. https://doi.org/10.1016/j.ctim.2021.102775
4. Suantawee T, Tantavisut S, Adisakwattana S et al. Oxidative stress, vitamin E, and antioxidant capacity in knee osteoarthritis. *Journal of Clinical and Diagnostic Research.* 2013; 7(9): 1855-1859. https://doi.org/10.7860/JCDR/2013/5802.3333
5. Atabaki M, Shariati-Sarabi Z, Tavakkol-Afshari J, Mohammadi M. Significant immunomodulatory properties of curcumin in patients with osteoarthritis; a successful clinical trial in Iran. *International Immunopharmacology.* 2020; 85: 106607. https://doi.org/10.1016/j.intimp.2020.106607
6. Buck AN, Vincent HK, Newman CB et al. Evidence-Based Dietary Practices to Improve Osteoarthritis Symptoms: *An Umbrella Review. Nutrients*. 2023: 15(13): 3050. https://doi.org/10.3390/nu15133050
7. Grässel S, Muschter D. Recent advances in the treatment of osteoarthritis. *F1000Research.* 2020; 9 (F1000 Faculty Rev): 325. https://doi.org/10.12688/f1000research.22115.1
8. Rahimnia AR, Panahi Y, Alishiri G, Sharafi M, Sahebkar A. Impact of Supplementation with Curcuminoids on Systemic Inflammation in Patients with Knee Osteoarthritis: Findings from a Randomized Double-Blind Placebo-Controlled Trial. *Drug Research (Stuttg)*. 2015; 65(10): 521-525. https://doi.org/10.1055/s-0034-1384536
9. Ozon EA, Iuga IDM, Mititelu M et al. Pharmacotechnical, Physico-Chemical, and Antioxidant Evaluation of Newly Developed Capsule Formulations. *International Journal of Molecular Sciences*. 2023; 24(14): 11426. https://doi.org/10.3390/ijms241411426
10. Kulkarni PD, Damle ND, Singh S et al. Double-blind trial of solid lipid Boswellia serrata particles (SLBSP) vs. standardized Boswellia serrata gum extract (BSE) for osteoarthritis of knee. *Drug Metabolism and Personalized Therapy.* 2020; 35(2): 20200104. https://doi.org/10.1515/dmpt-2020-0104
11. Bhattacharya I, Saxena R, Gupta V. Efficacy of vitamin E in knee osteoarthritis management of North Indian geriatric population. *Therapeutic Advances in Musculoskeletal Disease.* 2012; 4(1): 11-19. https://doi.org/10.1177/1759720X11424458
12. Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. *Frontiers in Pharmacology.* 2018; 9: 1162. https://doi.org/10.3389/fphar.2018.01162
13. 13. Hu HC, Lei YH, Zhang WH, Luo XQ. Antioxidant and Anti-inflammatory Properties of Resveratrol in Diabetic Nephropathy: A Systematic Review and Meta-analysis of Animal Studies. *Frontiers in Pharmacology.* 2022; 13: 841818. https://doi.org/10.3389/fphar.2022.841818
14. Ansari MY, Ahmad N, Haqqi TM. Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols. *Biomedicine & Pharmacotherapy.* 2020; 129: 110452. https://doi.org/10.1016/j.biopha.2020.110452
15. Nejadhosseinian M, Djalalinia S, Haerian H et al. The effects of antioxidants on knee osteoarthritis: A systematic review and meta-analysis. *Frontiers in Nutrition.* 2022; 9: 1026450. https://doi.org/10.3389/fnut.2022.1026450
16. 16. Menekşe S, Menekşe D. Comparison of the efficacy of oral and parenteral bisphosphonates in patients with postmenopausal osteoporosis: A single-center, retrospective clinical study. *Turkish Journal of Clinics and Laboratory.* 2022; 13(1): 146-152. https://doi.org/10.18663/tjcl.764506
17. Liu L, Luo P, Yang M, Wang J, Hou W, Xu P. The role of oxidative stress in the development of knee osteoarthritis: A comprehensive research review. *Frontiers in Molecular Biosciences.* 2022; 9: 1001212. https://doi.org/10.3389/fmolb.2022.1001212
18. Vincent HK, Percival SS, Conrad BP, Seay AN, Montero C, Vincent KR. Hyaluronic Acid (HA) Viscosupplementation on Synovial Fluid Inflammation in Knee Osteoarthritis: A Pilot Study. *The Open Orthopaedics Journal.* 2013; 7: 378-384. https://doi.org/10.2174/1874325001307010378
19. Papageorgiou SN. Visualising the results of clinical trials to draw conclusions. *Journal of Orthodontics.* 2022; 49(2): 240-243. https://doi.org/10.1177/14653125221101911
20. Kokkotis C, Moustakidis S, Baltzopoulos V, Giakas G, Tsaopoulos D. Identifying Robust Risk Factors for Knee Osteoarthritis Progression: *An Evolutionary Machine Learning Approach. Healthcare (Basel)*. 2021; 9(3): 260. https://doi.org/10.3390/healthcare9030260

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Platelet-serotonin Dynamics: Elucidating Their Role in Pulmonary Arterial Hypertension

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Abstract

Background and Objectives: Pulmonary arterial hypertension (PAH) is a significant complication in pediatric patients with congenital heart disease (CHD). The role of platelets and serotonin in the pathogenesis of PAH has been increasingly recognized. This study aims to investigate the correlation between platelet count, serotonin levels, and PAH in children with CHD, and to understand the impact of surgical intervention on these parameters.

Material and Methods: This study included 26 children with CHD and PAH (Group I) and an 11-child control group without PAH. Pre- and post-operative platelet counts, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), and serotonin levels in plasma and platelets were measured. Group I underwent surgical correction for CHD, and the control group received no such intervention. Data were analyzed to determine the relationships between these hematological and biochemical markers and PAH.

Results: Group I showed higher pre-operative platelet counts and serotonin levels compared to the control group. Post-surgical data indicated a significant decrease in platelet serotonin levels, aligning more closely with the control group. The study also observed lower plasma serotonin levels in the control group, suggesting altered serotonin metabolism in PAH patients.

Conclusion: The study suggests a strong association between elevated platelet counts, increased serotonin levels, and the presence of PAH in children with CHD. Surgical correction of CHD appears to normalize these parameters, indicating a potential pathophysiological link. These findings emphasize the need for further research to understand the underlying mechanisms and to explore targeted therapeutic strategies for PAH in pediatric CHD patients.

Keywords: children, congenital heart defects, pulmonary arterial hypertension, platelets, serotonin

Introduction

Pulmonary Arterial Hypertension (PAH) in the context of Congenital Heart Defects (CHD) represents a critical intersection of two complex cardiovascular pathologies, each contributing to a multifaceted clinical presentation and progression. The pathophysiology of PAH in CHD encompasses a range of mechanisms, from molecular alterations to functional changes in the heart and lungs [1].

In the pediatric population, CHDs such as atrial and ventricular septal defects, patent ductus arteriosus, and more complex anomalies like Eisenmenger syndrome are significant contributors to the development of PAH. These congenital anomalies create abnormal connections between the systemic and pulmonary circulations or defects within the heart chambers, leading to altered hemodynamics. The increased blood flow through these abnormal pathways, particularly those that shunt blood from the systemic to the pulmonary circulation, results in elevated pressure in the pulmonary artery—a condition termed pulmonary hypertension [2].

The initial response of the pulmonary vasculature to increased blood flow and pressure is often compensatory. This response can include hypertrophy of the vascular smooth muscle cells and endothelial proliferation. However, these changes eventually become maladaptive, leading to progressive narrowing and stiffening of the pulmonary arteries. This phenomenon, known as vascular

remodeling, is a hallmark of PAH and is driven by complex interactions between endothelial cells, smooth muscle cells, and the extracellular matrix [3].

A key aspect of the pathophysiology of PAH in CHD is endothelial dysfunction. Normally, the endothelium plays a critical role in maintaining vascular tone by balancing vasoconstrictive and vasodilative substances. In PAH, this balance is disrupted, favoring vasoconstriction, inflammation, and thrombosis. Endothelial dysfunction in PAH is characterized by reduced production of nitric oxide (NO) and prostacyclin, both potent vasodilators, and an increased expression of endothelin-1, a potent vasoconstrictor. These changes contribute significantly to increased pulmonary vascular resistance, a defining feature of PAH [4].

The role of genetic and molecular factors in the pathogenesis of PAH in the setting of CHD is also increasingly recognized. Research has identified several genetic mutations and polymorphisms that may predispose individuals to more severe vascular remodeling and PAH. For instance, mutations in the BMPR2 gene, which plays a role in the regulation of pulmonary vascular growth and inflammation, have been identified in some patients with PAH [5].

Over time, the persistent elevation in pulmonary arterial pressure due to these combined mechanisms places an increased workload on the right ventricle. In an attempt to compensate, the right ventricle undergoes hypertrophy and dilation, changes that initially help maintain cardiac output. However, these adaptations can become maladaptive, leading to right ventricular dysfunction and failure, which are major determinants of prognosis in PAH associated with CHD [5].

Another aspect of the pathophysiology of PAH in CHD is the development of Eisenmenger syndrome in some patients. This syndrome represents an advanced form of PAH where prolonged high pressure in the pulmonary circulation causes a reversal of the initial left-to-right shunt to a right-to-left shunt. This shunting leads to cyanosis as oxygen-poor blood bypasses the lungs and enters systemic circulation, a situation that significantly complicates the clinical management and prognosis of these patients [6].

The progressive nature of PAH in the setting of CHD is also characterized by a decreased response to vasodilator therapy, unlike idiopathic PAH. This resistance to treatment is partly due to the fixed component of the increased pulmonary vascular resistance due to structural changes in the pulmonary vasculature. These pathophysiological changes culminate in increased pulmonary arterial pressure, right ventricular overload, and ultimately heart failure. Understanding these mechanisms is crucial for the development of targeted therapies and the improvement of clinical outcomes in this patient population [7].

Platelets in PAH pathogenesis. In the pathogenesis of PAH, platelets play a pivotal and multifaceted role, contributing significantly to the progression of this complex vascular disorder. Central to the formation of in-situ thrombi in the small pulmonary arteries, a characteristic feature of PAH, platelet activation and aggregation lead to increased pulmonary vascular resistance and pressure. This thrombotic activity is further exacerbated by the release of serotonin, a potent vasoconstrictor and pro-fibrotic agent, from the dense granules within the platelets. Upon activation, platelets expel serotonin into the pulmonary circulation, instigating not only vasoconstriction but also stimulating smooth muscle cell proliferation, thereby contributing to the pathological remodeling of pulmonary arteries [8, 9].

Serotonin in PAH pathogenesis. Serotonin, also known as 5-hydroxytryptamine (5-HT), is a multifunctional monoamine neurotransmitter that exerts significant influence in various physiological systems, including the vascular system. Its role in vascular biology is complex and multifaceted, impacting vascular tone, endothelial function, and contributing to vascular pathologies such as atherosclerosis, pulmonary hypertension, and more [10, 11, 12].

The study of platelet and serotonin dynamics offers a promising avenue for unraveling the complex etiology of PAH, potentially leading to more targeted and effective therapeutic strategies. Such research is not only pivotal for advancing our fundamental knowledge but also holds significant clinical implications in improving the management and outcomes of patients suffering from this challenging condition.

The aim of this study is to investigate the dynamics of platelets and serotonin in the etiology of PAH.

