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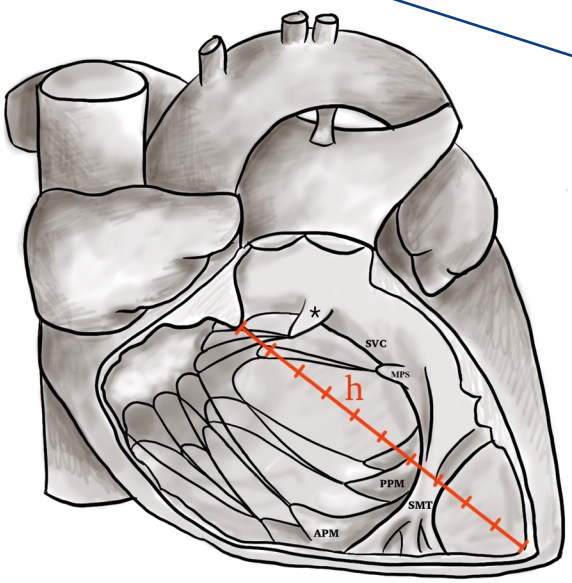

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Photo on the cover of the issue
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Editorial Office

Medical University of Gdańsk
European Journal of Translational Medicine
Dębinki 1 Street,
80-210 Gdańsk, Poland

Phone: +48 58 349 15 37

E-mail: ejtcm@gumed.edu.pl
ejtcm.gumed.edu.pl

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INVITED EDITORIAL

Cardiovascular risk in women: here, there and everywhere

Biljana Parapid

University Clinical Centre of Serbia, Division of Cardiology, Faculty of Medicine, University of Belgrade, Serbia

Abstract

While majority of women globally live in a misperception that breast cancer is the leading killer of all women, it sadly still is heart disease irrelevant of the corner of the world.

Keywords: cardiovascular risk · women · inequality · misogyny

Citation

Parapid B. Cardiovascular risk in women: here, there and everywhere. Eur J Transl Clin Med. 2025;8(2):5-8.

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While majority of women globally live in a misperception that breast cancer is the leading killer of all women, it sadly still is heart disease, irrelevant of the corner of the world [1]. The findings from 2 major epidemiological registries helped to identify the cardiovascular disease risk factors: the Seven Countries' Study (SCS) conducted across 3 continents (the United States, Japan, Finland, the Netherlands, Italy, Greece, and former Yugoslavia) and the Framingham Heart Study (FHS). The SCS included only men because in the post-WWII era, women were thought to be protected from heart disease, whereas the FHS did include women and men, though primarily of Caucasian descent. Notably, the maternal morbidity and mortality data collected from the SCS participants, are both insightful and valuable. A 40-year follow-up revealed that sons of mothers with confirmed heart disease and / or hypertension had greater cardiovascular morbidity and mortality [2]. Still, it was not until the Nurses' Health Study II (1991-2013) that the link between night shift work and an increased risk of developing diabetes and obesity was found [3-4]. Regrettably, sex-specific risk factors appeared for the first time in the US and European guidelines as late as 2019 and 2021, respectively [5-6]. The current American Heart Association's (AHA) "Life's Essential Eight" encompass smoking status, control of glycemia, cholesterol, blood pressure and weight (including diet and physical activity) and for the first time sleep habits [7]. However, it was abridged for cardiovascular risk in women recognizing peripartum as a window of opportunity for timely screening and prevention since hypertensive disorders of pregnancy, gestational diabetes, pre-

Corresponding author:
Biljana Parapid, University Clinical Centre of Serbia, Division of Cardiology, Faculty of Medicine, University of Belgrade, Serbia
e-mail: biljana_parapid@yahoo.com
Available online: ejtcm.gumed.edu.pl

term birth, placental abruption and small for gestational age were added as an additional concentric circle [8-9].

Irrelevant of the abundance of growing evidence and a recent AHA scientific statement on cardiovascular health in the transition from adolescence to emerging adulthood, different regional efforts are needed to optimize health literacy of women and promote collaborations of all involved [10-21]. In that spirit, Serbia has formally launched its campaign in 2018, followed by continuous research efforts, advocacy and introducing new concepts to the curricula of medicine residents' and cardiology fellows' [15, 22-25]. Women are the most underserved group the COVID-19 pandemic created, therefore we are optimizing their comprehensive cardiovascular care via the "Dr. Nanette Kass Wenger" Women's Heart Center and an additional program that, besides the features of similar programs based in the United States and Canada [26-27], also include a Lifestyle Clinic aiming to serve [14, 28-29] not only women, are the pandemic frontliners, but also all post-COVID-19 and long COVID patients [15].

In conclusion, as we are ending the first quarter of the 21st century it remains of paramount importance to continue to develop regional toolkits (e.g. the recently published by the American College of Cardiology) [30] and to keep fighting against misogyny. Regardless whether its promoters are men or women (via internalized misogyny masked as "cultural habits") [23] and even when systemic racism (such as the one reported in the US) is excluded, misogyny is recognized as the basis of barriers to timely diagnosis and treatment of women. The resultant more severe CVD in previously undiagnosed women can be observed at the Women's Heart Centers around the world. Misogyny requires both additional quantification in healthcare and the academia, as well as prompt and just sanctions for perpetrators for otherwise no progress shall be achieved.

Conflict of interest

None to declare pertaining to the given keynote lecture at the opening of the 38th National Student Cardiology Conference (XXXVIII Ogólnopolska Studencka Konferencja Kardiologiczna) on March 28, 2025 in Gdańsk (Poland).

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References

1. Martin SS, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, et al. 2024 Heart Disease and Stroke Statistics: A Report of US and Global Data From the American Heart Association. Circulation [Internet]. 2024;149(8). Available from: https://www.ahajournals.org/doi/10.1161/CIR.0000000000001209
2. Parapid B. Metabolic Syndrome and Cardiovascular Morbidity and Mortality In the National Cohorts of The Seven Countries Study-The 40 Years' Follow Up Results. Belgrade, Serbia Belgrade Univ Med. 2014.
3. Shan Z, Li Y, Zong G, Guo Y, Li J, Manson JE, et al. Rotating night shift work and adherence to unhealthy lifestyle in predicting risk of type 2 diabetes: results from two large US cohorts of female nurses. BMJ [Internet]. 2018;(363):k4641. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.k4641
4. Fujishiro K, Lividoti Hibert E, Schernhammer E, Rich-Edwards JW. Shift work, job strain and changes in the body mass index among women: a prospective study. Occup Environ Med [Internet]. 2017;74(6):410–6. Available from: https://oem.bmj.com/lookup/doi/10.1136/oemed-2016-103747
5. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol [Internet]. 2019;74(10):e177–232. Available from: https://www.jacc.org/doi/full/10.1016/j.jacc.2019.03.010?_ga=2.222884050.879693528.1622220635-165095556.1618604419
6. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J [Internet]. 2021;42(34):3227–337. Available from: https://academic.oup.com/eurheartj/article/42/34/3227/6358713
7. Lloyd-Jones DM, Allen NB, Anderson CAM, Black T, Brewer LC, Foraker RE, et al. Life's Essential 8: Updating and Enhancing the American Heart Association's Construct of Cardiovascular Health: A Presidential Advisory From the American Heart Association. Circulation [Internet]. 2022;146(5). Available from: https://www.ahajournals.org/doi/10.1161/CIR.0000000000001078
8. Lewey J, Beckie TM, Brown HL, Brown SD, Garovic VD, Khan SS, et al. Opportunities in the Postpartum Period to Reduce Cardiovascular Disease Risk After Adverse Pregnancy Outcomes: A Scientific Statement From the American Heart Association. Circulation [Internet]. 2024;149(7). Available from: https://www.ahajournals.org/doi/10.1161/CIR.0000000000001212

9. Wenger NK. The Feminine Face of Heart Disease 2024. Circulation [Internet]. 2024;149(7):489–91. Available from: https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.123.064460
10. Scott J, Agarwala A, Baker-Smith CM, Feinstein MJ, Jakubowski K, Kaar J, et al. Cardiovascular Health in the Transition From Adolescence to Emerging Adulthood: A Scientific Statement From the American Heart Association. J Am Heart Assoc [Internet]. 2025;14(9). Available from: https://www.ahajournals.org/doi/10.1161/JAHA.124.039239
11. Macut D, Micic D, Parapid B, Cvijovic G, Sumarac-Dumanovic M, Kendereski A, et al. Age and body mass related changes of cardiovascular risk factors in women with polycystic ovary syndrome. Vojnosanit Pregl [Internet]. 2002;59(6):593–9. Available from: https://doiserbia.nb.rs/Article.aspx?ID=0042-84500206593M
12. Gojnić M, Branković M, Maksimović M, Parapid B, Dugalić V, Jeremić K, et al. Postmenopausal palpable ovary and ovarian cancer. Clin Exp Obstet Gynecol [Internet]. 2011;38(3):265–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21995161
13. Parapid B. Gender dispariates and coronary artery disease: Women's health. In: Expert lectures 5 BASICS+ 7th (Belgrade Summit of Interventional Cardiologists) 2011 April 8-9. Belgrade, Serbia.; 2011.
14. Parapid B, Alasnag M, Hayes S, Samargandy S, Banerjee S, Alasnag M, et al. COVID19 impact on women on both sides of the frontline: The American College of Cardiology Women in Cardiology Section's International Working Group perspective. Srp Arh Celok Lek [Internet]. 2020;148(9–10):637–43. Available from: https://doiserbia.nb.rs/Article.aspx?ID=-0370-81792000095P
15. Parapid B, Kanjuh V, Kostic V, Polovina S, Dinic M, Loncar Z, et al. Women's health in Serbia - past, present, and future. Srp Arh Celok Lek [Internet]. 2021;149(11–12):745–54. Available from: https://doiserbia.nb.rs/Article.aspx?ID=0370-81792100105P
16. Bond RM, Gaither K, Nasser SA, Albert MA, Ferdinand KC, Njoroge JN, et al. Working Agenda for Black Mothers. Circ Cardiovasc Qual Outcomes [Internet]. 2021;14(2). Available from: https://www.ahajournals.org/doi/10.1161/CIRCOUTCOMES.120.007643
17. Parapid B, editor. Women's Heart Programs: A Bare Necessity of Diversity & Inclusion, not a Fashion Accessory of Innovation NOVAK 2022. In: Symposium of the Cardiology society of Serbia -Clinical update and perspectives of cardiology in Serbia; 28-30102022. Novi Sad, Serbia; 2022.
18. Parapid B, Rakić S. Oral Contraception: Beyond What Meets the Eye. Sorry, the Ovaries! Int J Cardiovasc Sci [Internet]. 2022;35(4):511–3. Available from: https://ijcscardiol.org/article/oral-contraception-beyond-what-meets-the-eye-sorry-the--ovaries/
19. Bond RM, Phillips K, Ivy KN, Ogueri V, Parapid B, Miller SC, et al. Cardiovascular Health of Black Women Before, During, and After Pregnancy: A Call to Action and Implications for Prevention. Curr Cardiovasc Risk Rep [Internet]. 2022;16(11):171–80. Available from: https://link.springer.com/10.1007/s12170-022-00703-0
20. Parapid B, editor. ESC 2023: Innovations in the treatment of cardiovascular diseases in women or not so much? In: Serbia Prevent; 28-29092023. Belgrade, Serbia.; 2023.
21. Manzo-Silberman S, Hawranek M, Banerjee S, Kaluzna-Oleksy M, Alasnag M, Paradies V, et al. Call to action for acute myocardial infarction in women: international multi-disciplinary practical roadmap. De Rosa S, editor. Eur Hear J Open [Internet]. 2024;4(6). Available from: https://academic.oup.com/ehjopen/article/doi/10.1093/ehjopen/oeae087/7819869
22. Djapanović V, editor. Risk Factors for the Development of Early Cardiovascular Disease in Women: Results of the University Clinical Center of Serbia Pilot Study. In: 62 Kongres Studenata Biomedicinskih Nauka sa Internacionalnim Učešćem. Kopaonik, Srbija 24-28.04.2023. Belgrade, Serbia: Medicinski Fakultet Univerziteta u Beogradu; 2023.
23. Parapid B, Grujić Ž, Bisenić O, Đurović M, Simić P, Đikanović-Tetiković B, et al. Sex-specific risk factor awareness, covert misogyny and long-term cardiovascular risk management: Pilot study. Med Istraz [Internet]. 2025;58(1):25–32. Available from: https://scindeks.ceon.rs/Article.aspx?artid=0301-06192501025P
24. Parapid B, Djikanovic-Tetikovic B, Podunavac-Kuzmanovic S, Bubanja D, Djurovic M, Djukic-Dejanovic S, et al. Women in science and equity in Serbia - so near, yet so far. Srp Arh Celok Lek [Internet]. 2024;152(11–12):630–7. Available from: https://doiserbia.nb.rs/Article.aspx?ID=0370-81792400095P
25. Parapid B, Gojnić-Dugalić M, Kanjuh V., Kass Wenger N. Koje su specifičnosti KV prevencije kod žena? In: Beleslin B, editor. Cardiologia preventiva: kroz 120 pitanja i odgovora. Beograd; 2023.
26. Lundberg GP, Mehta LS, Sanghani RM, Patel HN, Aggarwal NR, Aggarwal NT, et al. Heart Centers for Women. Circulation [Internet]. 2018;138(11):1155–65. Available from: https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.118.035351
27. Gulati M, Hendry C, Parapid B, Mulvagh SL. Why We Need Specialised Centres for Women's Hearts: Changing the Face of Cardiovascular Care for Women. Eur Cardiol Rev [Internet]. 2021;16. Available from: https://www.ecrjournal.com/articleindex/ecr.2021.49

28. Öz TK, Cader FA, Dakhil ZA, Parapid B, Kadavath S, Bond R, et al. International consensus statement on challenges for women in cardiovascular practice and research in the COVID-19 era. Minerva Cardiol Angiol [Internet]. 2023;70(6). Available from: https://www.minervamedica.it/index2.php?show=R05Y2022N06A0641
29. Parapid B, Bond RM. COVID-19: An Insult to Injury on Equity. Int J Cardiovasc Sci [Internet]. 2021;34(4):342-3. Available from: https://ijcscardiol.org/article/covid-19-an-insult-to-injury-on-equity/
30. Santos Volgman A, Mehta L, Aggarwal N, Bond R, Costello B, Gianos E. How to Start a Women's Heart Program: A Toolkit Washington, DC. 2025.

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RESEARCH ARTICLE

Exploring the relationship between fetal growth restriction and heart rate variability parameters

Igor Victorovich Lakhno

Kharkiv National Medical University, Kharkiv, Ukraine

Abstract

Background: Fetal growth restriction (FGR) is a multifactorial disturbance of fetal nutrition with short- and long--term consequences (e.g. autonomic malfunction and delayed neurological maturation). Fetal heart rate variability (HRV) is critically dependent on autonomic regulation. This study focused on identifying a correlation between neonatal biometry and HRV variables. **Material and methods:** This was a descriptive cross-sectional study of 48 women at 22-36 weeks of pregnancy. The fetal cardiac signals were obtained from the maternal abdominal wall via non-invasive fetal electrocardiography (NI-FECG). The stress index (SI) was selected for evaluation among all linear HRV variables. Cardiotocographic parameters (short-term variation (STV) and long-term variation (LTV)) were determined, along with cardiographic: AC (acceleration capacity) and DC (deceleration capacity). **Results**: FGR was detected in 9 women. The fetal growth was appropriate in 31 patients. 8 patients were excluded from the study. The detected variables of HRV in FGR were different, however statistical significance was impossible to determine (small number of cases). A strong linear correlation was detected between all the HRV variables: AC, DC, SI, STV, and LTV. Whereas, AC and DC had significant correlation with the 1-minute Apgar score. Multivariate regression analysis showed a statistically significant correlation of SI with the gestational age at birth. **Conclusions:** SI could be of use in the advancement of conventional FGR management and has potential for further research.

Keywords: fetal growth restriction · fetal non-invasive electrocardiography · heart rate variability

Citation

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Corresponding author:
Igor Victorovich Lakhno, Kharkiv National Medical University, Kharkiv, Ukraine
e-mail: igorlakhno71@gmail.com
Available online: ejtcm.gumed.edu.pl

Introduction

Fetal growth restriction (FGR) has short-term and long-term consequences on fetal and neonatal health and neurodevelopment. The fluctuations of fetal hemodynamics known as heart rate variability (HRV) reflect the fetus's ability to support nutrition and well-being. The HRV level is critically dependent on autonomic regulation [1]. The autonomic malfunction was found in FGR and reflected the delayed neurological maturation in growth-restricted fetuses [2].

Ultrasonography is the gold standard for FGR diagnosis [3]. However, several studies found that HRV variables were possible predictors of FGR [4-6] Studies performed over last decade revealed the potential for ultrasonic cardiotocographic (CTG) assessment of fetal heart rate fluctuations in low-resource settings [7]. The use of fetal HRV in fetal growth assessment was demonstrated. However, ultrasound is not neutral for the fetus, therefore it could not be used for continuous Holter monitoring. CTG is a mechanical reflection of cardiac rhythm, but not the initial fluctuations in myocardium. Non-invasive fetal electrocardiography (NI-FECG) is a potential technique for obtaining cardiac signals through the maternal abdominal wall. There are some challenges associated with low signal-to-noise ratio [8]. This method was found to be an alternative for cardiotocography anterpartum and during labor [9]. Systems for remote monitoring of fetal HRV via NI-FECG could be a good option for low-resource settings. This technique does not incur any major expense and helps calculate several sensitive parameters of fetal distress. Remote monitoring is appropriate during wartime [10].

NI-FECG captures primary electrophysiological processes in the heart. The variations in the duration of cardiac cycles reflect the continual changes in sympathovagal balance. Nowadays, both linear and non-linear methods are used to proceed with HRV. Some of them refer to sympathetic or parasympathetic regulation. The growing fetus demonstrates increased HRV in the process of neurological maturation. The known marker of fetal neurological maturation is reactivity to its motile activity in a non-stress test. Autonomic regulation is disturbed in fetal deterioration. Abnormally increased sympathetic tone is a marker of fetal compromise. Stress index (SI) is an integrative variable that expresses the load on regulatory systems influenced by the sympathetic branch of the autonomic function. Reflecting the central sympathetic circuit of hemodynamic regulation, SI is one of the most sensitive variables of sympathetic activity [2, 11]. The phase rectified signal averaging – acceleration capacity and deceleration capacity (AC/DC) are useful in the assessment of fetal well-being [12]. The technique for AC/DC calculation is dependent on RR extraction in NI-FECG and is a sensitive marker for fetal deterioration detected in long- or short-term recordings. Several parameters were obtained from CTG (Daws-Redman criteria) – short-term variations (STV) and long-term variations (LTV). They are very familiar and known to be useful in diagnosing fetal distress in the event of a nonreactive non-stress test [13]. This research was motivated by the speculation of decreased autonomic regulation in FGR. Probably, the pathological fetal environment reflecting abnormal HRV variables causes insufficient fetal growth. This study focused on detecting the correlation between neonatal biometry parameters and HRV variables.

Material and methods

This descriptive cross-sectional study was performed among pregnant women who were admitted to Kharkiv Municipal Perinatal Center between 1 April 2024 and 30 June 2024. The data were obtained from the hospital records system. Ethics approval was received from the Research Council and Ethical Committee of Kharkiv National Medical University (No. 25.0224p). Informed consent was obtained from all the patients. Patients from the Department of Maternal-Fetal Medicine were selected at random, using the automated numbers technique. FGR was diagnosed in case fetal weight was lower than the 10th percentile according to ultrasound. The ultrasonic investigations were performed longitudinally following current clinical protocols [14]. Inclusion criteria were: healthy pregnancy with appropriate fetal growth, FGR. Whereas the exclusion criteria were: chromosomal abnormalities, multiple pregnancy, possible preterm birth, pre-eclampsia, gestational diabetes mellitus, pre-existing medical disorders (e.g. diabetes mellitus, metabolic syndrome, cardiac diseases, renal disease, thyrotoxicosis).

All of the fetal HRV variables were obtained from an RR-interval time series recorded from the maternal abdominal wall via the Cardiolab Baby Card NI-FECG device (XAI Medica, Kharkiv, Ukraine). The recordings lasted 30-60 minutes. The SI was selected for evaluation among all linear HRV variables and calculated according to the formula below.

$$SI = \frac{AMo\ (\%)}{(2 \times Mo \times Var)}$$

$$Var = NNmax - NNmin$$

AMo (the most frequent NN interval value or the highest column in the histogram) – the number of NN intervals included in the pocket corresponding to the mode measured in percentages (%).

The obtained fetal RR interval time series was transformed into a cardiotocographic (CTG) tracing and the following CTG parameters were determined: short-term variation (STV) and long-term variation (LTV). The AC/DC variables were also detected [15]. All recordings were performed while the patients were resting in a recumbent position after eating. The study

protocol also included recording several other parameters: gestational age at the time of investigation of HRV, gestational age at birth, neonatal biometry at birth [neonate's body weight (gram), body length (sm), head circumference (sm)] and 1-minute Apgar score.

The Statistical Package for Social Sciences (SPSS) program (version 25.0., IBM Corp., Armonk, USA) was used for statistical analysis. The results were presented as means and standard deviations for numerical variables, whereas for categorical data as frequencies and percentages. The relation of the numerical variables to normal distribution was evaluated using skewness values and histograms. An independent sample t-test was used to compare the numerical variables that matched normal distribution. Variables that did not conform to normal distribution were analysed with the Mann-Whitney U test. The Chi-Square (or Fisher's exact) test was used for comparing categorical variables. Depending on their distribution, Spearman or Pearson correlation analysis was used to assess the correlations between numerical variables. For multivariate examinations, a logistic regression analysis with the entered model was used. Sample size was calculated using confidence level 95% and margin of error 5%. A p-value of < 0.05 was considered statistically significant.