Materials and Methods

The study was authorized by ethic committee of Karaganda Medical University No. 37 dd. 29.03.2022. In this study, informed consent was obtained from the parents of all participating patients. Participant recruitment was conducted at the Karaganda City Cardiosurgery Center (Kazakhstan). The study cohort was stratified into two distinct groups. The I group comprised children diagnosed with CHDs complicated by PAH before and after surgical treatment. The II group included the control, consisting of healthy children free from CHD and PAH. Inclusion criteria for the I group encompassed the presence of a medically confirmed CHD, the presence (or absence, for the second group) of pulmonary hypertension, no current infectious complications, and an age range from 0 to 7 years, alongside parental or legal guardian consent for the child's involvement in the research. For the II group, the criteria were the absence of significant somatic pathologies, including CHD and PAH, no active infectious or inflammatory conditions, and an age range of 0 to 7 years, in addition to parental or guardian consent. The inclusion of a control group in our study was essential to establish a comparative reference point for evaluating the specific effect of the serotonin system on pulmonary hypertension. By comparing outcomes between the intervention group and the control group, we could effectively control for confounding variables and discern the true impact of serotonin dynamics on disease progression.

The sample size for our study was meticulously determined based on statistical considerations, including the desired level of confidence, margin of error, and expected variability of the data. This rigorous approach ensures that our study is adequately powered to detect significant differences or relationships with confidence.

Our primary research question was to investigate the influence of the serotonin system on the development of pulmonary hypertension in children with congenital heart defects. This question stems from a recognized gap in the literature regarding the understanding of the pathophysiological mechanisms underlying pulmonary hypertension in this specific patient population.

For laboratory analysis, several parameters were evaluated: the quantity of blood platelets and their specific attributes (including mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit) (PCT), along with the concentration of serotonin in both blood serum and platelets. The process involved using citrated whole blood for platelet

extraction and EDTA-plasma for obtaining Platelet Poor Plasma (PPP).

The hematological investigations involved assessing the platelet count and various platelet indices (mean platelet volume, the coefficient of variation in platelet volume, plateletcrit) using the Hematology analyzer Mindray 3200. The extraction of platelets was carried out from the citrated plasma.

For the immunoassay analysis, particularly for measuring serotonin levels, the study employed ELISA kits manufactured by Cloud-Clone Corp.

In terms of statistical methodology, normal quantitative data were expressed using the mean (M) and standard deviations (SD), providing a comprehensive statistical overview of the collected data.

Results

Group I, consisting of 26 pediatric patients, exhibited a median age of 9 months. The age spectrum extended from a minimum of 1 month to a maximum of 6 years. This group demonstrated an equitable gender distribution, with a 50:50 ratio of male and female participants. Each child in this group was clinically diagnosed with congenital heart disease. The prevalence of specific cardiac anomalies varied: atrial septal defect (ASD) was observed in 38% of the cases, ventricular septal defect (VSD) in 7%, patent ductus arteriosus (PDA) in 2%, and a combination of these defects in an additional 7%. Regarding pulmonary arterial hypertension (PAH), 11.5% of the subjects presented with a high degree of severity, whereas the remainder exhibited moderate to mild levels . Surgical intervention was undertaken for all individuals to mitigate the risk of right ventricular failure.

Pre-operative hematological and biochemical parameters were recorded. The average platelet count stood at 328.28 thousand/µl, with a median value of 344.00 thousand/µl. Additional parameters, such as median mean platelet volume (MPV) at 7.30 fl, platelet distribution width (PDW) at 15.70%, and plateletcrit (PCT) at 0.23%, were noted (p-value = 0.005). Serotonin levels, both in plasma and platelets, were quantified before surgery. Plasma serotonin fluctuated between 2.31 ng/ml and 193.54 ng/ml, averaging at 41.72 ng/ml. Platelet serotonin concentrations varied from 1.90 ng/ml to 230.21 ng/ml, with an average of 21.09 ng/ml (p-value = 0.05).

Table 1 Characteristics of the Study Groups

Characteristic	Group I	Group II
Age Range	1 month - 6 years	6 months - 7 years
Gender Distribution	50% male, 50% female	45% male, 55% female
Diagnosis	Congenital Heart Disease	None (No Heart Disease)
Prevalence of Cardiac Anomalies	- Atrial Septal Defect (ASD): 38%	Control group
	- Ventricular Septal Defect (VSD): 7%	Control group
	- Patent Ductus Arteriosus (PDA): 2%	Control group
	- Combination of Defects: 7%	Control group
Pulmonary Arterial Hypertension (PAH)	- 11.5% with high severity	Control group
	- 88.5% moderate to mild levels	Control group
Surgical Intervention	Yes	No

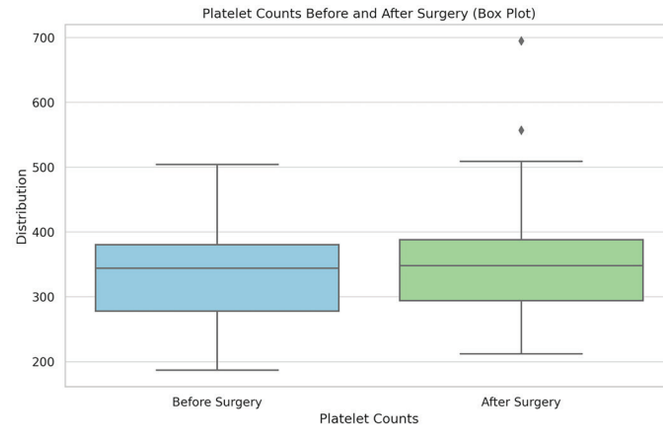

Figure 1 - The number of people with ASD in the respondent's environment

Figure 2 - The number of people with ASD in the respondent's environment

Figure 3 - The number of people with ASD in the respondent's environment

Post-surgical assessments revealed significant changes. The average platelet count increased to 377.25 thousand/µl, with a median of 348.00 thousand/µl. There were slight alterations in MPV (median of 7.70 fl), PDW (15.60%), and PCT (0.25%) (p-value = 0.005). Postoperative plasma serotonin levels were observed in the range of 4.10 ng/ml to 174.19 ng/ml, with a mean value of 50.80 ng/ml. In contrast, platelet serotonin concentrations were noted to be between 1.30 ng/ml and 14.20 ng/ml, averaging at 8.70 ng/ml (p-value = 0.05).

The control group, comprising 11 children aged between 6 months and 7 years (median age 3 years), included 45% male and 55% female subjects. Baseline hematological measurements in this cohort showed an average platelet count of 287.45 thousand/µl, with a median of 299.00 thousand/µl. The control group's median MPV was registered at 8.30 fl, PDW at 14.20%, and PCT at 0.22% (p-value = 0.005). Serotonin levels in this group also varied, with plasma levels ranging from 7.30 ng/ml to 41.05 ng/ml (mean 17.24 ng/ml) and platelet serotonin concentrations spanning from 10.58 ng/ml to 34.76 ng/ml (mean 20.26 ng/ml, p-value = 0.05).

Discussion

In the realm of cardiovascular physiology, platelets and serotonin, particularly platelet-derived serotonin, play a critical role. Beyond their well-known functions in hemostasis, these elements are integral to the development of the cardiovascular system and the pathogenesis of PAH.

In PAH, where endothelial dysfunction is prominent, platelets adhere to the damaged endothelial cells, amplifying vasoconstriction and fostering vascular remodeling – processes that are central to the disease's pathogenesis. Beyond their role in coagulation and vasoconstriction, platelets also partake in inflammatory processes. They release pro-inflammatory cytokines and engage in crosstalk with leukocytes, thus maintaining an inflammatory milieu within the pulmonary vasculature, which is increasingly acknowledged as a critical component in the development of PAH [13, 14, 15].

Additionally, platelets secrete growth factors such as Platelet-Derived Growth Factor (PDGF) and Transforming Growth Factor-Beta (TGF-β), both of which are implicated in the pathological remodeling of pulmonary arteries. These growth factors promote the proliferation and migration of smooth muscle cells and fibroblasts, culminating in the detrimental remodeling that typifies PAH [16].

One of the critical functions of serotonin in vascular biology is the regulation of vascular tone. Serotonin exerts its effects on blood vessels through a diverse array of receptors, predominantly through the 5-HT1 and 5-HT2 receptor families. The 5-HT1 receptors, particularly the 5-HT1B and 5-HT1D subtypes, are known to mediate vasoconstriction in certain blood vessels, including coronary arteries. Conversely, the 5-HT2 receptors, especially 5-HT2A, are associated with both vasoconstriction and vasodilation, depending on the vascular bed and the state of the endothelium. The net effect of serotonin on vascular tone is context-dependent, influenced by factors such as the type of blood vessel, the local concentration of serotonin, and the relative expression of its receptors [17, 18].

In healthy endothelium, serotonin typically induces vasodilation, mediated through the release of endothelium-derived relaxing factors like nitric oxide (NO) and prostacyclin. This vasodilatory effect is often attenuated in conditions where endothelial function is impaired, such as in atherosclerosis or hypertension, leading to a more pronounced vasoconstrictive response to serotonin [18].

The endothelium plays a crucial role in maintaining vascular homeostasis. Serotonin influences endothelial function in several ways. It modulates the expression of various endothelial adhesion molecules, which are pivotal in the recruitment of leukocytes during inflammation. Furthermore, serotonin can stimulate endothelial cell proliferation and migration, processes essential for angiogenesis and vascular repair [18].

In pathological conditions these normally reparative actions can contribute to disease progression. For instance, in atherosclerosis, serotonin may promote the proliferation and migration of endothelial cells and smooth muscle cells, contributing to plaque formation and vascular remodeling. Additionally, serotonin's interaction with platelets, which are rich in serotonin content, facilitates thrombosis by enhancing platelet aggregation. This pro-thrombotic effect of serotonin further implicates it in the pathogenesis of various cardiovascular diseases [18, 19, 20].

A particularly notable aspect of serotonin's role in vascular biology is its contribution to pulmonary vascular remodeling, a key feature of PAH. In the pulmonary vasculature, serotonin can induce smooth muscle cell proliferation and hypertrophy, leading to the narrowing of pulmonary arteries and increased pulmonary vascular resistance. This effect is mediated through various serotonin receptors, with the 5-HT2B receptor being particularly implicated in pulmonary smooth muscle cell proliferation [17, 18].

The serotonin transporter (SERT) also plays a crucial role in this context. Enhanced SERT activity in pulmonary arterial smooth muscle cells leads to increased uptake of serotonin, promoting cell proliferation and contributing to vascular remodeling [21].

Experimental studies, both in vitro and in animal models, have provided substantial evidence supporting serotonin's role in vascular biology. These studies have shown how alterations in serotonin signaling can contribute to various vascular diseases. For instance, mice lacking the 5-HT2B receptor exhibit reduced pulmonary vascular remodeling in response to hypoxia, highlighting the receptor's role in PAH [17, 18, 19, 20].

Beyond its role in vascular pathology, serotonin also contributes to normal vascular development and angiogenesis. Serotonin receptors are expressed in embryonic vascular tissues, suggesting a role for serotonin in vascular patterning and development. In angiogenesis, serotonin can stimulate the proliferation and migration of endothelial cells, essential for the formation of new blood vessels. This role has potential therapeutic implications in conditions where angiogenesis is desirable, such as in wound healing or ischemic heart disease.

The analysis revealed a notable difference in platelet levels between the control group and children with CHDs complicated with PAH. Specifically, the platelet count in the control group was approximately 30% lower than that observed in the CHD-PAH cohort. This disparity suggests a potential correlation between elevated platelet levels and the pathogenesis of PAH. Platelets, known for their primary role in hemostasis and thrombosis, also contribute significantly to inflammatory and proliferative processes, which are central to the development of PAH. Their involvement in PAH pathogenesis likely encompasses various mechanisms, including the modulation of vascular tone, promotion of endothelial dysfunction, and facilitation of smooth muscle cell proliferation.