Results

A total of 48 females at 22-36 weeks of pregnancy were enrolled. FGR was detected in 9 patients. The fetal growth was appropriate in 31 patients. 8 patients were excluded from the study due to diagnosis of gestational diabetes mellitus (3 cases), severe pre-eclampsia (3 cases), and potential preterm birth (2 cases). The average age of the patients in the study cohort was 24.1 ± 6.8 years and the mean body mass index was 26.5 ± 7.2 (units). NI-FECG tracing was successfully recorded in 100.0% of the patients. The detected variables in the FGR were different (Table 1). However, the number of patients was too small to determine statistical significance. The gestational age at investigation, AC, DC, STV, LTV, gestational age at birth, body weight, body length, head circumference, and Apgar score were lower in patients with FGR. SI was higher in growth-restricted fetuses.

Table 2 shows a significant or moderate correlation between the gestational age at investigation and all other parameters: AC ($r = 0.44$; $p = 0.005$), DC ($r = 0.43$; $p = 0.006$), SI ($r = -0.44$; $p = 0.004$), STV ($r = 0.47$; $p = 0.002$), LTV ($r = 0.51$; $p = 0.001$), gestational age at birth ($r = 0.34$; $p = 0.029$), body weight ($r = 0.34$; $p = 0.033$), body length ($r = 0.4$; $p = 0.011$); head circumference ($r = 0.4$; $p = 0.011$), and the Apgar score ($r = 0.46$; $p = 0.03$). A strong or moderate correlation was detected between all the HRV variables: AC, DC, SI, STV, and LTV. AC and DC both demonstrated a significant correlation with the 1-minute Apgar score. However, no correlation was found between HRV variables and neonatal biometry at birth.

Discussion

These results showed a linear correlation between gestational age and fetal HRV, and the link between SI and gestational age at birth following logistic regression parameters. The linear correlation showed similarities and mutual origin between AC, DC, SI, STV, and LTV. Correlations were found between AC and Apgar score, as well as DC and Apgar score, demonstrating the usefulness of these variables in diagnosing fetal distress. However, the linear correlation did not provide evidence of a potential connection between fetal HRV and neonatal biometry parameters. This outcome may have been influenced by the significant time interval between fetal NI-FECG recording and the time of birth. Probably, fetal HRV reflected environmental conditions responsible for fetal growth, maturation, and well-being during and after recording. Since the non-stress test performed via CTG has no predictive value, the prognostic ability of NI-FECG is of interest [16]. The relationships between fetal growth and maturation throughout gestation were clearer. The verification of a possible link with body length and head circumference requires further study on a larger cohort. This suggests that this temporal HRV index may be associated with the anthropometric parameters of newborns.

FGR is a multifactorial disturbance of fetal nutrition causing short- and long-term consequences. Severe maternal comorbidities or gestational complications were not included in this study. Such methodology helped determine the relations between fetal HRV and growth without any repercussions from maternal autonomic dysfunction. However, the aetiological reason for FGR is still under question or undetermined in the majority of cases. Idiopathic FGR is associated with placental disorders [17]. Therefore, screening for FGR is a key point in the management of patients. The humanitarian crisis caused by armed conflict necessitates the use of low-resource techniques. During armed conflict, access to obstetric care can be limited (e.g. due to the urgent need to relocate or to stay in a bomb shelter), thus increasing the need for reliable wireless fetal monitoring technologies. Our results demonstrated a certain potential for NI-FECG in measuring fetal growth, further research involving a larger study cohort is required. This technique could promote better wireless monitoring in the event of fetal arrhythmia or any suspicion of fetal deterioration.

The generalizability of this study is limited by the small number of observed FGR cases. Lower gestational age at birth in FGR was to be expected. The long time interval between NI-FECG and delivery could be also a limitation for this research.

Table 1. Descriptive statistics of the variablesin the study cohort

Parameter (units)		n	Mean	SD	Minimum	Maximum	Mean ± SD
Gestational age at investigation (weeks)	Appropriate	31	30.97	5.47	20	36	30.97 ± 5.47
	FGR	9	28	4.44	23	36	28 ± 4.44
AC (ms)	Appropriate	31	1.93	0.51	1.28	3.71	1.93 ± 0.51
	FGR	9	1.57	0.72	0.77	3.07	1.57 ± 0.72
DC (ms)	Appropriate	31	2.3	0.65	1.24	4	2.3 ± 0.65
	FGR	9	1.81	0.93	0.78	3.61	1.81 ± 0.93
SI (conventional units)	Appropriate	31	929.48	433.83	326	2167	929.48 ± 433.83
	FGR	9	1370.11	952.09	251	3102	1370.11 ± 952.09
STV (ms)	Appropriate	31	7.12	2.44	2.5	13	7.12 ± 2.44
	FGR	9	5.74	3.31	1.2	11.6	5.74 ± 3.31
LTV (ms)	Appropriate	31	36.12	10.72	17.1	58.7	36.12 ± 10.72
	FGR	9	32.54	18.58	9.3	71	32.54 ± 18.58
Gestational age at birth (weeks)	Appropriate	31	37.61	2.12	32	41	37.61 ± 2.12
	FGR	9	32.89	4.7	26	38	32.89 ± 4.7
Body weight (grams)	Appropriate	31	3189.68	790.39	1800	5530	3189.68 ± 790.39
	FGR	9	1598.89	914.84	410	2660	1598.89 ± 914.84
Body length (cm)	Appropriate	31	51.55	5.49	39	62	51.55 ± 5.49
	FGR	9	38.44	7.5	27	48	38.44 ± 7.5
Head circumference (cm)	Appropriate	31	34.03	1.97	30	38	34.03 ± 1.97
	FGR	9	27.22	5.31	19	34	27.22 ± 5.31
Apgar score (points)	Appropriate	31	7.87	1.12	5	9	7.87 ± 1.12
	FGR	9	5.11	3.1	0	8	5.11 ± 3.1

AC – acceleration capacity; DC – deceleration capacity; LTV – long-term variations; SD – standard deviation; SI – stess index; STV – short-term variation

Table 2. The correlation and significance between the detected variables in the study cohort

Parameter		Gestational age at investigation	AC	DC	SI	STV	LTV	Gestational age at birth	Body weight	Body length	Head circumference	Apgar score
Gestational age at investigation (weeks)	Correlation	1	**0.44**	**0.43**	**-0.44**	**0.47**	**0.51**	**0.34**	**0.34**	**0.4**	**0.4**	**0.46**
	p		.005	.006	.004	.002	.001	.029	.033	.011	.011	.003
AC (ms)	Correlation	**0.44**	1	**0.87**	**-0.64**	**0.86**	**0.84**	0.21	0.05	0.16	0.23	**0.34**
	p	.005		< .001	< .001	< .001	< .001	.186	.78	.337	.162	.032
DC (ms)	Correlation	**0.43**	**0.87**	1	**-0.62**	**0.91**	**0.84**	0.19	0.04	0.14	0.2	**0.37**
	p	.006	< .001		< .001	< .001	< .001	.231	.802	.39	.226	.018
SI (conventional units)	Correlation	**-0.44**	**-0.64**	**-0.62**	1	**-0.72**	**-0.8**	-0.13	0.07	-0.1	-0.15	-0.24
	p	.004	< .001	< .001		< .001	< .001	.428	.668	.551	.369	.131
STV (ms)	Correlation	**0.47**	**0.86**	**0.91**	**-0.72**	1	**0.96**	0.1	0.01	0.13	0.19	0.27
	p	.002	< .001	< .001	< .001		< .001	.538	.975	.413	.233	.088
LTV (ms)	Correlation	**0.51**	**0.84**	**0.84**	**-0.8**	**0.96**	1	0.08	-0.04	0.09	0.16	0.2 3
	p	.001	< .001	< .001	< .001	< .001		.631	.808	.56	.334	.148
Gestational age at birth (weeks)	Correlation	**0.34**	0.21	0.19	-0.13	0.1	0.08	1	**0.83**	**0.82**	**0.67**	**0.72**
	p	.029	.186	.231	.428	.538	.631		< .001	< .001	< .001	<.001
Body weight (grams)	Correlation	**0.34**	0.05	0.04	0.07	0.01	-0.04	**0.83**	1	**0.92**	**0.84**	**0.68**
	p	.033	.78	.802	.668	.975	.808	< .001		< .001	< .001	< .001
Body length (sm)	Correlation	0.4	0.16	0.14	-0.1	0.13	0.09	**0.82**	**0.92**	1	**0.85**	**0.74**
	p	.011	.337	.39	.551	.413	.56	< .001	< .001		< .001	< .001
Head circumference (sm)	Correlation	0.4	0.23	0.2	-0.15	0.19	0.16	**0.67**	**0.84**	**0.85**	1	**0.71**
	p	.011	.162	.226	.369	.233	.334	< .001	< .001	< .001		< .001
Apgar score (points)	Correlation	**0.46**	**0.34**	**0.37**	-0.24	0.27	0.23	**0.72**	**0.68**	**0.74**	**0.71**	1
	p	.003	.032	.018	.131	.088	.148	< .001	< .001	< .001	< .001	

AC – acceleration capacity; DC – deceleration capacity; LTV – long-term variations; SI – stess index; STV – short-term variation

Table 3. Multivariate logistic regression model with SI coefficient

Variable	Unstandardized coefficients	Standardized coefficients	Standard error	T	p	95% confidence interval for B	
Model	B	Beta				Lower bound	Upper bound
(Constant)	7313.09		1549.82	4.72	< .001	4143.7	10482.47
Gestational age at investigation	-8.37	-0.07	15.68	-0.53	.598	-40.43	23.7
AC	102.67	0.1	281.71	0.36	.718	-473.44	678.77
DC	154.43	0.19	254.29	0.61	.548	-365.59	674.46
STV	-64.8	-0.29	92.03	-0.7	.487	-253	123.4
LTV	-21.3	-0.45	14.36	-1.48	.149	-50.66	8.07
Gestational age at birth	-104.64	-0.6	47.47	-2.2	.036	-201.72	-7.57
Body weight	0.4	0.69	0.22	1.83	.078	-0.05	0.84
Body length	3.33	0.04	34.94	0.1	.925	-68.13	74.78
Head circumference	-106.37	-0.73	51.97	-2.05	.05	-212.65	-0.09
Apgar score	87.68	0.3	79.13	1.11	.277	-74.14	249.49

AC – acceleration capacity; DC – deceleration capacity; LTV – long-term variations; STV – short-term variations

In addition, the findings were not corroborated by repeating NI-FECG prior to delivery. Receiver operating characteristic analysis could be the next stage in investigating efficient diagnostic algorithms based on fetal NI-FECG. Since FGR is a model for the study of fetal programming, the investigation of fetal autonomic maldevelopment could help to create novel antenatal markers for the diseases of adulthood. FGR is associated with an increased rate of cardiac abnormalities and arrhythmia. The systems based on NI-FECG can improve wireless fetal monitoring [2, 18].

Conclusions

SI demonstrated a correlation with gestational age at birth. This variable could be of use in the advancement of conventional management of FGR and has potential for further research.

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Conflict of interest

The author declares no conflict of interest.

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Data availability

All data and materials are available from the author.

References

1. Frasch MG. Heart Rate Variability Code: Does It Exist and Can We Hack It? Bioengineering [Internet]. 2023 Jul 10;10(7):822. Available from: https://www.mdpi.com/2306-5354/10/7/822
2. Hoyer D, Żebrowski J, Cysarz D, Gonçalves H, Pytlik A, Amorim-Costa C, et al. Monitoring fetal maturation—objectives, techniques and indices of autonomic function. Physiol Meas [Internet]. 2017 May 1;38(5):R61–88. Available from: https://iopscience.iop.org/article/10.1088/1361-6579/aa5fca
3. Nardozza LMM, Caetano ACR, Zamarian ACP, Mazzola JB, Silva CP, Marçal VMG, et al. Fetal growth restriction: current knowledge. Arch Gynecol Obstet [Internet]. 2017 May 11;295(5):1061–77. Available from: http://link.springer.com/10.1007/s00404-017-4341-9
4. Smith V, Nair A, Warty R, Sursas JA, da Silva Costa F, Wallace EM. A systematic review on the utility of non-invasive electrophysiological assessment in evaluating for intra uterine growth restriction. BMC Pregnancy Childbirth [Internet]. 2019 Dec 5;19(1):230. Available from: https://bmcpregnancychildbirth.biomedcentral.com/articles/10.1186/s12884-019-2357-9
5. Zizzo AR, Hansen J, Petersen OB, Mølgaard H, Uldbjerg N, Kirkegaard I. Growth-restricted human fetuses have preserved respiratory sinus arrhythmia but reduced heart rate variability estimates of vagal activity during quiescence. Physiol Rep [Internet]. 2022 Nov 21;10(22). Available from: https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.15458
6. Stroux L, Redman CW, Georgieva A, Payne SJ, Clifford GD. Doppler-based fetal heart rate analysis markers for the detection of early intrauterine growth restriction. Acta Obstet Gynecol Scand [Internet]. 2017 Nov 27;96(11):1322–9. Available from: https://obgyn.onlinelibrary.wiley.com/doi/10.1111/aogs.13228
7. Stroux L, Martinez B, Coyote Ixen E, King N, Hall-Clifford R, Rohloff P, et al. An mHealth monitoring system for traditional birth attendant-led antenatal risk assessment in rural Guatemala. J Med Eng Technol [Internet]. 2016 Nov 16;40(7–8):356–71. Available from: https://www.tandfonline.com/doi/full/10.1080/03091902.2016.1223196
8. Odendaal HJ, Kieser E, Crockart IC, Brink LT, Du Plessis C, Nel DG. Clinical associations of fetal heart rate accelerations as derived from transabdominal fetal electrocardiograms. Eur J Obstet Gynecol Reprod Biol [Internet]. 2022 Dec;279:5–11. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0301211522005437
9. Velayo CL, Funamoto K, Silao JNI, Kimura Y, Nicolaides K. Evaluation of Abdominal Fetal Electrocardiography in Early Intrauterine Growth Restriction. Front Physiol [Internet]. 2017 Jun 26;8. Available from: http://journal.frontiersin.org/article/10.3389/fphys.2017.00437/full
10. Lakhno I. The Insight into Obstetric Care near the Front Line in Kharkiv. Acta medica Litu [Internet]. 2022 Sep 26;29(2):187–95. Available from: https://www.zurnalai.vu.lt/AML/article/view/28051
11. Lakhno I. The Use of Fetal Noninvasive Electrocardiography. Scientifica (Cairo) [Internet]. 2016;2016:1–6. Available from: http://www.hindawi.com/journals/scientifica/2016/5386595/
12. Stampalija T, Casati D, Monasta L, Sassi R, Rivolta M, Muggiasca M, et al. Brain sparing effect in growth-restricted fetuses is associated with decreased cardiac acceleration and deceleration capacities: a case–control study. BJOG An Int J Obstet Gynaecol [Internet]. 2016 Nov 23;123(12):1947–54. Available from: https://obgyn.onlinelibrary.wiley.com/doi/10.1111/1471-0528.13607
13. Hoyer D, Schmidt A, Gustafson KM, Lobmaier SM, Lakhno I, van Leeuwen P, et al. Heart rate variability categories of fluctuation amplitude and complexity: diagnostic markers of fetal development and its disturbances. Physiol Meas [Internet]. 2019 Jun 1;40(6):064002. Available from: https://iopscience.iop.org/article/10.1088/1361-6579/ab205f
14. Leal CRV, Rezende KP, Macedo E do CP de, Rezende G de C, Corrêa Júnior MD. Comparison between Protocols for Management of Fetal Growth Restriction. Rev Bras Ginecol e Obs / RBGO Gynecol Obstet [Internet]. 2023 Feb 28;45(02):096–103. Available from: https://journalrbgo.org/article/comparison-between-protocols-for-management-of-fetal-growth-restriction/
15. Ponsiglione AM, Cosentino C, Cesarelli G, Amato F, Romano M. A Comprehensive Review of Techniques for Processing and Analyzing Fetal Heart Rate Signals. Sensors [Internet]. 2021 Sep 13;21(18):6136. Available from: https://www.mdpi.com/1424-8220/21/18/6136
16. Kapaya H, Jacques R, Anumba D. Comparison of diurnal variations, gestational age and gender related differences in fetal heart rate (FHR) parameters between appropriate-for-gestational-age (AGA) and small-for-gestational-age (SGA) fetuses in the home environment. Frasch MG, editor. PLoS One [Internet]. 2018 Mar 9;13(3):e0193908. Available from: https://dx.plos.org/10.1371/journal.pone.0193908

17. Kingdom JC, Audette MC, Hobson SR, Windrim RC, Morgen E. A placenta clinic approach to the diagnosis and management of fetal growth restriction. Am J Obstet Gynecol [Internet]. 2018 Feb;218(2):S803–17. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002937817323402
18. Liu B, Ridder A, Smith V, Thilaganathan B, Bhide A. Feasibility of antenatal ambulatory fetal electrocardiography: a systematic review. J Matern Neonatal Med [Internet]. 2023 Dec 31;36(1). Available from: https://www.tandfonline.com/doi/full/10.1080/14767058.2023.2204390

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RESEARCH ARTICLE

Hormonal profiles and the diagnostic utility of serum dihydrotestosterone in polycystic ovary syndrome: a comparative case-control study

Taif Raheem Hmoud[1], Murtadha M. Jawad[2],

Redha Dawud Abd Alredha[3]

[1] Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, Kufa, Iraq
[2] College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, Kufa, Iraq
[3] Department of Pathological Analysis Techniques, College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, An Najaf, Iraq

Abstract

Background: Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder characterized by hyperandrogenism, anovulation, and metabolic disturbances. Dihydrotestosterone (DHT), a potent androgen formed via the 5α-reductase pathway, may play a role in the clinical manifestations of PCOS. This study aimed to compare serum DHT levels in women with PCOS versus healthy controls and to evaluate its diagnostic value. **Methods:** In this case-control study, women aged 18-45 years were recruited from outpatient clinics at the Maysan Specialized Surgical Hospital (Al-Amarah, Iraq). PCOS was diagnosed according to the Rotterdam criteria, while age-matched healthy volunteers with regular menstrual cycles served as controls. All participants underwent comprehensive clinical assessments, including anthropometric measurements. Fasting venous blood samples were collected between 8:00 and 10:00 AM, and serum was isolated for biochemical analysis. Serum DHT concentrations were measured using a competitive ELISA. Statistical analyses, including group comparisons and receiver operating characteristic (ROC) curve analysis, were performed using IBM SPSS Statistics. **Results:** Participants with PCOS had significantly higher serum DHT levels (4256 ± 233 ng/L) compared to controls (3776 ± 186 ng/L, $p < 0.001$). ROC curve analysis identified an optimal DHT cutoff value of approximately 408.3 ng/L, yielding a sensitivity of 90% and specificity of 100% for PCOS diagnosis. **Conclusion:** Elevated serum DHT is a key feature of PCOS and demonstrates excellent diagnostic accuracy. These findings support the clinical utility of DHT as a reliable biomarker for early PCOS detection and may facilitate targeted therapeutic interventions.

Keywords: polycystic ovary syndrome (PCOS) · hyperandrogenism · dihydrotestosterone (DHT) · 5α-reductase · diagnostic biomarker

Corresponding author:
Taif Raheem Hmoud, Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, Kufa, Iraq
e-mail: taifa7989@gmail.com
Available online: ejtcm.gumed.edu.pl

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine disorders affecting women of reproductive age, with a multifaceted presentation that includes hyperandrogenism, chronic anovulation, and polycystic ovarian morphology [1]. Hyperandrogenism is a central feature in PCOS and is typically assessed by measuring serum levels of androgens such as total testosterone and free testosterone. However, dihydrotestosterone (DHT), a potent androgen metabolite of testosterone, has garnered increasing interest due to its higher affinity for androgen receptors and its significant biological activity [2].

DHT is formed from testosterone via the catalytic action of the 5α-reductase enzyme. This conversion is critical because DHT is approximately 2-3 times more potent than testosterone in activating androgen receptors, thereby exerting more pronounced effects on target tissues [3]. Clinically, elevated DHT levels have been linked to manifestations such as hirsutism, acne, and androgenic alopecia – symptoms that are commonly observed in women with PCOS [4]. Despite these observations, the specific role and diagnostic utility of DHT in PCOS remain underexplored compared to other androgens.

The mechanisms leading to increased DHT levels in PCOS are multifactorial. A dysregulated gonadotropin milieu – marked by an elevated luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio – stimulates ovarian theca cells to enhance androgen production [4]. Furthermore, insulin resistance and the resultant hyperinsulinemia, which are common in PCOS, can amplify androgen synthesis by upregulating the activity of key steroidogenic enzymes, including 5α-reductase [5]. This hyperinsulinemic state not only accelerates the conversion of testosterone to DHT but also reduces hepatic production of sex hormone-binding globulin (SHBG), thereby increasing the levels of free and bioactive DHT.

Previous research in PCOS has predominantly focused on total and free testosterone measurements, often neglecting the potential diagnostic significance of DHT [6]. Given the potent androgenic properties of DHT, its measurement might provide a more sensitive indicator of hyperandrogenism. Emerging evidence suggests that DHT could serve as a reliable biomarker, offering enhanced diagnostic precision in identifying PCOS compared to traditional androgen assays [7]. Moreover, correlations between elevated DHT levels and the severity of clinical hyperandrogenic symptoms underscore its potential utility in both diagnosis and in assessing disease severity.

The present study is designed to investigate the serum DHT levels in women diagnosed with PCOS relative to healthy controls, and to evaluate its diagnostic performance using receiver operating characteristic (ROC) curve analysis. By focusing on DHT, we aim to clarify its contribution to the pathophysiology of PCOS and to establish its potential role as a diagnostic biomarker. This focus is particularly timely given the clinical challenges in diagnosing PCOS and the need for more precise and reliable biomarkers that can guide therapeutic strategies.