Following surgical correction of congenital heart defects, a marked reduction in pulmonary hypertension was observed, accompanied by a nearly threefold decrease in platelet serotonin concentration. This finding underscores the significant role of serotonin in the pathogenesis of PAH. Serotonin, a potent vasoconstrictor and mitogen for smooth muscle cells, contributes to vascular remodeling, a hallmark of PAH. Elevated serotonin levels in platelets may enhance their release upon activation, further exacerbating vascular changes. Additionally, serotonin's influence on platelet aggregation could amplify the prothrombotic state, characteristic of PAH, promoting pulmonary vascular remodeling and increased pulmonary vascular resistance.

Furthermore, the study observed that the concentration

of serotonin in plasma was, on average, twice as low in the control group compared to the CHD-PAH group (figure 5). This finding indicates a systemic alteration in serotonin metabolism in patients with PAH, possibly reflecting its increased uptake and storage in platelets or its augmented production and release in response to chronic hypoxemia and endothelial dysfunction characteristic of CHD.

These observations suggest a complex interplay between platelets, serotonin, and the pulmonary vasculature in the context of PAH associated with CHD. However, it is imperative to acknowledge that these findings represent a preliminary exploration into a multifaceted and intricate pathophysiological process. Further research is essential to delineate the specific mechanisms by which platelets and serotonin contribute to the onset and progression of PAH in the context of congenital heart disease. Such studies should aim to elucidate the molecular pathways involved, evaluate the impact of various therapeutic interventions targeting platelet function and serotonin metabolism, and ultimately guide the development of more effective treatments for PAH in pediatric patients with CHD. The integration of advanced molecular techniques, longitudinal clinical studies, and interdisciplinary collaboration will be crucial in advancing our understanding of this complex disease process.

Conclusion

Our research highlights serotonin's role within the complex etiology of pulmonary hypertension. It is essential for future research to delve deeper into this relationship, examining the multifaceted mechanisms by which serotonin may contribute to pulmonary hypertension and validating these findings in broader cohorts. In essence, while our research opens new avenues for exploring serotonin and platelets dynamics as a biomarkers in pulmonary hypertension, it firmly establishes the foundation for subsequent, more extensive investigations to confirm these observations and their clinical relevance.

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References

1. Weatherald J, Reis A, et al. Pulmonary arterial hypertension registries: past, present and into the future. *Eur Respir Rev.* 2019; 28(154):1-2. DOI: 10.1183/16000617.0128-2019
2. Zijlstra W. Survival differences in pediatric pulmonary arterial hypertension: Clues to a better understanding of outcome and optimal treatment strategies. *Am Coll Cardiol.* 2014; 3(20):2159-2169. DOI: 10.1016/j.jacc.2014.02.575
3. Pascall E, Tulloh RMR. Pulmonary hypertension in congenital heart disease. *Future Cardiol.* 2018; 14(4):343–353. doi: 10.2217/fca-2017-0065
4. Iacobazzi D, Suleiman MS, et al. Cellular and molecular basis of RV hypertrophy in congenital heart disease. *Heart*. 2016; 102(1):12–17. doi: 10.1136/heartjnl-2015-308348
5. Rosenzweig EB, Krishnan U. Congenital Heart Disease-Associated Pulmonary Hypertension. *Clin Chest Med.* 2021; 42:9–18. DOI: 10.1016/j.ccm.2020.11.005
6. Diller GP, Kempny A, et al. Survival prospects of treatment naive patients with Eisenmenger: a systematic review of the literature and report of own experience. *Heart.* 2016; 100(17):1366–1372. DOI: 10.1136/heartjnl-2014-305690
7. Stępnowska E, Lewicka E, et al. Prognostic factors in pulmonary arterial hypertension: Literature review. *Adv Clin Exp Med.* 2017; 26(3):549–553. DOI: 10.17219/acem/61855
8. Davizon-Castillo P, Allawzi A, Sorrells M, et al. Platelet activation in experimental murine neonatal pulmonary hypertension. *Physiological Reports.* 2020; 8(5):1-14. doi: 10.14814/phy2.14386
9. Dees C, Akhmetshina A, et al. Platelet-derived serotonin links vascular disease and tissue fibrosis. *ExpMed*. 2011; 208:961–972. DOI: 10.1084/jem.20101629
10. Santos-Gomes J, Gandra I, Adão R, et al. An Overview of Circulating Pulmonary Arterial Hypertension Biomarkers. *Frontiers in Cardiovascular Medicine*. 2022; 9:1-26. https://doi.org/10.3389/fcvm.2022.924873
11. Shajib MS, Khan WI. The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol* (Oxf). 2015; 213:561–574. DOI: 10.1111/apha.12430
12. Kelley L. Biomarkers for pediatric pulmonary arterial hypertension – a call to collaborate. Frontiers in Pediatrics. *Pediatric Pulmonology.* 2014; 2:1-12. https://doi.org/10.3389/fped.2014.00007
13. Gaheen R, El Amrousy D, et al. Plasma copeptin levels in children with pulmonary arterial hypertension associated with congenital heart disease. *Eur J Pediatr.* 2021; 180:2889–2895. DOI: 10.1007/s00431-021-04060-9
14. Lannan KL, Phipps RP, et al. Thrombosis, platelets, microparticles and PAH: more than a lot. *Drug Discovery Today.* 2014; 13(83):1-6. doi: 10.1016/j.drudis.2014.04.001

15. Mese T, Guven B, Yilmazer MM, et al. Platelet activation markers in children with congenital heart disease associated with pulmonary arterial hypertension. Congenital Heart Disease. 2018; 13(4):506–511. DOI: 10.1111/chd.12616
16. Ismail EAR, Youssef OI. Platelet-Derived Microparticles and Platelet Function Profile in Children With Congenital Heart Disease. *Clinical and Applied Thrombosis/Hemostasis.* 2013; 19(4):424–432. DOI: 10.1177/1076029612456733
17. West JD, Carrier EJ, et al. Serotonin 2B receptor antagonism prevents heritable pulmonary arterial hypertension. *PLoS One.* 2016; 10(11):1-18. DOI: 10.1371/journal.pone.0148657 https://doi.org/10.1371/journal.pone.0148657
18. Hutcheson JD, Setola V, et al. Serotonin receptors and heart valve disease. *Pharmacol Ther.* 2011; 132:146–157. DOI: 10.1016/j.pharmthera.2011.03.008
19. Gray EA. Assessment of the serotonin pathway as a therapeutic target for pulmonary hypertension. *Synchrotron Rad.* 2013; 20:756-764. DOI: 10.1107/S0909049513021213
20. Sadoughi A, Roberts KE, Preston IR, et al. Use of selective serotonin reuptake inhibitors and outcomes in pulmonary arterial hypertension. *Chest*. 2013; 144:531–541. DOI: 10.1378/chest.12-2081
21. Mindubayeva F, Niyazova Y, Nigmatullina R, et al. Membrane serotonin transporter as a biomarker of pulmonary arterial hypertension in children with congenital heart defect. *Research Journal of Pharmacy and Technology.* 2020; 5(13):2435-2438. DOI:10.5958/0974-360X.2020.00436.9

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Comparative Evaluation of the Use of the Double Cementation Method and Modular Metal Augments for the Replacement of Bone Defects in Revision Knee Arthroplasty

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Abstract

Background: During revision arthroplasty of the knee joint, defects of the femur and tibia may occur. One common method to replace these defects is the use of modular metal augments, but this method has certain disadvantages. Therefore, we suggest using the double cementation method.

Objective: This study aims to compare the effectiveness of the double cementation method and modular metal augments in replacing bone defects during revision knee replacement.

Material and Methods: We examined 150 patients diagnosed with periprosthetic infection who were treated at the National Scientific Center of Traumatology and Orthopedics named after Academician N.D.Batpenov from 2021 to 2024. For a randomized study, 36 patients were selected, divided into 2 groups of 18 people. In the main group, the double cementing method was used to replace defects of the femur and tibia during revision knee arthroplasty; in the control group, metal augments were used. A follow-up examination was conducted on all patients one year after the surgery.

Results: No significant differences were found between the groups in terms of the number of hospital beds spent (p = 0.11), bed days spent in the intensive care unit after surgery (p = 0.44), duration of surgery (p = 0.18), amount of intraoperative blood loss (p = 0.18), knee joint function according to the Knee Society Score (p = 0.23) and Oxford Knee Score (p = 0.09). In the main group, signs of radiographic instability were detected in 1 case (5.6%), in the control group, there were revealed 5 (27.8%) cases. The number of cases of periprosthetic infection in the main group was 1 case (5.6%), in the control group were 3 cases (16.7%).

Conclusion: The double cementation method is less likely to cause radiography lines of illumination at the cement/bone boundary and may be recommended for high-risk postoperative infections. Additionally, it may be more cost-effective than using metal augments.

Keywords: double cementation method, modular metal augments, revision arthroplasty, knee joint, bone defects.

Introduction

The issue of revision interventions in patients with knee replacement remains highly relevant due to the annual increase in their number [1,2]. Statistical collections indicate a rise in the number of revision endoprostheses of the knee joint in the Republic of Kazakhstan. In 2013, there were 26 cases of revision arthroplasty, while in 2020, the number increased to

175 people [3]. Audit operations are observed in many countries worldwide. In Germany, 13,961 revision arthroplasties of the knee joint were performed in 2021, with infectious complications accounting for 14.5% to 15.0% of all revisions between 2019 and 2021, according to the German Registry of Endoprosthetics [4-7]. In Australia, Ackerman et al. reported 43,188 revision knee replacement surgeries between 2007 and 2017 [8].

During knee joint revision arthroplasty, defects in the femur and tibia are common [9]. Proper positioning and stability of the endoprosthesis depend on the replacement of these defects [10]. Currently, various methods are used to replace bone defects during revision knee replacement, including cementing, cementing with reinforced screws, factory cement spacers with augments, modular metal augments, metaphysical bushings with pressed coating of porous titanium and structural cones of porous tantalum, autologous bone grafting, allogeneic bone grafting, impact bone grafting, structural bone allografts, mega-endoprostheses, or individual endoprostheses [9, 11-13].

The most common method for replacing defects is through modular metal augmentation. However, this method has certain disadvantages, such as metal abrasion and corrosion, as well as loosening of endoprosthesis components [11-16]. Additionally, noncement-based methods for replacing bone defects in the knee have the main problem of being unable to locally deliver antibacterial drugs to the infected joint or to those at high risk of postoperative infection [17]. The developed method of double cementation can serve as an alternative to the use of metal augments, avoiding their disadvantages.

The purpose of this study was to compare the use of the developed double cementation method with the traditional method of replacing defects with modular metal augments during revision arthroplasty of the knee joint.

Hypotheses of the study: the use of the double cementating method for replacing defects of the femur and tibia during revision knee joint replacement is equally effective with the traditional method using metal augments.

Materials And Methods

Ethics

The study was conducted in accordance with international ethical standards and principles of the Helsinki Declaration and was approved by the Local Ethics Commission of our hospital (Protocol No. 4 of October 19, 2021). All patients participating in the study signed an informed consent to be included in the study.

Main characteristics of the compared groups

The total number of examined patients was 150 people. A randomized clinical trial selected 36 patients who were treated at the of our center, according to the criteria for inclusion in the study. The criteria for inclusion in the study were: Patients with aseptic instability of the knee arthroplasty, a history of surgery for knee replacement, the patient's age between 40 and 79, the patient's consent to the treatment, the absence of severe concomitant diseases affecting the results of treatment. The exclusion criteria were: The age of patients under 40 and over 79, periprosthetic infection, hemiparesis on the side of the proposed operation, neoplasms of other localizations with or without metastases, as well as the patient's' refusal of surgery.

Patients were included in the study after prior consultation with related specialists.

A main group and a control group of 18 patients each were formed. The distribution into groups was carried out using the sealed envelope method.

In the main group, patients underwent revision knee arthroplasty using the double cementation method. In the control group, patients underwent revision knee arthroplasty using the standard method of defect replacement – the use of modular metal augments.