Materials and methods

Study design and ethical considerations

This case-control study was conducted at the Maysan Child and Birth Hospital (Al-Amarah, Iraq) between October 8th, 2024 and January 31st, 2025. The study protocol was approved by the Institutional Review Board (7-37-4480) and adhered to the ethical principles outlined in the Declaration of Helsinki [8]. All participants provided written informed consent prior to enrollment.

Participant recruitment and election

Participants were recruited from the outpatient endocrinology and gynecology clinics at Maysan Child and Birth Hospital. Women aged 18-45 years were considered for inclusion. PCOS diagnosis was made according to the Rotterdam criteria [9], which require the presence of at least two of the following: oligo/anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology on ultrasound. Controls were healthy volunteers with regular menstrual cycles and no clinical signs of hyperandrogenism. Exclusion criteria for all participants included the use of hor-

monal medications within the preceding three months, pregnancy, and any diagnosed endocrine disorders (e.g. thyroid dysfunction, hyperprolactinemia).

Clinical assessment and anthropometry

Participants underwent a detailed clinical evaluation that included a structured interview and physical examination. Anthropometric measurements were recorded following standard protocols (Lohman et al., 1988) [10]. Height was measured to the nearest 0.1 cm using a calibrated stadiometer (Cat. No. ST-100, Seca GmbH, Germany), and body weight was assessed using a digital scale (Cat. No. DS-200, Tanita Corporation, Japan) to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Sample collection and processing

Venous blood samples were collected between 8:00 and 10:00 AM after an overnight fast. Blood was drawn into plain serum separator tubes (Vacutainer®, BD Biosciences, USA) and allowed to clot at room temperature for 30 minutes. Samples were then centrifuged at 3 000 rpm for 10 minutes, and the serum was aliquoted into polypropylene tubes (Cat. No. PP-500, Eppendorf, Germany). The serum samples were immediately stored at –80°C until further analysis.

Biochemical measurements

Fasting blood glucose (FBG) was measured using the glucose oxidase method on an automated analyzer (Bio Research for Medical Diagnostics, Jordan). Serum insulin was quantified via emiluminescent fluorescence immunoassay on an automated analyzer (A. Menarini Diagnostics S.r.l., Italy), and insulin resistance was evaluated using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) formula:

$$\frac{\text{fasting insulin (uIU/ml)} \times \text{fasting glucose (mg/dl)}}{405}$$

Reproductive hormones (LH, FSH, testosterone, estradiol, and progesterone) were measured by AFIAS-10 automated fluorescence immunoassay system, on an automated analyzer (AFIAS-10, A. Menarini Diagnostics S.r.l., Italy), ensuring intra- and inter-assay variability below 8% and 10%, respectively. Serum epiregulin levels were quantified using a Human Epiregulin ELISA Kit (R&D Systems, USA), following standard ELISA procedures, with optical density measured at 450 nm using a microplate reader (BioTek ELx800, Agilent, USA). Assay sensitivity and variability were maintained as per manufacturer specifications and all samples were analyzed in duplicate, with necessary dilutions for values exceeding the assay's dynamic range.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 25.0 (IBM Corp., USA). Continuous variables are reported as mean ± standard deviation (SD), whereas the categorical variables as frequencies and percentages. The normality of continuous data was evaluated using the Shapiro-Wilk test. For normally distributed data, group comparisons were performed using the Student's t-test; otherwise, the Mann-Whitney U test was applied. Categorical data were compared using the Chi-square test (χ^2). Receiver operating characteristic (ROC) curve analysis was employed to assess the diagnostic performance of serum DHT, with sensitivity, specificity, and the area under the curve (AUC) reported. A p-value < 0.05 was considered statistically significant.

Results and discussion

The demographic characteristics of the control and study groups are summarized in Table 1. There was no statistically significant difference between the PCOS and control groups in terms of age or BMI. As shown in Table 2, menstrual irregularity was significantly more prevalent among PCOS patients, while marital status and history of PCOS showed no significant difference between groups (Table 2). The hormonal profile comparisons significantly elevated levels of LH, FSH, and prolactin in the PCOS group (Table 3). Notably, LH levels were approximately threefold higher in PCOS patients. Serum dihydrotestosterone (DHT) levels were significantly elevated in PCOS participants compared to controls (Table 4). The diagnostic performance of DHT is presented in Table 5. ROC analysis demonstrated a high sensitivity (86%) and perfect specificity (100%) at a DHT cutoff value of > 4084.3 ng/L. The ROC curve shows excellent diagnostic accuracy (AUC = 93%, $p < 0.001$) (Figure 1).

In this study we aimed to evaluate the hormonal profile of women with polycystic ovary syndrome (PCOS) compared to controls and to evaluate the diagnostic utility of serum dihydrotestosterone (DHT) in this population. Our findings confirm the well-documented hyperandrogenic state in PCOS and offer compelling evidence for the use of serum DHT as a diagnostic biomarker.

Our demographic analysis revealed that the control and PCOS groups were well-matched in terms of age and body mass index (BMI), with mean values of 28.8 ± 7.8 years versus 25.3 ± 4.5 years and 27.4 ± 3.6 kg/m^2 versus 28.1 ± 4.8 kg/m^2, respectively. These findings are in line with previous research that has shown minimal demographic differences between PCOS and non-PCOS cohorts when stringent inclusion criteria are applied. However, the markedly higher prevalence of menstrual irregularity in the PCOS group – where only 13%

Table 1. Demographic characteristics of the control and PCOS groups

Characteristic	Control n = 60	PCOS n = 60	p
Age (years)			
Mean ±SD	28.8 ± 7.8	25.3 ± 4.5	0.09 (I, NS)
Range	18 – 45	18 – 36	
BMI (kg/m²)			
Mean ±SD	27.4 ± 3.6	28.1 ± 4.8	0.43 (I, NS)
Range	20 – 36.7	22 – 46	

I – independent samples t-test; NS – not significant; SD – standard deviation.
Statistical significance was indicated by ***p < 0.001.

Table 2. Menstrual cycle regularity, marital status and PCOS history in control and PCOS groups

Characteristic	Control n = 60	PCOS n = 60	X2	p
Menstrual cycle				
Regular, n (%)	51 (85%)	8 (13%)	61.6	< 0.001***
Irregular, n (%)	9 (15%)	52 (87%)		
Marital Status				
Married, n (%)	37 (62%)	41 (68%)	0.5	0.4^{NS}
Not married, n (%)	23 (38%)	19 (32%)		
History of PCOS				
Positive, n (%)	16 (27%)	21 (35%)	0.97	0.3^{NS}
Negative, n (%)	44 (73%)	39 (65%)		

I – independent samples t-test; SD – standard deviation.
Statistical significance was indicated by ***p < 0.001.

Table 3. Mean hormone levels in control and PCOS groups

Characteristic	Control n = 60	PCOS n = 60	p
LH (mIU/ml)			
Mean ±SD	3.9 ± 1.09	11.8 ± 5.8	< 0.001 (I***)
Range	1.9 – 6.3	1.5 – 36	
FSH (mIU/ml)			
Mean ±SD	5.11 ± 1.4	6.09 ± 2.2	0.03 (I*)
Range	2.2 – 7.8	1.5 – 12.1	
Prolactin (ng/ml)			
Mean ± SD	17.6 ± 6.4	23.9 ± 7.7	0.002 (I**)
Range	7.8 – 34	12 – 47	

I – independent samples t-test; SD – standard deviation.
Statistical significance was indicated by ***p < 0.001.

Table 4. Serum dihydrotestosterone levels in control and PCOS groups

Characteristic	Control n = 60	PCOS n = 60	p
Dihydrotestosterone (ng/L)			
Mean ± SD	3776 ± 186	4256 ± 233	< 0.001 (I***)
Range	3443 – 4092	3744 – 4657	

I – independent samples t-test; SD – standard deviation.
Statistical significance was indicated by ***p < 0.001.

Table 5. ROC curve analysis for serum dihydrotestosterone in PCOS diagnosis

Variables	Cut-off value	Sens**%	Spec%	PPV**	NPV	AUC%	P-value (AUC = 0.05)
Dihydrotestosterone (ng/L)	> 4084.3	86	100	100	78	93	< 0.001**

AUC – area under curve; NPV – negative predictive value; PPV – positive predictive value; Sens – Sensitivity; Spec – Specificity

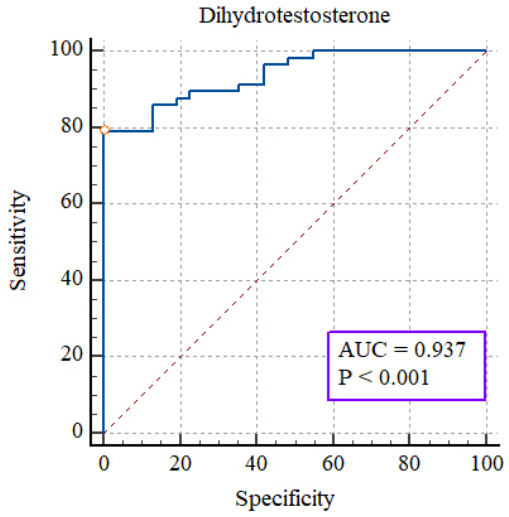

Figure 1. ROC curve analysis for serum dihydrotestosterone in PCOS diagnosis
AUC – area under the curve

of patients reported regular cycles compared to 85% of controls – confirms the clinical phenotype associated with PCOS as reported by Panidis et al. [11].

The endocrine profile of our PCOS cohort further underscored the syndrome's characteristic hormonal imbalances. Significantly elevated luteinizing hormone (LH) levels (11.8 ± 5.8 mIU/ml vs. 3.9 ± 1.09 mIU/ml, $p < 0.001$) and increased follicle-stimulating hormone (FSH) and prolactin levels were observed, which corroborate earlier findings. For example, Legro et al. (1999) reported mean LH levels in PCOS patients of approximately 12.5 ± 5.0 mIU/ml, a value strikingly similar to our results. The modest yet significant increase in FSH (6.09 ± 2.2 mIU/ml vs. 5.11 ± 1.4 mIU/ml, $p = 0.03$) and prolactin (23.9 ± 7.7 ng/ml vs. 17.6 ± 6.4 ng/ml, $p = 0.002$) further supports the presence of an altered gonadotropin milieu in PCOS, although the degree of alteration may vary between populations [12].

Of particular note in our study is the substantial increase in serum DHT levels among PCOS patients (4256 ± 233 ng/L) compared to controls (3776 ± 186 ng/L, $p < 0.001$). We found that women with polycystic ovary syndrome (PCOS) exhibited significantly higher serum DHT levels (4256 ± 233 ng/L) compared to controls (3776 ± 186 ng/L, $p < 0.001$), reaffirming the hyperandrogenic state characteristic of PCOS. DHT, a potent androgen derived from testosterone through the action of the 5α-reductase enzyme, is increasingly recognized as a key mediator of the clinical manifestations of PCOS, such as hirsutism and acne [13].