Comparison and comparability in the two formed groups were carried out according to the following criteria: gender, age, size of defects, the number of revision operations performed on this joint, including the revision performed as part of the study.

A follow-up examination of all patients was performed 1 year after surgery. The following indicators were evaluated: the number of hospital beds; the number of bed days spent in the intensive care unit; the duration of the operation; the amount of intraoperative blood loss; assessment of knee joint function, radiographic stability, the number of cases of periprosthetic infection. Knee joint function was assessed using the Knee Society Score scale (KSS) and the Oxford Knee Score questionnaire (OKS). The evaluation of the radiological stability of the knee joint was carried out using the Modern Knee Society Radiographic Evaluation System.

Surgical techniques

Main group – the double cementation method. The double cementation method is a method of revision knee arthroplasty, in which polymerized bone cement acts as Augments to replace defects in the femur and tibia. During revision knee arthroplasty, after removal of unstable components of the knee arthroplasty and careful debridement of tissues, the size of defects in the femur and tibia is assessed. Next, an endoprosthesis of the required size is selected and the first layer of bone cement is applied to the components of the endoprosthesis, acting as augments. Upon completion of polymerization of the first layer of bone cement, a second layer of bone cement is applied on top of the endoprosthesis and bone cement augments and the components of the endoprosthesis are installed. After the postoperative wound is sutured in layers [18]. The process of applying the double cementation method is also shown in Figure 1. An example of preoperative and postoperative knee radiographs is shown in Figure 2.

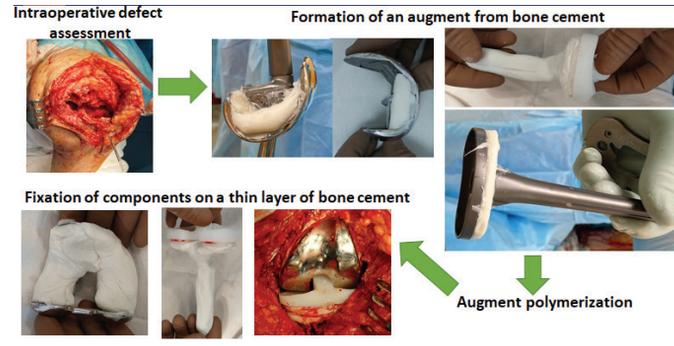

Figure 1 - The process of forming cement augments on endoprosthesis components and their subsequent fixation on a thin layer of bone cement (the order of the process is indicated by arrows)

Control group – Modular metal augments. Modular metal augments for this period of time are presented in the form of metal blocks and wedges [11]. After removal of unstable components of the knee arthroplasty and careful debridement of tissues, the size of the defect of the femur and tibia is assessed. After selecting the required size of the endoprosthesis, the necessary metal augments are fitted (Figure 3).

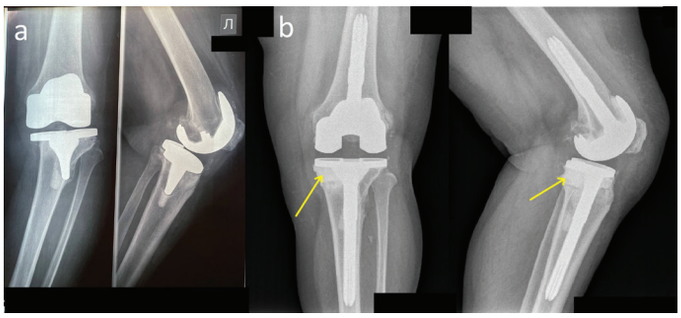

Figure 2 - Radiographs of the knee joint (a) - preoperatively, instability of both endoprosthesis components and migration of the tibial component are identified; (b) - postoperatively, the fitted endoprosthesis and cement augmentation are identified (indicated by arrows)

Next, the components of the endoprosthesis, together with augments, are installed in the bone on a thin layer of bone cement. After the postoperative wound is sutured in layers.

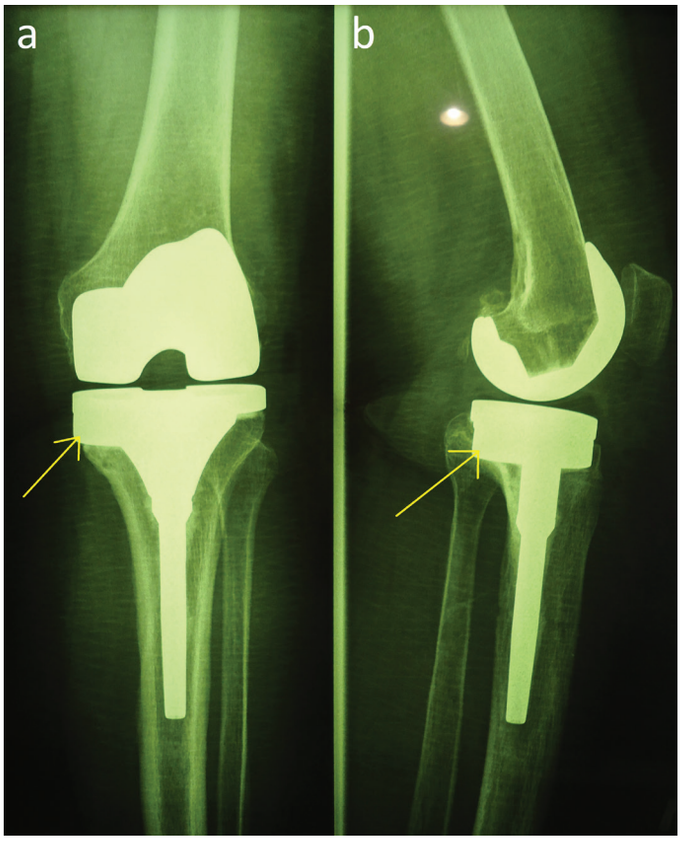

Figure 3 - Radiographs of the knee joint in frontal (a) and lateral (b) projections after revision endoprosthesis with the standard technique - use of modular metal augmentation (indicated by arrows)

Statistical analysis

Descriptive statistical methods were used for processing the statistical data. The nonparametric Mann-Whitney criterion was used to determine the significance of quantitative differences between the groups. To assess the significance of qualitative parameters when comparing treatment results in both groups, we used a nonparametric method of calculating Pearson's criterion $\chi 2$ (chi-squared). Differences between the groups were considered significant at $p<0.05$.

The statistical data was processed using Microsoft Excel from the Microsoft Office 2016 package and Statistica 12.0 software for statistical analysis developed by Statsoft [19].

Results

In the both groups, there were 4 men (22.2%) and 14 women (77.8%).

The average age of patients in the main group was 63.6 years ($\sigma = 9.7$; CI = 58.68 – 68.32), and in the comparison group 61.4 years ($\sigma = 5.4$; CI = 58.71 – 64.09).

The sizes of defects in the articular surfaces of the femur and tibia were estimated according to the Anderson Orthopaedic Research Institute (1997) scale [20]. In the main group, bone defects were distributed as follows: F1 – 4 (22.2%), F2A – 6 (33.3%), F2B – 8 (44.4%), T2A – 7 (38.9%), T2B – 11 (61.1%). In the control group, bone defects were distributed as follows: F1 – 5 (27.8%), F2A – 5 (27.8%), F2B – 8 (44.4%), T2A – 5 (27.8%), T2B – 13 (72.2%).

The average number of revision operations performed on the knee joint, including the revision performed as part of the study, in the main group was 1.5 ($\sigma = 0.6$; CI = 1.19 – 1.81) while in the control group – 2.2 ($\sigma = 0.7$; CI = 1.82 – 2.52).

Despite the presence of a small difference in age and size of defects between the two groups, no statistically significant differences were found. Statistically significant differences were revealed in the number of revision operations performed on the knee joint, including the revision performed as part of the study. In the group of metal modular augments, there were on average 0.7 more revisions than in the double cementation group ($p = 0.02$).

A comparison of the formed groups is also shown in Table 1.

The average number of hospital bed days in the main group was 14.7 days ($\sigma = 3.2$; CI = 13.13 – 16.31), and in the comparison group 18.1 days ($\sigma = 6.3$; CI = 14.95 – 21.17).The average number of bed days spent in the intensive care unit in the main group was 0.8 days ($\sigma=0.4$; CI = 0.57 – 0.99), in the comparison group also 0.8 days ($\sigma=0.9$ CI = 0.37 – 1.29).There was no statistically significant difference in the average number of bed days spent in the hospital ($p = 0.11$) or in the intensive care unit ($p = 0.44$) in the groups.

When comparing the time spent on the operation, the following results were obtained: in the main group, the average operation time was 97 minutes ($\sigma= 18.6$; CI = 87.75 – 106.25), in the comparison group 97.8 minutes ($\sigma= 19.4$; CI = 87.75 – 106.25). There was no statistically significant difference in the duration of the operation in these groups ($p = 0.18$). Also, when comparing the number of blood loss in the general group, there was no statistically significant difference ($p = 0.18$). The total increase in the main group averaged 339.5 ml ($\sigma= 205.9$; CI = 237.09 – 441.86), and in the control group 512.5 ml ($\sigma= 364.1$; CI = 331.40 – 693.60).

Comparative results of knee and functional scores in the both groups on the Knee Society Score and Oxford Knee Score scale 1 year after surgery are presented in Table 2. The average number of knee scores on the Knee Society Score scale in the main group was 85.6 ($\sigma= 10.3$; CI = 80.47 – 90.69), in the control group 81.3 ($\sigma= 12$; CI = 75.33 – 87.30).The average number of functional scores on the Knee Society Score scale in the main group was 80.6 ($\sigma= 14.4$; CI = 73.37 – 87.74), and in the control group 73.7 ($\sigma= 17.4$; CI = 65,04 – 82,33).The average number of points on the Oxford Knee Score scale in the main group was 17.8 ($\sigma= 8.6$; CI = 13.50 – 22.08), in the control group 22.3 ($\sigma=11.7$; CI = 16.43 – 28.07).The assessment showed that there were no statistically significant differences in scores on the presented scales between the group of the double cementation method and the group of modular metal augments.

An assessment of the radiographic stability of the endoprosthesis components at the bone/cement boundary

Table 1 Comparison of the formed groups

Groups	Knee Society Score Knee Scores	Knee Society Score Functional Scores	Oxford Knee Score
Main group	85.6 ±10.3 (CI = 80.47 – 90.69)	**80.6** ±14.4 (CI = 73.37 – 87.74)	**18.1** ±8.8 (CI = 13.75 – 22.47)
Control group	**81,2** ±12,4 **(CI = 75.07 – 87.38)**	**73.7** ±17.4 (CI = 65.04 – 82.33)	**22.7** ±11.8 (CI = 16.79 – 28.58)
p-value	**0.23**	**0.18**	**0.09**
Mann-Whitney U-test	**U = 123.5**	**U = 119.5**	**U = 108.5**

Table 2 Comparative results of evaluation of knee joint function 1 year after surgery

Comparison criteria	Double cementation method	Modular metal augments
Number of men	4 (22.2%)	4 (22.2%)
Number of women	14 (77.8%)	14 (77.8%)
Patients' average age	63.6 years (σ = 9,7; CI = 58.68 – 68.32)	61.4 years (σ = 5.4; CI = 58.71 – 64.09).
The size of femur defects	F1 – 4 (22.2%), F2A – 6 (33.3%), F2B – 8 (44.4%)	F1 – 5 (27.8%), F2A – 5 (27.8%), F2B – 8 (44.4%)
The size of tibial defects	T2A – 7 (38.9%), T2B – 11 (61.1%)	T2A – 5 (27.8%), T2B – 13 (72.2%)
The average number of postponed revision operations	1.5 (σ = 0.6; CI = 1.19 – 1.81)	2,2 (σ = 0.7; CI = 1.82 – 2.52)

showed that in the main group, signs of radiographic instability were detected in 1 case (5.6%). In the control group, there were revealed 5 (27.8%) cases of radiographic instability of the endoprosthesis components in the area of installation of metal augments.

The number of cases of periprosthetic infection in the main group was 1 case (5.6%), in the control group were 3 cases (16.7%) (Figure 4). The Mann-Whitney U-test score showed that there were no statistically significant differences between the groups in the number of cases of periprosthetic infection (p = 0.29).

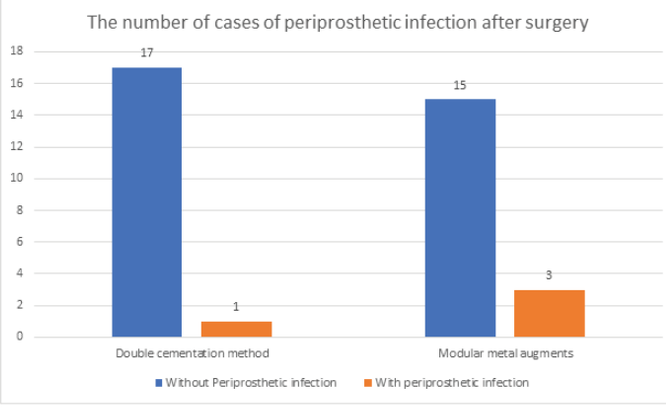

Figure 4 - Graph of the number of cases of periprosthetic infection in both groups

Discussion

According to several authors, modular metal augments can provide a more durable revision for bone defects up to 20 mm or type 2 and 3 according to AORI [9,11,21,22]. Huten et al. concluded that the use of metal augments is advisable for elderly patients and those with low motor activity [9,23].

Patel et al. also described the use of metal augments for AORI type 2 defects in their study. The study analyzed 102 patients who underwent revision knee replacement over an 11-year follow-up period. According to researchers, endoprostheses had a 92% survival rate without significant complications [11].

However, some studies have shown the drawbacks of using metal augments. Similarly, Innocenti et al. noted that the use of solid metal augments can increase the load on the adjacent bone, potentially decreasing the endoprosthesis characteristics [15]. In a study by Lee et al. on 37 patients (39 knee joints), it was concluded that the use of metal augments can lead to instability of the endoprosthesis components, which may result in the need for revision [14]. Panegrossi and co-authors conducted a study which found that the use of metal fragments can cause corrosion and abrasion of metal [16].

Studies by Cnudde et al. and Kumar et al. describe the application of a new layer to an old layer of bone cement using the 'cement-in-cement' technique. This involves removing the femoral component of the hip arthroplasty from a well-fixed femoral cement mantle. Afterwards, a new cement foot is installed in the original mantle. According to the assessment conducted by the authors, there was no significant difference in the survival of the leg and the risk of repeated revision for all reasons. The authors also noted that this technique shows promising results and has several advantages: reduced surgical intervention time, less blood loss during surgery, less bone loss, and reduced financial costs for the treated case [24, 25].

The fixation of factory cement augments of the tibia, in conjunction with factory cement spacers of the knee joint, implies the fixation of these components on bone cement. This procedure is prescribed in the operating instructions. The presented type of augment is approved for use by the Food and Drug Administration (FDA) [13].

The described report of the case of Balgazarov and co-authors showed successful long-term results (4.5 years) the use of the double cementation method to replace femoral and tibial defects against the background of recurrent periprosthetic knee infection shows the possibility of using this method during the treatment of periprosthetic infection and at high risk of postoperative infection [26].

A similar method was also described in the report of the Gililland and co-authors' case. The authors used an additional layer of bone cement in order to adjust the rotation of the femoral component of the endoprosthesis and achieve the correct positioning of both components of the endoprosthesis [27].

The main advantage of bone cement is that it can act as a means of delivering an antibacterial drug for local antibiotic

therapy. In their study, Lawrie and co-authors showed that the optimal method of local exposure to microorganisms is the addition of tobramycin and vancomycin to bone cement containing gentamicin [28].

A separate point in revision arthroplasty is to highlight the economic burden of these surgical interventions. According to a study by Fang and co-authors, revision knee arthroplasty requires more hospital resources and costs compared to primary arthroplasty [29]. Steele and co-authors described in their study that the cost of revision arthroplasty depends on the number and type of replacement components of the endoprosthesis [30]. Reducing the use of modular metal augments and the use of bone cement augments can significantly reduce the cost of revision knee replacement.

The negative aspects of our study are an insufficiently long follow-up period to confirm positive long-term treatment results and assess the risk of periprosthetic knee infection, the need to expand the sample of patients and the need to evaluate the use of the method for type 3 AORI defects.

Conclusion

A comparative assessment of the use of the double cementation method and modular metal augments during revision knee replacement revealed no significant differences in the number of hospital beds spent, bed days spent in the intensive care unit after surgery, in the duration of surgery, in the amount of intraoperative blood loss, in knee joint function according to the Knee Society Score and Oxford Knee Score. The developed method of double cementation can be used for revision knee replacement along with modular metal augments. The use of the double cementation method may be recommended at a high risk of postoperative infection, since bone cement can act as a method of delivering an antibacterial drug for local infection prevention. Also, the use of bone cement may be more economically advantageous than the use of modular metal augments.

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References

1. Cram P, Lu X, Kates SL, Singh JA, Li Y, Wolf BR. Total knee arthroplasty volume, utilization, and outcomes among Medicare beneficiaries, 1991-2010. *JAMA*. 2012 Sep 26;308(12):1227-36. https://doi.org/10.1001/2012.jama.11153
2. Nham FH, Patel I, Zalikha AK, El-Othmani MM. Epidemiology of primary and revision total knee arthroplasty: analysis of demographics, comorbidities and outcomes from the national inpatient sample. *Arthroplasty*. 2023 Apr 2;5(1):18. https://doi.org/10.1186/s42836-023-00175-6
3. Bermagambetova G.N., Dzhaksybekova G.K. Osnovnye pokazateli travmatologo-ortopedicheskoj pomoshhi naseleniju Respubliki Kazahstan v 2020 godu. 20 let Nacional'nomu nauchnomu centru travmatologii i ortopedii imeni akademika Batpenova N.D. [in Russian]. Nur -Sultan: tipografija «Print Centr Julija», Kostanaj, 2021 – 85 p.
4. Grimberg A, Lützner J, Melsheimer O, Morlock M, Steinbrück A. German Arthroplasty Registry (Endoprothesenregister Deutschland - EPRD) - Annual Report 2022. Berlin: EPRD Deutsche Endoprothesenregister gGmbH; 2023. 172 p.
5. Grimberg A, Jansson V., Lützner J, Melsheimer O, Morlock M, Steinbrück A. German Arthroplasty Registry (Endoprothesenregister Deutschland - EPRD) - Annual Report 2021. Berlin: EPRD Deutsche Endoprothesenregister gGmbH; 2022. 189 p.
6. Grimberg A, Jansson V., Lützner J, Melsheimer O, Morlock M, Steinbrück A. German Arthroplasty Registry (Endoprothesenregister Deutschland - EPRD) - Annual Report 2020. Berlin: EPRD Deutsche Endoprothesenregister gGmbH; 2021. 127 p.
7. Grimberg A, Jansson V., Lützner J, Melsheimer O, Steinbrück A. German Arthroplasty Registry (Endoprothesenregister Deutschland - EPRD) - Annual Report 2019. Berlin: EPRD Deutsche Endoprothesenregister gGmbH; 2020. 118 p.
8. Ackerman IN, Busija L, Lorimer M, de Steiger R, Graves SE. Monitoring the lifetime risk of revision knee arthroplasty over a decade : a population-level analysis of Australian national registry data. *Bone Joint J*. 2022 May;104-B(5):613-619. https://doi.org/10.1302/0301-620X.104B5.BJJ-2021-1219.R1
9. Qiu YY, Yan CH, Chiu KY, Ng FY. Review article: Treatments for bone loss in revision total knee arthroplasty. *J Orthop Surg (Hong Kong)*. 2012 Apr;20(1):78-86. https://doi.org/10.1177/230949901202000116
10. Mozella AP, Cobra HAAB. Bone Defects in Revision Total Knee Arthroplasty. *Rev Bras Ortop (Sao Paulo)*. 2021 Apr;56(2):138-146. https://doi.org/10.1055/s-0040-1713392
11. Lei PF, Hu RY, Hu YH. Bone Defects in Revision Total Knee Arthroplasty and Management. *Orthop Surg*. 2019 Feb;11(1):15-24. https://doi.org/10.1111/os.12425
12. Ponzio DY, Austin MS. Metaphyseal bone loss in revision knee arthroplasty. *Curr Rev Musculoskelet Med*. 2015 Dec;8(4):361-7. https://doi.org/10.1007/s12178-015-9291-x
13. Lachiewicz PF, Wellman SS, Peterson JR. Antibiotic Cement Spacers for Infected Total Knee Arthroplasties. *J Am Acad Orthop Surg*. 2020 Mar 1;28(5):180-188. https://doi.org/10.5435/JAAOS-D-19-00332
14. Lee KJ, Bae KC, Cho CH, Son ES, Jung JW. Radiological Stability after Revision of Infected Total Knee Arthroplasty Using Modular Metal Augments. *Knee Surg Relat Res*. 2016 Mar;28(1):55-61. https://doi.org/10.5792/ksrr.2016.28.1.55
15. Innocenti B, Fekete G, Pianigiani S. Biomechanical Analysis of Augments in Revision Total Knee Arthroplasty. *J Biomech Eng*. 2018 Nov 1;140(11):111006. https://doi.org/10.1115/1.4040966
16. Panegrossi G, Ceretti M, Papalia M, Casella F, Favetti F, Falez F. Bone loss management in total knee revision surgery. *Int Orthop*. 2014 Feb;38(2):419-27. https://doi.org/10.1007/s00264-013-2262-1