The mechanism driving the elevation of DHT in PCOS appears to be multifactorial. One of the central factors is the increased activity of 5α-reductase, which converts testosterone into DHT. In PCOS, ovarian theca cells are hyperstimulated (primarily by elevated LH levels) which upregulates the expression and activity of steroidogenic enzymes, including 5α-reductase [14]. Additionally, insulin resistance (a common feature in PCOS) leads to compensatory hyperinsulinemia. Elevated insulin levels are known to enhance ovarian androgen synthesis by stimulating the activity of enzymes such as CYP17A1 and 5α-reductase, while concurrently reducing hepatic synthesis of sex hormone-binding globulin (SHBG) [15]. This dual action not only boosts the production of androgens but also increases the proportion of free, bioactive androgens available to exert their effects.

Our findings are consistent with previous studies reporting hyperandrogenism in PCOS. For instance, Zeng et al. documented elevated androgen levels in PCOS patients, although most investigations have focused on total testosterone rather than DHT specifically [16]. By concentrating on DHT, our study provides novel insights into the hyperandrogenic profile of PCOS, suggesting that increased 5α-reductase activity and the resultant DHT accumulation may be more directly linked to the clinical severity of the syndrome.

At the molecular level, hyperinsulinemia plays a crucial role by upregulating genes encoding steroidogenic enzymes. This promotes an enhanced conversion of testosterone to DHT, which may further exacerbate the metabolic and reproductive disturbances observed in PCOS [17]. Moreover, the strong correlation between DHT levels and the clinical markers of hyperandrogenism in our cohort underscores the potential of DHT as a reliable biomarker for disease diagnosis and progression.

Clinically, the strong diagnostic performance of serum DHT, as evidenced by our ROC curve analysis (with an optimal cutoff yielding 90% sensitivity and 100% specificity), highlights its utility as a non-invasive diagnostic tool for PCOS. Early detection of hyperandrogenism using DHT measurements could facilitate timely intervention, potentially mitigating long-term complications such as metabolic syndrome and infertility. Furthermore, our study suggests that targeting insulin resistance (through lifestyle interventions or pharmacotherapy) may reduce DHT levels and ameliorate hyperandrogenic symptoms, thereby offering a promising therapeutic avenue. The novelty of our study lies in the focused evaluation of DHT, rather than the more commonly assessed total testosterone, providing additional insight into the androgenic profile of PCOS.

Despite its strengths, this study has several limitations. The case-control design precludes any inference of causality. The sample size, while adequate for initial comparisons, may limit the generalizability of our findings. Furthermore, immunoassays for DHT quantification are practical, yet may be less precise than liquid chromatography-tandem mass spectrometry (LC-MS/MS) [18]. Future studies should include longitu-

dinal design and larger, more diverse populations to validate these findings.

Conclusions

In conclusion, our study confirms that women with PCOS exhibit a distinct hormonal profile characterized by elevated LH, FSH, prolactin, and particularly DHT levels. The diagnostic performance of serum DHT is promising, offering a novel tool for the accurate identification of PCOS. These results not only align with previous literature but also contribute new insights into the pathophysiology and potential diagnostic strategies for PCOS.

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Conflicts of interest

The authors declare that they have no competing interests.

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Availability of data and materials

The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

References

1. Azziz R. Polycystic Ovary Syndrome. Obstet Gynecol [Internet]. 2018;132(2):321–36. Available from: https://journals.lww.com/00006250-201808000-00009
2. Pfaff DW, Baum MJ. Hormone-dependent medial preoptic/lumbar spinal cord/autonomic coordination supporting male sexual behaviors. Mol Cell Endocrinol [Internet]. 2018;467:21–30. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0303720717305646
3. Naamneh Elzenaty R, du Toit T, Flück CE. Basics of androgen synthesis and action. Best Pract Res Clin Endocrinol Metab [Internet]. 2022;36(4):101665. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1521690X22000525
4. Taieb A, Feryel A. Deciphering the Role of Androgen in the Dermatologic Manifestations of Polycystic Ovary Syndrome Patients: A State-of-the-Art Review. Diagnostics [Internet]. 2024;14(22):2578. Available from: https://www.mdpi.com/2075-4418/14/22/2578
5. Moghetti P. Insulin resistance and polycystic ovary syndrome. Curr Pharm Des [Internet]. 2016;22(36):5526–34. Available from: https://www.ingentaconnect.com/content/ben/cpd/2016/00000022/00000036/art00004
6. Abdelazim I, Alanwar A, AbuFaza M, Amer O, Bekmukhambetov Y, Zhurabekova G, et al. Elevated and diagnostic androgens of polycystic ovary syndrome. Menopausal Rev [Internet]. 2020;19(1):1–5. Available from: https://www.termedia.pl/doi/10.5114/pm.2020.95293
7. Bizuneh AD, Joham AE, Teede H, Mousa A, Earnest A, Hawley JM, et al. Evaluating the diagnostic accuracy of androgen measurement in polycystic ovary syndrome: a systematic review and diagnostic meta-analysis to inform evidence-based guidelines. Hum Reprod Update [Internet]. 2025;31(1):48–63. Available from: https://academic.oup.com/humupd/article/31/1/48/7762932
8. Goodyear MDE, Krleza-Jeric K, Lemmens T. The Declaration of Helsinki. BMJ [Internet]. 2007;335(7621):624–5. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.39339.610000.BE
9. Teede HJ, Tay CT, Laven JJE, Dokras A, Moran LJ, Piltonen TT, et al. Recommendations From the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome. J Clin Endocrinol Metab [Internet]. 2023;108(10):2447–69. Available from: https://academic.oup.com/jcem/article/108/10/2447/7242360
10. Panidis D, Tziomalos K, Papadakis E, Chatzis P, Kandaraki EA, Tsourdi EA, et al. Associations of menstrual cycle irregularities with age, obesity and phenotype in patients with polycystic ovary syndrome. Hormones [Internet]. 2015;14(3):431–7. Available from: https://link.springer.com/10.14310/horm.2002.1593

11. Saei Ghare Naz M, Mousavi M, Mahboobifard F, Niknam A, Ramezani Tehrani F. A Meta-Analysis of Observational Studies on Prolactin Levels in Women with Polycystic Ovary Syndrome. Diagnostics [Internet]. 2022;12(12):2924. Available from: https://www.mdpi.com/2075-4418/12/12/2924
12. Housman E, Reynolds R V. Polycystic ovary syndrome: A review for dermatologists. J Am Acad Dermatol [Internet]. 2014;71(5):847.e1-847.e10. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0190962214014340
13. Chang WY, Azziz R. Pathogenesis of Hyperandrogenism in Polycystic Ovary Syndrome. In: Polycystic Ovary Syndrome [Internet]. Totowa, NJ: Humana Press; p. 281–94. Available from: http://link.springer.com/10.1007/978-1-59745-108-6_17
14. Zhu J, Chen Z, Feng W, Long S, Mo Z-C. Sex hormone-binding globulin and polycystic ovary syndrome. Clin Chim Acta [Internet]. 2019;499:142–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S000989811932039X
15. Zeng X, Xie Y, Liu Y, Long S, Mo Z. Polycystic ovarian syndrome: Correlation between hyperandrogenism, insulin resistance and obesity. Clin Chim Acta [Internet]. 2020;502:214–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0009898119321187
16. Ressler IB, Grayson BE, Ulrich-Lai YM, Seeley RJ. Diet-induced obesity exacerbates metabolic and behavioral effects of polycystic ovary syndrome in a rodent model. Am J Physiol Metab [Internet]. 2015;308(12):E1076–84. Available from: https://www.physiology.org/doi/10.1152/ajpendo.00182.2014
17. Greaves RF, Jolly L, Hartmann MF, Ho CS, Kam RKT, Joseph J, et al. Harmonisation of serum dihydrotestosterone analysis: establishment of an external quality assurance program. Clin Chem Lab Med [Internet]. 2017;55(4). Available from: https://www.degruyter.com/document/doi/10.1515/cclm-2016-0394/html

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RESEARCH ARTICLE

The length and topographical location of septal papillary muscles in adult human hearts

Adam Kosiński[1], Agata Kaczyńska[1], Grzegorz Piwko[1], Damian Jeżyk[2], Paulina Słonimska[1], Karina Zajączkowska[3], Małgorzata Zawrzykraj[1], Miłosz Andrzej Zajączkowski[1]

[1] Division of Clinical Anatomy, Medical University of Gdańsk, Poland
[2] Division of Anatomy and Anthropology, Gdańsk University of Physical Education and Sport, Gdańsk, Poland
[3] Division of Physical Activity for Women's Health, Gdańsk University of Physical Education and Sport, Gdańsk, Poland

Abstract

Background: This study aimed to evaluate the relative length and anatomical position of septal papillary muscles, including the papillary muscle of the conus arteriosus (MCA) and other septal papillary muscles (MPS), as these may have significant clinical implications. **Material and methods:** We examined 111 formalin-fixed human hearts from individuals aged 49-97 years, with no pathological lesions or malformations. The right ventricle was opened with a V-shaped incision, and measurements were taken along the posterior angle from the annulus fibrosus to the apex. The ventricle height was divided into ten levels for topographical assessment. Relative muscle lengths were calculated as percentages of ventricular height. **Results:** MCA was present in all specimens, predominantly as a poorly developed structure (67.57% with relative length 1-5%). MPS were absent in 28 hearts, with only tendinous cords present. When developed, MPS showed similar proportions to MCA. The latter was primarily located at the third level from the annulus fibrosus, while MPS and associated cords showed greater topographical variability (levels 1-7). **Conclusions:** Septal papillary muscles demonstrate considerable morphological and topographical variability. The predominance of poorly developed muscles and the presence of tendinous cords alone suggest evolutionary variations in septal muscular organization. These findings provide important anatomical insights for cardiac interventions.

Keywords: tricuspid valve · right ventricle · cardiac morphology · septal papillary muscles · conus arteriosus muscle

Citation

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Corresponding author:
Miłosz Andrzej Zajączkowski, Division of Clinical Anatomy, Medical University of Gdańsk, Poland
email: milosz.zajaczkowski@gumed.edu.pl

Available online: ejtcm.gumed.edu.pl

Introduction

Although not a new area of research, cardiac morphology remains indispensable in the dynamic development of interventional cardiology and cardiac surgery. Understanding the detailed anatomy of individual cardiac structures is essential for recognizing pathological variations that may occur within the heart. While modern visualization techniques provide valuable insights, comprehensive autopsy studies enable detailed morphological observations that remain crucial for clinical practice. Such studies may, among other things, help determine whether a specific configuration of a heart's structure represents the norm (or its variant) or a pathology.

In 1863, Luschka described the muscle located on the heart's septum and proposed the name "papillary muscle of the conus arteriosus" (*musculus coni*, MCA) [1]. Some controversies arise around this structure's nomenclature. Some authors have used the above-mentioned term, although the terms "muscle of Luschka" or "muscle of Lancisi" are can also be found in the literature [2].