17. Berger C, Parai C, Tillander J, Bergh P, Wennergren D, Brisby H. High Risk for Persistent Peri-Prosthetic Infection and Amputation in Mega-Prosthesis Reconstruction. *J Clin Med.* 2023 May 20;12(10):3575. https://doi.org/10.3390/jcm12103575
18. Batpen A.N., Balgazarov S.S., Belokobylov A.A., Ramazanov Zh.K., Rimashevsky D.V., Serikbayev V.D., Abilov R.S., Dolgov A.A., Moroshan A.V., Balgazarov A.S., Kriklivyi A.A., Kurmangaliyev Ye.-D. T., Aubakirov M.G., Ali A.E., Ustazov K.A. Patent for invention "Method of revision endoprosthetics of the knee joint by double cementation". «Academician N.J. Batpenov National Scientific Center of Traumatology and Orthopedics» Republican state enterprise on the right of economic management of the Ministry of Health of the Republic of Kazakhstan. 2023.
19. Koychubekov B.K. Biostatistika: ucheb. posobiye. – Almaty: Izdatel'stvo «Evero», 2015. –152 p.
20. Engh GA, Parks NL. The management of bone defects in revision total knee arthroplasty. *Instr Course Lect*. 1997;46:227-36.
21. Vasso M, Beaufils P, Cerciello S, Schiavone Panni A. Bone loss following knee arthroplasty: potential treatment options. *Arch Orthop Trauma Surg.* 2014 Apr;134(4):543-53. https://doi.org/10.1007/s00402-014-1941-8
22. Kang KS, Tien TN, Lee MC, Lee KY, Kim B, Lim D. Suitability of Metal Block Augmentation for Large Uncontained Bone Defect in Revision Total Knee Arthroplasty (TKA). *J Clin Med*. 2019 Mar 19;8(3):384. https://doi.org/10.3390/jcm8030384
23. Huten D. Femorotibial bone loss during revision total knee arthroplasty. *Orthop Traumatol Surg Res*. 2013 Feb;99(1 Suppl):S22-33. https://doi.org/10.1016/j.otsr.2012.11.009.
24. Cnudde PH, Kärrholm J, Rolfson O, Timperley AJ, Mohaddes M. Cement-in-cement revision of the femoral stem: analysis of 1179 first-time revisions in the Swedish Hip Arthroplasty Register. *Bone Joint J.* 2017 Apr;99-B(4 Supple B):27-32. https://doi.org/10.1302/0301-620X.99B4.BJJ-2016-1222.R1
25. Kumar A, Porter M, Shah N, Gaba C, Siney P. Outcomes of Cement in Cement Revision, in Revision Total Hip Arthroplasty. *Open Access Maced J Med Sci.* 2019 Dec 12;7(23):4059-4065. https://doi.org/10.3889/oamjms.2019.710
26. Balgazarov S, Belokobylov A, Batpen A, Ramazanov Z, Dolgov A, Rimashevskiy D, et al. The First Stage of Knee Revision Arthroplasty in Periprosthetic Infection with Replacement of a Large Defect Double Cementing Method: A Case Report. *Int Med Case Rep J*. 2023 Sep 6;16:513-520. https://doi.org/10.2147/IMCRJ.S420109
27. Gililland JM, Carlson VR, Fehring K, Springer BD, Griffin WL, Anderson LA. Balanced, Stemmed, and Augmented Articulating Total Knee Spacer Technique. *Arthroplast Today.* 2020 Oct 1;6(4):981-986. https://doi.org/10.1016/j.artd.2020.08.006
28. Lawrie CM, Jo S, Barrack T, Roper S, Wright RW, Nunley RM, et al. Local delivery of tobramycin and vancomycin in primary total knee arthroplasty achieves minimum inhibitory concentrations for common bacteria causing acute prosthetic joint infection. *Bone Joint J.* 2020 Jun;102-B(6_Supple_A):163-169. https://doi.org/10.1302/0301-620X.102B6.BJJ-2019-1639.R1
29. Fang CJ, Shaker JM, Ward DM, Jawa A, Mattingly DA, Smith EL. Financial Burden of Revision Hip and Knee Arthroplasty at an Orthopedic Specialty Hospital: Higher Costs and Unequal Reimbursements. *J Arthroplasty*. 2021 Aug;36(8):2680-2684. https://doi.org/10.1016/j.arth.2021.03.044
30. Steele JR, Ryan SP, Jiranek WA, Wellman SS, Bolognesi MP, Seyler TM. Cost of Aseptic Revision Total Knee Arthroplasty at a Tertiary Medical Center. *J Arthroplasty.* 2021 May;36(5):1729-1733. https://doi.org/10.1016/j.arth.2020.11.028

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Epidemiology of Congenital Heart Disease in Kazakhstan: Data from the Unified National Electronic Healthcare System 2014-2021

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Abstract

The aim of this study was to investigate the epidemiology of congenital heart disease (CHD) in Kazakhstan, using the data from the Unified National Electronic Healthcare System (UNEHS) for the period of 2014-2021. This retrospective cohort study included all patients diagnosed with CHD in Kazakhstan and registered in the UNEHS between January 2014 and December 2021. CHDs were defined based on ICD-10 codes Q20-Q26. Incidence, prevalence, and all-cause mortality rates were calculated per 100,000 population. Survival analysis was performed using Cox proportional hazards regression modeling and Kaplan-Meier method. The cohort consisted of 68,371 CHD patients, of whom 61,285 (89.6%) had a single CHD type, 40,767 (59.6%) were diagnosed before the age of 1 year, and 5,225 (7.6%) died over the study period. Incidence of CHD decreased from 64.6 to 47.3 cases per 100,000 population in males, and from 68.7 to 42.5 cases in females between 2014 and 2020. All-cause mortality rates per 100,000 population increased from 3.3 to 4.7 cases among males, and from 2.7 to 3.7 among females between 2014 and 2020. Survival analysis showed that in patients diagnosed with CHD before 1 year of age, risk of death was significantly associated with male sex (hazard ratio [HR] 1.17), multiple CHD types (HR 1.70), and no performed surgery (HR 0.57). In patients diagnosed with CHD after 1 year of age, risk factors were male sex (HR 1.65), multiple CHD types (HR 1.55), and no performed surgery (HR 1.82).

Keywords: Big Data, Epidemiology, Heart Defects, Congenital, Kazakhstan.

Introduction

Congenital heart disease (CHD) is a condition present at birth, which can be defined as a structural malformation of the heart or great vessels [1]. CHD is the most common congenital condition reaching an estimated incidence of 17.9 cases per 1,000 live births [2]. In 2017, a global estimate of CHD prevalence reached 11,998,283 people, which corresponds to an age-standardized prevalence rate of 170.6 cases per 100,000 population [3]. Over 97% of children diagnosed with CHD survive beyond the age of 18 years, but the risk of death before the age of 68 years for this population is around 3.2 times higher, compared to people without CHD [4]. Across geographical regions, the highest incidence rates of CHD were observed in lower-income countries in Africa and Asia, reaching over 30 cases per 1,000 population in Central African Republic (33.8), Burundi (30.6), and Somalia (31.9). Conversely, the lowest incidence rates were under 10 cases per 1,000 population and were found in the higher-income countries like France (8.6), Portugal (6.7), and Qatar (6.2) [2]. Approximately 35% of CHD cases are diagnosed after infancy, up until late adulthood [5].

According to the systematic analysis for the Global Burden of Disease Study 2017 by Zimmerman et al. [3], the estimated number of deaths attributable to CHD in 2017 was 261,247, a 34.5% decrease from the estimated 398,580 deaths in 1990. Out of those, 180,624 (69%) deaths occurred in infants under the age of 1 year, which corresponds to 131.0 deaths per 100,000 infants in 2017. Infant mortality rates were the lowest in high income regions, such as Western Europe with 29.2 deaths per 100,000 infants, and Australasia with 26.3 deaths per 100,000 infants. The highest mortality rates were documented in North Africa and Middle east at 211.7 deaths per 100,000 infants, and in Oceania at 226.4 deaths per 100,000 infants. Age-standardized mortality in individuals of all ages decreased from 6.3 to 3.9 deaths per 100,000 population between 1990 and 2017, which constitutes a 39.0% decrease.

Although epidemiological data on the hospitalized CHD patients, especially in pediatrics cohorts, are now widely reported in both high-income and developing countries, there is little research done in Kazakhstan using such data. One example is the study by Sermanizova et al. [6]. There, it was found that the incidence of CHD in newborns under 1 year had been steadily increasing across the country from 4.4 cases per 1,000 population in 2003 to 8.9 in 2012. However, this study dealt only with the incidence of CHD in Kazakhstan in children under 5 years of age, regional variation of incidence, and categorization by CHD types. To better understand the epidemiology of CHD in Kazakhstan, further research is required.

In 2014, the unified national electronic health system (UNEHS) was established in Kazakhstan, which created a unique opportunity to study various health conditions on a national scale, including CHD. UNEHS aggregates patient data from various electronic sources, such as inpatient electronic registries of hospitalized patients, outpatient electronic registries of dispensary patients, and others, used by medical facilities in the country. Hence, the aim of this study is to explore the incidence, prevalence, all-cause mortality, and survival patterns of patients with CHD in Kazakhstan from 2014 to 2021 using patient data from the UNEHS.

Materials and Methods

Study population

This is a retrospective cohort study, which included patients diagnosed with CHD and registered in the inpatient registry of the Unified National Electronic Health System (UNEHS) between January 1st 2014 and December 31st 2021. Individual patient records containing socio-demographic and clinical data are aggregated in the UNEHS database using International Classification of Diseases 10 (ICD-10) coding. CHD were defined as ICD-10 codes Q20-26.

The patients were divided into groups of those diagnosed with a single type of CHD, and those who were diagnosed with 2 or more types, labeled ‘single and ‘multiple’, respectively.

Exposures and covariates

Patients’ records extracted from the UNEHS database contained the following information: date of birth, sex, date of diagnosis, ICD-10 codes for the main diagnosis, date of death, dates of admission and discharge, date of surgery, type of surgery, and an anonymized population registry number (RPN). Where appropriate, the date of death was retrieved using RPN's linkage with the Population Registry. Age was divided into 2 categories: under 1 year, and more than 1 year old at the time of earliest CHD diagnosis.

Outcome assessment

For each year of follow-up between 2014 and 2021, incidence, period prevalence, and all-cause mortality were analyzed for CHD patients. The incidence rate per 100,000 population was derived by dividing the number of incident cases in a year by Kazakhstan's total population of all ages in that year. Similar to this, the number of patients surviving at the end of a year and the number of fatalities in that year were divided by the total population at risk, respectively, to determine period prevalence and mortality rates per 100,000 population. Population statistics were procured from Taldau Statistics [7]. The follow-up period was defined as the period from the date of CHD diagnosis to December 31st, 2021, or until the date of death.

Statistical methods

For categorical variables, data are summarized as patient numbers and percentages. The median and interquartile range (IQR) are used to summarize continuous variables. Chi square and Mann–Whitney U tests were used for bivariate analysis. Cox proportional hazards regression modeling and the Kaplan-Meier method were used for survival analysis. Cox modeling was used to produce crude and adjusted hazard ratios (HR) with 95% confidence intervals (CI). Separate Cox regression models were built for patients of age below and above 1 year. This was done due to a significant interaction between age at diagnosis and most other predictors. The Kaplan-Meier method was used to calculate survivor functions for CHD patients based on age at diagnosis, sex, number of malformations, surgery, and residence. The log-rank test was used to determine the significance of the difference between the survival curves.

All statistical analyses were performed using STATA 15 MP2 Version (STATA Corporation, College Station, TX). P values are two-sided and reported as statistically significant at <=0.05 for all analyses. Nazarbayev University Institutional Research Ethics Committee (NU-IREC) approved this project to be exempt from further NU IREC oversight (NU-IREC 505/06122021). The study was performed according to both international and local ethics guidelines and regulations as well as declaration of Helsinki.

Results

General characteristics of the cohort

The final cohort consisted of 68,371 patients diagnosed with CHD. Among them, 61,285 were diagnosed with a single CHD type, while 7,086 were diagnosed with multiple CHD types (ranging from 2 to 7). In Table 1, the cohort's demographic details are shown. The median age at diagnosis of the cohort was 0.3 (0.0 - 7.5) years, with a higher median age among patients with a single type of CHD, compared to the patients with multiple CHD types. Among the patients, 59.6% were diagnosed with CHD within the first year of life. Moreover, almost 80% of cases with multiple CHDs were diagnosed in children under 1 year of age. Regarding surgical interventions, a statistically significant difference was also revealed. In cases with multiple CHDs, the presence of surgical interventions was 40%, compared to the 34.7% in cases with a single CHD. The percentage of deaths among multiple CHDs reached 11.5%, compared to 7.2% in the single CHD group.

The most common cardiac defects among single CHD were atrial septal defect (ASD) (25.9%), ventricular septal defect (VSD) (21.3%), and patent ductus arteriosus (PDA) (12.0%) (Table 2).

Table 1 Baseline characteristics of patients with CHD registered in UNEHS in 2014-2021

	Total	Single CHD	Multiple CHDs	p-value
Total	68,371 (100.0)	61,285 (89.6)	7,086 (10.4)	
Sex, N (column %)				
Female	34,809 (50.9)	31,240 (51.0)	3,569 (50.4)	0.332
Male	33,562 (49.1)	30,045 (49.0)	3,517 (49.6)	
Age at diagnosis, years median (IQR)	0.3 (0.0 – 7.5)	0.4 (0.0 – 8.9)	0.1 (0.0 – 0.6)	<0.001
Age at death, years median (IQR)	0.7 (0.2 – 19.8)	0.8 (0.2 – 30.9)	0.3 (0.1 – 1.2)	<0.001
Age at diagnosis, N (column %)				
<=1 year	40,767 (59.6)	35,230 (57.5)	5,537 (78.1)	<0.001
>1 year	27,604 (40.4)	26,055 (42.5)	1,549 (21.9)	
Residence, N (column %)				
Urban	44,196 (64.6)	39,612 (64.6)	4,584 (64.7)	0.927
Rural	24,175 (35.4)	21,673 (35.4)	2,502 (35.3)	
Surgery, N (column %)				
No	44,261 (64.7)	40,009 (65.3)	4,252 (60.0)	<0.001
Yes	24,110 (35.3)	21,276 (34.7)	2,834 (40.0)	
Number of surgeries, median (IQR)	1 (1 – 2)	1 (1 – 2)	2 (1 – 3)	<0.001
Death				
No	63,146 (92.4)	56,874 (92.8)	6,272 (88.5)	<0.001
Yes	5,225 (7.6)	4,411 (7.2)	814 (11.5)	

Abbreviations: CHD - congenital heart disease, IQR - interquartile range.

Table 2 The most common congenital heart defects among patients registered in UNEHS in 2014-2021

Diagnoses with the corresponding ICD-10 codes	Number of patients with the given diagnosis as a single malformation, N (%)	Number of patients who received surgical interventions with the given diagnosis, N (%)
Q21.1 Atrial septal defect	15,849 (25.9)	7,712 (32.0)
Q21.0 Ventricular septal defect	13,025 (21.3)	6,066 (25.2)
Q25.0 Patent ductus arteriosus	7,372 (12.0)	3,842 (15.9)
Q24.8 Other specified congenital malformations of heart	4,846 (7.9)	751 (3.1)
Q21.8 Other congenital malformations of cardiac septa	2,956 (4.8)	268 (1.1)
Q20.8 Other congenital malformations of cardiac chambers and connections	2,559 (4.2)	234 (1.0)
Q24.9 Congenital malformation of heart, unspecified	2,383 (3.9)	399 (1.7)
Q21.3 Tetralogy of Fallot	1,692 (2.8)	1,095 (4.5)
Q22.1 Congenital pulmonary valve stenosis	1,080 (1.8)	953 (4.0)
Q21.2 Atrioventricular septal defect	913 (1.5)	530 (2.2)

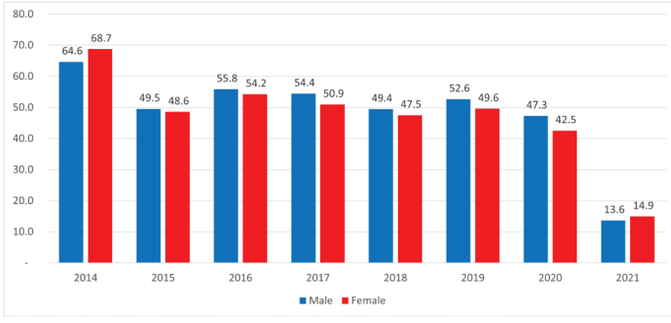

Figure 1 - Incidence rate per 100,000 population stratified by sex & year of diagnosis for CHD cohort registered in UNEHS in 2014-2021

Among them, surgery was performed on 32% of the ASD patients, 25.2% of the VSD patients, and 15.9% of the PDA patients.

Incidence, prevalence, and mortality

The incidence of CHD has decreased between 2014 and 2020 from 64.6 to 47.3 cases per 100,000 population in males, and from 68.7 to 42.5 cases in females. The estimates for 2021 are significantly lower, reaching 13.6 and 14.9 cases per 100,000 for male and female populations, respectively (Figure 1).

The period prevalence increased from 66.3 male and 71.0 female prevalent cases per 100,000 population in 2014 to 342.5 male and 339.2 female cases per 100,000 population in 2021 (Figure 2).

In terms of mortality, there is a steady increase between 2014 and 2020, whereby the rates per 100,000 population rise from 3.3 to 4.7 cases among males, and from 2.7 to 3.7 among females. Similar to the incidence, the mortality for 2021 are lower than those for earlier years, dropping to 1.1 male and 0.7 female cases per 100,000 population (Figure 3).

Given in Figure 4 are the incidence and mortality rates per 100,000 median population in Kazakhstan in the period between

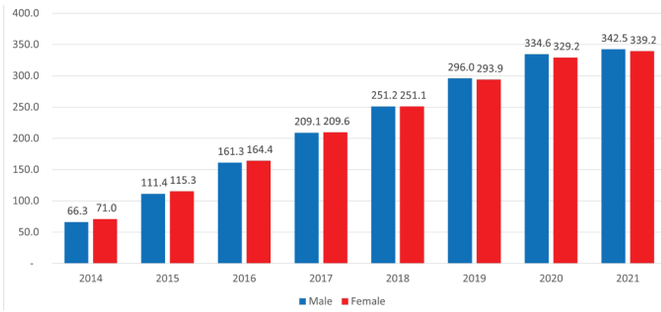

Figure 2 - Period prevalence rate per 100,000 population stratified by sex & year of diagnosis for CHD cohort registered in UNEHS in 2014-2021

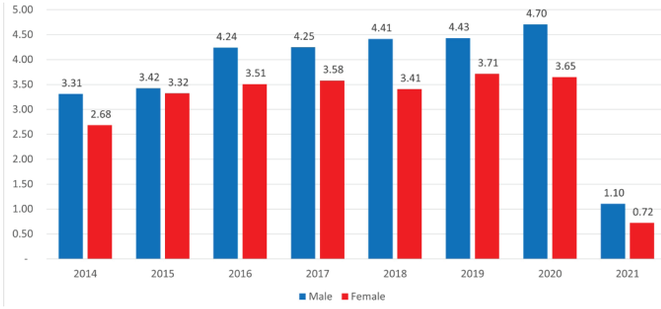

Figure 3 - Mortality rate per 100,000 population stratified by sex & year of death for CHD cohort registered in UNEHS in 2014-2021

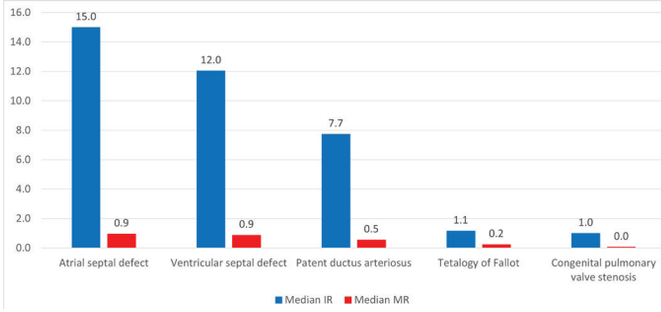

Figure 4 - Median incidence and mortality rates of most common diagnosed CHD types for CHD cohort registered in UNEHS in 2014-2021

2014 and 2021 for 5 most common specific CHD types: ASD, VSD, PDA, tetralogy of Fallot (ToF), and congenital pulmonary valve stenosis (CPVS), listed in the decreasing order of prevalence. Incidence rates are 15.0 for ASD, 12.0 for VSD, 7.7 for PDA, 1.1 for ToF, and 1.0 for CPVS per 100,000 population. Mortality rates are 0.9 for ASD, 0.9 for VSD, 0.5 for PDA, 0.2 for ToF, and <0.1 for CPVS per 100,000 total population in Kazakhstan.

Survival analysis

Significant differences in the risk of death are observed in patients diagnosed with multiple CHD compared to those with a single CHD (HR: 1.70, 95% CI: 1.51 – 1.92, p<0.001) among those diagnosed before the age of 1 years of age. A 43% lower risk of death is observed among those who did not undergo surgical intervention compared to those who did (HR: 0.57, 95% CI: 0.51 – 0.63, p<0.001). Males had a 17% higher risk of death compared to females (HR: 1.17, 95% CI: 1.04 – 1.32, p<0.001) (Table 3).

Among those diagnosed after the age of 1 year of age males had a 65% higher risk of death than females (HR: 1.65, 95% CI: 1.42 – 1.95, p<0.001). Those with multiple CHDs had a 55% higher risk compared to those with a single CHD (HR: 1.55, 95% CI: 1.20 – 1.99, p=0.001). No performed surgery was associated with an 82% higher risk of mortality compared to those who underwent surgery (HR: 1.82, 95% CI: 1.53 – 2.18, p<0.001) (Table 4).

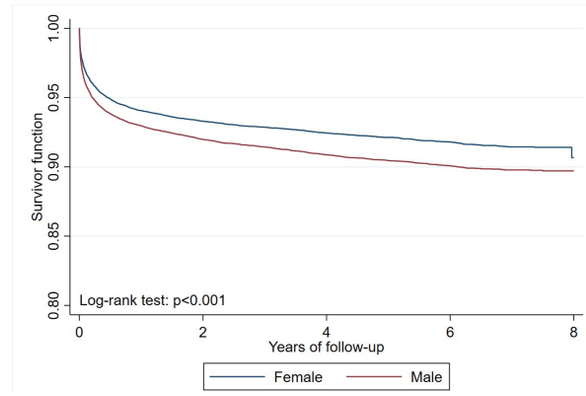

Figure 5- Kaplan-Meier plot of survivor function stratified by sex for the CHD cohort registered in UNEHS in 2014-2021.

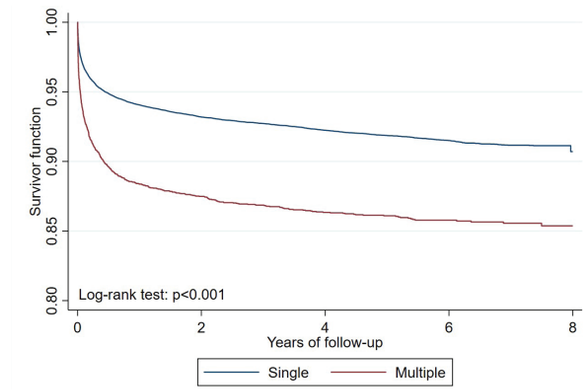

Figure 6 - Kaplan-Meier plot of survivor function stratified by the number of malformations for the CHD cohort registered in UNEHS in 2014-2021.

Table 3 Cox proportional hazards regression models of associations between risk factors & risk of all-cause death for CHD cohort registered in UNEHS in 2014-2021 diagnosed before 1 year of age

	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Sex				
Female	Ref.		Ref.	
Male	1.02 (0.94 – 1.09)	0.679	1.17 (1.04 – 1.32)	0.007
Malformation type				
Single	Ref.		Ref.	
Multiple	1.69 (1.54 – 1.85)	<0.001	1.70 (1.51 – 1.92)	<0.001
Surgery				
Yes	Ref.		Ref.	
No	0.47 (0.44 – 0.51)	<0.001	0.57 (0.51 – 0.63)	<0.001
Residence				
Urban	Ref.		Ref.	
Rural	1.69 (1.57 – 1.81)	<0.001	1.22 (0.90 – 1.67)	0.205

Abbreviations: CI - confidence interval, HR - hazard ratio.

Table 4 Cox proportional hazards regression models of associations between risk factors & risk of all-cause death for CHD cohort registered in UNEHS in 2014-2021 diagnosed after 1 year of age

	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Sex				
Female	Ref.		Ref.	
Male	1.40 (1.27 – 1.56)	<0.001	1.65 (1.42 – 1.95)	<0.001
Malformation type				
Single	Ref.		Ref.	
Multiple	1.19 (0.97 – 1.45)	0.099	1.55 (1.20 – 1.99)	0.001
Surgery				
Yes	Ref.		Ref.	
No	1.65 (1.48 – 1.83)	<0.001	1.82 (1.53 – 2.18)	<0.001
Residence				
Urban	Ref.		Ref.	
Rural	1.17 (1.05 – 1.29)	0.004	0.89 (0.67 – 1.18)	0.426

Abbreviations: CI - confidence interval, HR - hazard ratio.