In recent years, relatively few research articles have focused on the septal papillary muscles (*musculi papillares septales*, MPS) that contribute to the valvular apparatus of the right atrioventricular orifice. Szostakiewicz-Sawicka, Wenink and Restivo et al. noticed the significant role of the muscles located in the immediate vicinity of the musculus coni as supportive elements to the anterior commisure and the cuspis septalis of the tricuspid valve [3-5]. For this reason, Wenink suggested the term "the medial papillary complex" which, with a slight modification („the medial papillary muscle complex"), is also used by Restivo [4-5]. According to these authors, the muscles constitute a complex directly connected with the muscle of the arterial cone. Loukas et al. stresses the researchers' limited interest in MCA, although the knowledge of the heart's normal anatomy is essential for the correct understanding of cardiac diseases [2].

The aim of this study was to estimate the relative length of all MPS, including the muscles of the arterial cone, as well as remaining septal muscles and the height at which the basis of the muscles are located, which may have significant clinical relevance.

Materials and methods

The observations were conducted on 111 human hearts of both sexes, from individuals aged 49-97 years, with no pathological lesions or malformations, fixed in a solution of formalin and ethanol. The hearts were obtained from the collection of the Division of Clinical Anatomy at the Medical University of Gdańsk (Poland). All experimental procedures were approved by the local Independent Bioethics Committee for Scientific Research (decision No. 74/2012).

Classic anatomic methods were used: the right ventricle (RV) was opened with a V-incision from the aortic orifice of the pulmonary trunk (along the anterior interventricular groove), and then along the lateral border of the RV towards the right atrioventricular valve. The RV height was measured along its posterior angle: from the fibrous annulus of the tricuspid valve (FA) to the apex of the RV. The ventricular height was divided into ten equal levels (1-10, counted from FA towards the apex) serving for determining the height of location of the examined structures (Figure 1). Relative lengths of the muscles were measured and normalized to the height of the RV (Figure 2). These results were then categorized into the following ranges for analysis: 1-5%, 6-10%, 11-15%, 16-20%, and > 20%.

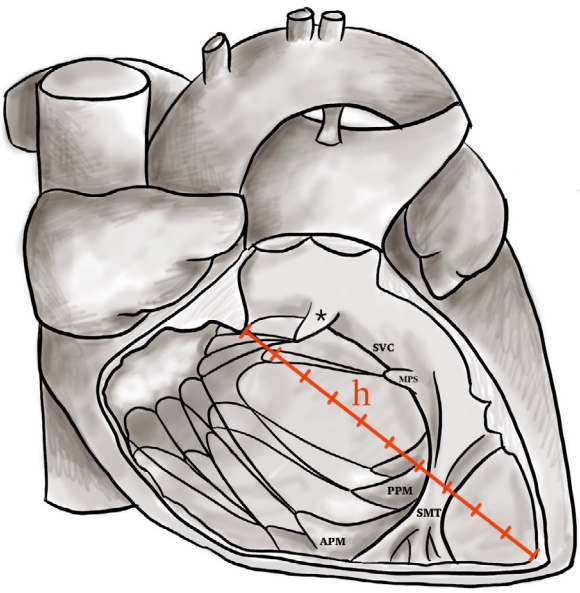

Figure 1. The division of interventricular septum into 10 levels
h – the height of the right ventricle; SVC – supraventricular crest; MPS – septal papillary muscles; MPP – posterior papillary muscle; APM – anterior papillary muscle; SMT – septomarginal trabecula; ★ – papillary muscle of the conus arteriosus

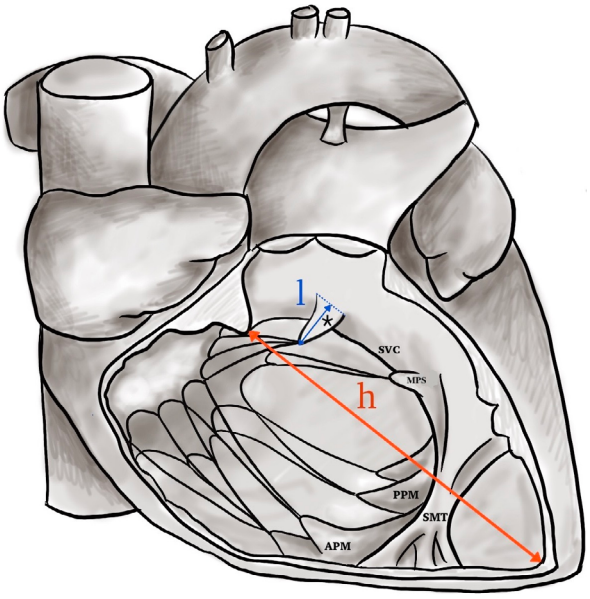

Figure 2. Relative height of the papillary muscles (l/h)
l – the length of papillary muscle of the conus arteriosus;
h – the height of the right ventricle; SVC – supraventricular crest;
MPS – septal papillary muscles; MPP – posterior papillary muscle;
APM – anterior papillary muscle; SMT – septomarginal trabecula;
★ – papillary muscle of the conus arteriosus

Statistical analysis

When evaluating the results, a non-parametric Pearson's chi-square test of independence was used. The software used to perform analyses was R 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad 5 (GraphPad Software, Inc. La Jolla California, USA). P values < 0.05 were considered statistically significant.

Results

All examined structures were divided into 2 groups, as suggested by Jeżyk et al.: "constant" (with MCA present in each specimen) and "variable" (included MPS) similarly to other papillary muscles, are essential elements of the heart valvular system [6]. Damage to their structure may lead to a considerable life risk. Of all the papillary muscles, the septal papillary muscles are characterized by the greatest topographical and morphological variability. However, information about these muscles is scarce and fragmentary. The objective of this study was to ascertain their occurrence and the region in which they are placed in the inter-ventricular septum. One hundred and eleven human hearts were examined. The hearts belonged to the Clinical Anatomy Department of the Medical University of Gdańsk. They were fixed in formalin with ethanol and came from middle-aged and older individuals of both sexes, devoid of pathological changes and birth defects. During the tests, classic anatomical methods were applied. The region where the papillary muscles are found covers a sizeable surface of the septum, from the conus arteriosus up to the back angle of the right chamber. Depending on their location the following septal papillary muscles (musculi papillares septales, MPS).

Relative length of MCA

MCA was observed in all examined hearts. In most cases it was a poorly developed structure (of a relative length of 1-5%), constituting a minor convexity (Figure 3). In 3 hearts only, its relative length reached the highest value of 16-20% of the RV's height. (Table 1).

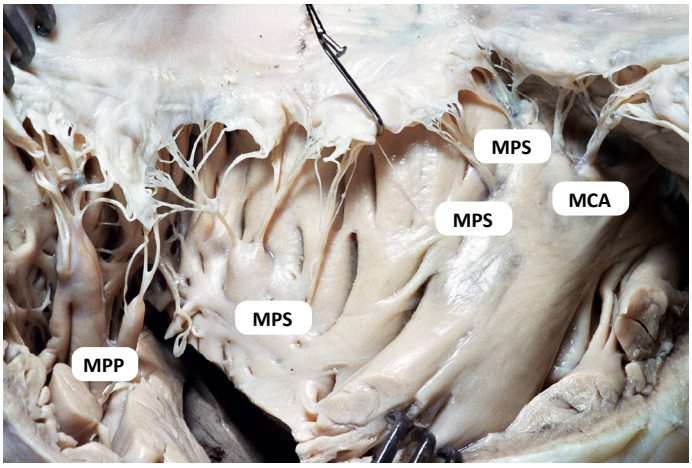

Figure 3. Gross anatomical view of the opened right ventricle in an adult human heart, showing the exposed cavity with papillary muscles. An example of a poorly developed MCA (relative length of 1-5%)
MPS – septal papillary muscles; MCA – papillary muscle of the conus arteriosus; MPP – posterior papillary muscle

Table 1. Relative length of MCA in adult human hearts expressed in percent of the ventricle's height (AL_{MCA})

AL_{MCA}(%)	Hearts	
	n	%
1-5	75	67.57
6-10	22	19.82
11-15	11	9.91
16-20	3	2.70
20	0	0
Total	111	100

Relative length of MPS

MPS are generally poorly developed (Table 2). In 28 hearts (25.23%) we did not dermine the presence of MPS. In such cases only tendinous cords ran from the intraventricular septum and supplied specific parts of the tricuspid valve cusp. Developed muscles were of convex or conical shape, lying in the medial or posterior part of the septum in the vicinity of the posterior papillary muscle.

Table 2. Relative length of MPS in adult human hearts expressed in percent of the ventricle's height (ALMPS)

AL_{MPS} (%)	MPS muscles	
	n	%
1-5	86	63.24
6-10	36	26.47
11-15	10	7.35
16-20	4	2.94
20	0	0
Total	136	100

As the above data indicate, very poorly developed MPS (with a relative length of 1-5%) clearly predominated, constituting the majority of cases and making up over 60% of all muscles observed. Comparing data related to the length of the MCA (Table 1) with the length of the MPS (Table 2), we might conclude that the value distribution for both muscles was alike (Figure 4). This is confirmed by Pearson's chi-square analysis (Pearson's) = 1.8095; df = 3; p value = 0.61.

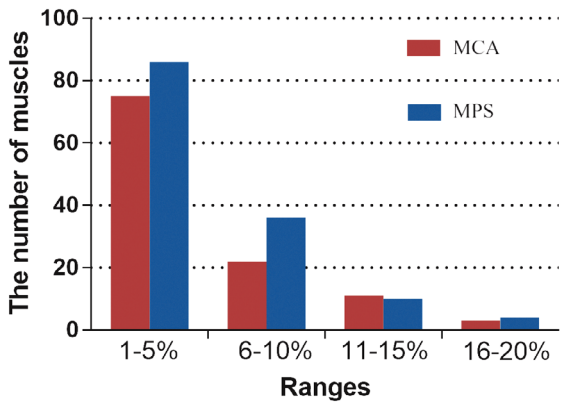

Figure 4. Relative length of MCA and MPS in adult human hearts – summary

The height of location of MPS' base on the intraventricular septum

In order to estimate the location of the MPS, the height of the right ventricle (previously divided into 10 equal levels) was used as the reference system for determining their position along the intraventricular septum. Particular muscles belonging to a certain level were observed.

The height of location of MCA's base on the intraventricular septum

In most cases the MCA was located on the 3rd level of the FA (Figure 5).