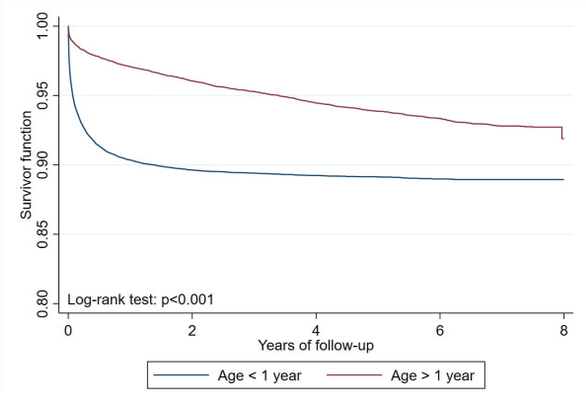

Figure 7 - Kaplan-Meier plot of survivor function stratified by age at diagnosis for the CHD cohort registered in UNEHS in 2014-2021.

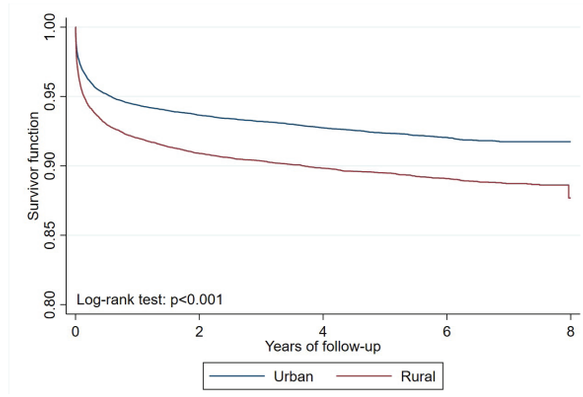

Figure 9 - Kaplan-Meier plot of survivor function stratified by residence for the CHD cohort registered in UNEHS in 2014-2021.

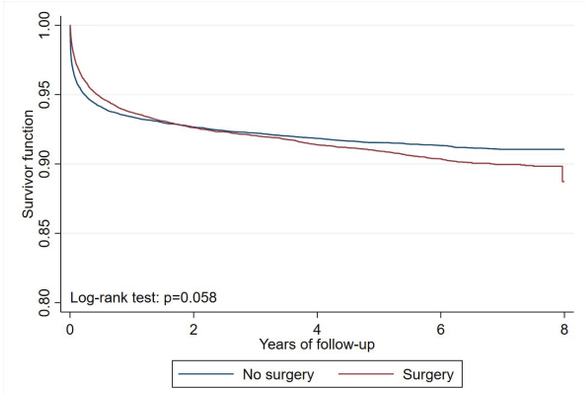

Figure 8 - Kaplan-Meier plot of survivor function stratified by surgery for the CHD cohort registered in UNEHS in 2014-2021.

Figures 5-9 depict survivor function plots constructed using the Kaplan-Meier method with the p-values from the log-rank test, indicating the significance of the difference between the survivor curves. Males exhibit generally poorer survival compared to females (Figure 5). Figure 6 shows that those diagnosed with multiple CHDs exhibit a significantly lower survival rate than those with a single CHD type. Survival in patients diagnosed before 1 year of age is significantly lower than that of patients diagnosed after 1 year of age (Figure 7). Finally, Figure 9 demonstrates that survival of rural residents is significantly lower than that of the urban residents.

Discussion

This study investigated the epidemiology of CHD in Kazakhstan. It is the first study in the Central Asian region performed on a national scale utilizing extensive administrative health data. In this study, we investigated demographic characteristics, incidence, period prevalence, all-cause mortality, and survival in CHD patients whose electronic health records were documented in the UNEHS in Kazakhstan from 2014 to 2021.

Compared to Saad et al. [8], who reported in a cross-sectional population-based study in Northern Ireland that 68% of patients were diagnosed with a single CHD type, and 32% had multiple CHD types, we found that in out cohort, 90% of the patients had single CHD types at the earliest hospitalization.

In Kazakhstan, the incidence of CHD over the study period varied between 0.65 and 0.47 for males, and between 0.69 and 0.42 for females per 1,000 live births, according to our research. This rate is considerably lower than the 4-14 per 1,000 live births CHD incidence commonly reported in large epidemiologic studies [9-11]. Since 40.4% of CHD diagnoses are made after the first year of life, the lower incidence may be explained by a potentially high prevalence of undiagnosed cases. Furthermore, the observed abrupt decrease in the number of documented cases of CHD in 2020-2021 might be explained by the burden on the healthcare system imposed by the COVID-19 pandemic and a subsequent diminished detection or capture of cases.

The challenges in comparing CHD prevalence across study results and countries owing to methodological differences are well understood [8]. Since our study cohort included people of all ages, our prevalence findings differ from those of many other studies that focused on children under the age of one. In addition, our estimates reflect period prevalence between 2014 and 2021, and do not cover cases diagnosed in earlier years due to the absence of corresponding data. Thus, a more comprehensive measure of prevalence that would encompass a longer period, would yield higher numbers.

In our study, single CHD such as ASD, VSD, and PDA were the most common. This is consistent with the findings of Liu et al. [1], where these 3 CHD types were found to account for 61% of all CHD cases worldwide. Among the patients with a single CHD type, ASD accounted for 25.9% of cases, compared to 15% globally; VSD accounted for 21.3% of cases, compared to 36% globally; and PDA accounted for 12.0% of cases, compared to 10.2% globally. Tetralogy of Fallot was found in 2.8% of the patients, compared to 4.4% worldwide [1]. The inclusion or removal of multi-code CHD in the analysis, potential variations in the inclusion of milder forms, or self-correcting forms make it difficult to draw exact comparisons [8].

In this study, the mortality rate in 2020 was 4.74 for males and 3.65 for females per 100,000 population respectively. According to Wu et al. [2], the mortality rate among CHD patients in a middle socio-demographical index (SDI) and low-middle SDI regions in 2017 were 3.5 and 4.4 per 100,000 population, respectively. The observed higher mortality rates and risks of death among male patients compared to female patients is in line with the findings by Wu et al. [2]. A higher risk of death was also observed in patients with multiple CHD types. Among the patients diagnosed before 1 year of age, the risk of death was 70% higher in those who had multiple CHDs compared to those with a single CHD type. According to Cleves et al. [12], first-year survival calculated as the percentage of the cohort decreases from 94.3% for infants with an isolated CHD to 55.6% for infants with 3 or more additional CHDs.

In infants under the age of 1 year diagnosed with CHD, the risk of death was 43% lower if no surgery was performed. Conversely, among the patients diagnosed later in life, not receiving surgery was associated with an 82% higher risk of all-cause mortality. According to Mandalenakis et al. [13], the most recent period cohort (2010-2017) had a worse outcome among children with CHD who underwent cardiac surgery, compared to earlier birth periods. The researchers concluded that this is most likely due to an increase in the detection of mild CHD conditions, which don't require treatment and have little to no effect on a child's health.

Finally, rural residence was associated with a 32% higher risk of death in the cohort, compared to urban residence. This may be attributable to higher access to healthcare facilities in urban settings, specifically because all large cardiological and cardiac-surgical hospitals dealing with CHD are located in the cities.

This study has several limitations. Firstly, it is the use of secondary data, which is influenced by measurement accuracy and documentation practices outside of the researchers` control. Lack of information on therapies, clinical data, and instrumental data is another major problem (echocardiography, severity of the disease, etc.). It is also worth noting that in this study, to avoid double counting in calculation of most common CHDs, we only use the cases with a 'single' CHD, which constitutes 90% of the cohort. Additionally, the cohort is highly heterogeneous in terms of age due to numerous cases of CHD diagnosis late in adult life. Finally, available data covers live births only, with no data on those who have died at birth and no preterm diagnosis data.

Conclusion

The results showed an increase in the mortality and period prevalence, but not the incidence in patients with CHD. The most common congenital heart defects were ASD, VSD, and PDA, similar to the global estimates. The risk of mortality was significantly associated with male sex, multiple CHD types, and CHD-related surgery. Among infants diagnosed before the age of 1 year, the risk of death was significantly higher in cases where surgery was performed. Among the patients diagnosed later in life, the risk of death was significantly higher if no surgery was performed. Future research should be aimed at identifying additional characteristics (social, economic, clinical) that may affect the epidemiological indicators of CHD in the country.

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References

1. Liu Y, Chen S, Zühlke L, Black GC, Choy M, Li N, et al. Global birth prevalence of congenital heart defects 1970–2017: updated systematic review and meta-analysis of 260 studies. *Int J Epidemiol.* 2019;48(2):455-463. https://doi.org/10.1093/ije/dyz009
2. Wu W, He J, Shao X. Incidence and mortality trend of congenital heart disease at the global, regional, and national level, 1990–2017. *Medicine*. 2020;99(23):e20593. https://doi.org/10.1097/MD.0000000000020593
3. Zimmerman MS, Smith AGC, Sable CA, Echko MM, Wilner LB, Olsen HE, et al. Global, regional, and national burden of congenital heart disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Child Adolesc Health*. 2020;4(3):185-200. https://doi.org/10.1016/S2352-4642(19)30402-X
4. Dellborg M, Giang KW, Eriksson P, Liden H, Fedchenko M, Ahnfelt A, et al. Adults with congenital heart disease: trends in event-free survival past middle age. *Circulation*. 2023;147(12):930-938. https://doi.org/10.1161/CIRCULATIONAHA.122.060834

5. Bouma BJ, Mulder BJM. Changing landscape of congenital heart disease. *Circ Res.* 2017;120(6):908-922. https://doi.org/10.1161/CIRCRESAHA.116.309302
6. Sermanizova G, Seisembekov T, Nakipov Z. Epidemiological characteristics of congenital heart diseases in Kazakhstan. *Sci World*. 2014;6(10):31-34. Available from: http://scienceph.ru/f/science-and-world--6-%2810%29-june-vol.-i.pdf
7. National Statistical Bureau. Taldau statistics. Available from: https://www.taldau.stat.gov.kz [Accessed 27 August 2023].
8. Saad H, Casey F, Dolk H, Loane M. Prevalence and trends of congenital heart defects among live births from 2005 to 2014 in Northern Ireland. *Cardiol Young*. 2022:1-7. https://doi.org/10.1017/S1047951122001937
9. Bernier PL, Stefanescu A, Samoukovic G, Tchervenkov CI. The challenge of congenital heart disease worldwide: epidemiologic and demographic facts. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu.* 2010;13(1):26-34. https://doi.org/10.1053/j.pcsu.2010.02.005
10. Hoffman JI, Kaplan S. The incidence of congenital heart disease. *J Am Coll Cardiol.* 2002;39(12):1890-1900. https://doi.org/10.1016/s0735-1097(02)01886-7
11. Egbe A, Uppu S, Stroustrup A, Lee S, Ho D, Srivastava S. Incidences and sociodemographics of specific congenital heart diseases in the United States of America: An evaluation of hospital discharge diagnoses. *Pediatr Cardiol.* 2014;35(6):975-982. https://doi.org/10.1007/s00246-014-0884-8
12. Cleves MA, Ghaffar S, Zhao W, Mosley BS, Hobbs CA. First-year survival of infants born with congenital heart defects in Arkansas (1993-1998): A survival analysis using registry data. *Birth Defects Res Part A Clin Mol Teratol.* 2003;67(9):662-668. https://doi.org/10.1002/bdra.10119
13. Mandalenakis Z, Giang KW, Eriksson P, Liden H, Synnergren M, Wåhlander H, et al. Survival in children with congenital heart disease: have we reached a peak at 97%? *J Am Heart Assoc.* 2020;9(22):e017704. https://doi.org/10.1161/JAHA.120.017704

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