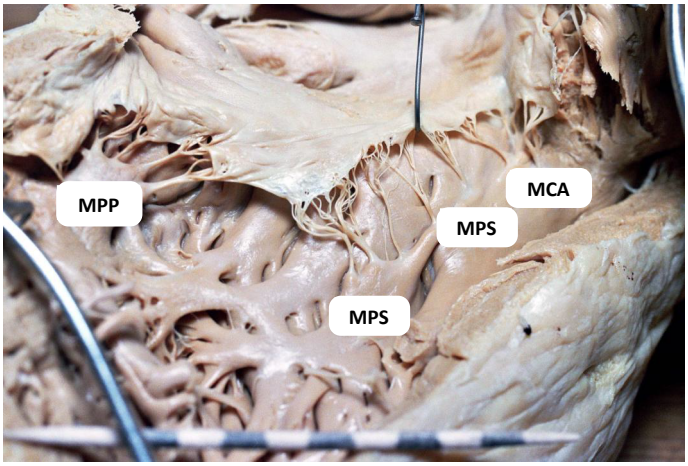

Figure 5. Gross anatomical view of the opened right ventricle in an adult human heart, showing the exposed cavity with papillary muscles
MPS – septal papillary muscles; MCA – papillary muscle of the conus arteriosus; MPP – posterior papillary muscle

The height of the location of MCA's base on the intraventricular septum is presented in Table 3.

Table 3. The height of the location of MCA's base on intraventricular septum in adult human hearts

Level of location	MCA muscles	
	n	%
2	15	13.5
3	93	83.8
4	3	2.7
Total	111	100

The height of location of MPS's base on the intraventricular septum

The observations revealed the presence of variable number of MPS muscles often accompanied by tendinous cords occurring in different proportion.

The muscles and / or the tendinous cords were mostly observed on upper levels, although they could also be found on the 6th and 7th levels (Figure 6). In total, 492 such structures were reported, of which 136 constituted the MPS, and 356 – the cords.

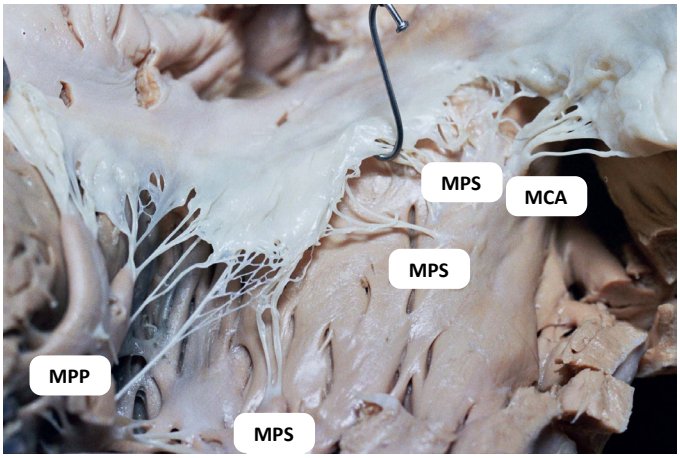

Figure 6. Gross anatomical view of the opened right ventricle in an adult human heart, showing the exposed cavity with papillary muscles
MPS – septal papillary muscles; MCA – papillary muscle of the conus arteriosus; MPP – posterior papillary muscle

The height of location of MPS' base on the septum (and/or the height of the location of the tendinous cords diverging) is presented in Table 4.

Table 4. The height of location of MPS's base and/or corresponding tendinous cords on the intraventricular septum in adult human hearts

Level of location	MPS muscles +/- tendinous chords	
	n	%
1	90	18.3
2	130	26.4
3	112	22.8
4	121	24.6
5	32	6.5
6	5	1.0
7	2	0.4
Total	492	100

When we compare the data from both tables, i.e. the height of the MCA base (Table 3) with the height of the MPS base (Table 4), we may conclude that the location of these muscles on the ventricular septum is variable (Figure 7) as confirmed by Pearson's chi-square analysis (Pearson's) = 155.676; df = 6; p value < 0.001.

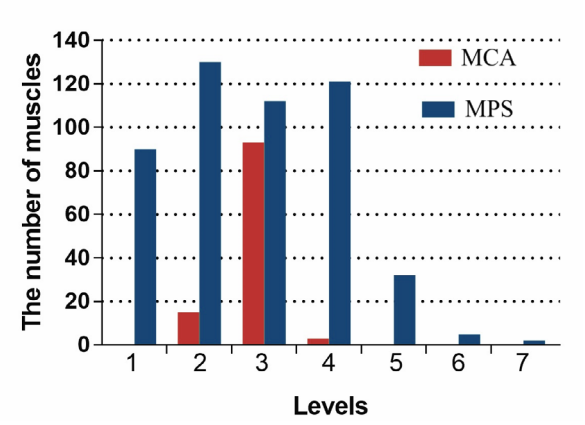

Figure 7. Height of the base of the MCA and MPS (and/or tendinous cords) in adult human hearts – summary

In 28 hearts MPS were not observed, although corresponding tendinous cords co-creating the valvular apparatus were present. In remaining 83 hearts we noted the presence of the total 136 MPS (Table 2). As already mentioned, the MPS were bound to the medial or posterior part of the septum, in the vicinity of the posterior papillary muscle. In total 356 tendinous cords were observed diverging directly from the septum and associated MPS. Some of them occurred alone with no developed MPS (28 hearts), other accompanied usually poorly developed muscles. The presence of various number of MPS muscles (often additionally accompanied by the cords), and even the presence of tendinous cords alone confirms the variability of this group of septal papillary muscles.

Discussion

Papillary muscles related to the septum constitute a part of the heart's valvular apparatus, thereby co-deciding about its proper functioning [3, 7-12]. Therefore, understanding the detailed anatomy of the MPS complex is crucial not only for anatomical classification but also for clinical applications such as imaging interpretation, catheter-based interventions, and valve repair procedures. Their morphology can influence conduction pathways and arrhythmogenic

potential in the right ventricular outflow tract (RVOT) [10, 13-14]. Advanced cardiac CT and MRI have become the reference methods for examining the complex geometry of the RVOT, thereby enabling further study of sub-structures such as septal papillary muscles and their potential morphological impact [15]. Structural damage to these muscles can pose considerable clinical risk [16-18]. Nevertheless, some authors emphasize that the attention given to MPS complex of septal papillary muscles is still insufficient [2].

The substantial topographical and morphological diversity of MCA and MPS has led to nomenclature ambiguity [19-20]. Jeżyk et al. observed a specific distinction within the septal muscles: a fixed group and a variable one. In the former group they included an always-present muscle located anteriorly on the septum, which due to its location is often referred to as the muscle of the arterial cone (*m. coni arteriosi* or *m. subarteriosus*) (MCA). The attitudinally based approach to cardiac anatomy introduced by Anderson et al. [21] redefines papillary muscle orientation in terms of spatial relationships rather than traditional directional terminology. In the latest attitudinally based description of right ventricular anatomy by Crucean et al. [20] the papillary muscles are characterized according to their spatial relationships with the septal, inferior, and antero-superior leaflets of the tricuspid valve, reflecting their true topographical orientation within the RV. In the available literature, MCA was most often regarded as the MPS. Jeżyk et al. proposed a distinction in which this muscle (MCA) belongs to the constant group, whereas the remaining MPS form a variable group [6]. In previous reports, these additional MPS were inconsistently described as accessory muscles (*m. accesorius*) or as the septum's own muscles (*m. papillaris proprius septi*) or were simply ignored. In our study, we adopted the same classification as Jeżyk et al. [6] similarly to other papillary muscles, are essential elements of the heart valvular system. Damage to their structure may lead to a considerable life risk. Of all the papillary muscles, the septal papillary muscles are characterized by the greatest topographical and morphological variability. However, information about these muscles is scarce and fragmentary. The objective of this study was to ascertain their occurrence and the region in which they are placed in the inter-ventricular septum. One hundred and eleven human hearts were examined. The hearts belonged to the Clinical Anatomy Department of the Medical University of Gdańsk. They were fixed in formalin with ethanol and came from middle-aged and older individuals of both sexes, devoid of pathological changes and birth defects. During the tests, classic anatomical methods were applied. The region where the papillary muscles are found covers a sizeable surface of the septum, from the conus arteriosus up to the back angle of the right chamber. Depending on their location the following septal papillary muscles (musculi papillares septales, MPS. According to this classification, the absence of a developed muscle was recorded as a tendinous cord.

In the available literature, authors typically use absolute values to estimate the length of papillary muscles [5, 16, 22]. Whereas, the relative length of the muscles, as used in this study, may provide a basis for broadening the scope of observations through comparative anatomical studies. Expressing muscle length in proportion to the ventricular height reflects its potential mechanical influence on leaflet tethering and right ventricular geometry. This proportional analysis is conceptually consistent with parameters used in previous experimental and clinical studies to predict residual tricuspid regurgitation, where altered papillary muscle positioning and tethering angles have been shown to correlate with postoperative outcomes [14, 23-24]. Therefore, this method may offer functionally relevant insight into the interplay between septal muscle morphology and tricuspid valve competence. The relative height used in such studies provides for differences within the size of particular hearts. Further discussion is needed to determine whether "muscle height" rather than "muscle length" would be a more appropriate term for the septal papillary muscles. In this study we used the former term. Topographic relations and the term "ventricle's height" used in the work as a point of reference, might support the former interpretation. However, we decided to use the latter term since it occurs in available literature, although referring to absolute values.

Huwyler figuratively described the size of the MCA in human hearts reporting that it reaches at most the dimension of a cherry stone, whereas Rouviere presented it as a small, conical muscle located above the moderator band [25-26]. Although most of the previous studies have focused on morphological and gross shape variations, our analysis extends this approach by offering a more detailed quantitative comparison of relative papillary muscle lengths [27]. This approach complements previous morphometric investigations by introducing a normalized assessment of septal papillary muscle dimensions relative to the RV height, enabling inter-individual comparison independent of heart size.

Saha and Roy reported the mean length of the septal papillary muscle as 0.95 ± 0.38 cm, typically arising from the upper one-third of the RV, whereas Sinha et al. observed a comparable mean value of 0.92 ± 0.54 cm [28-29]. Our findings are consistent with these observations. Farzana et al. noted an age-related increase in the muscle's mean length, from 0.51 cm in young adults to 0.81 cm in older individuals [30]. Bhadoria reported that all septal papillary muscles were uniformly conical in shape in all 50 (100%) hearts examined in their study [31]. Varghese described them as having a conical or broad-apex shape. He does not describe any cylindrical muscles on the septum [32]. Testut et al., described the muscle of the arterial cone as a round hump of 6-8 mm in length, often occurring at a site, where the supraventricular crest descends [33]. In turn, Mikhaylov wrote that the length of MCA in his material ranged from 2 to 14 mm, and the height of most muscles amounted from 2 to 6 mm, whereas Musso reported the dimension of 4 to 18 mm [16, 22].

Both MCA and MPS were in most cases observed as poorly developed structures. This was also confirmed by statistical analysis. Contemporary anatomical and electrophysiological studies describe marked variability in the development of right ventricular papillary muscles, therefore good anatomical knowledge is required [34]. The right ventricle can be separated into an inlet, an outlet, and an apical compartment. The inlet and outlet are separated by the septomarginal trabeculae, while the apex is situated below the moderator band. A lead position in the right ventricular apex is less desirable, last but not least due to the thin myocardial wall. Many leads supposed to be implanted in the apex are in fact fixed rather within the trabeculae in the inlet, which are sometimes difficult to pass. In the RVOT the septal papillary muscle is typically the least prominent element of the right ventricular apparatus and may even be absent as a distinct structure (reported absent in ~56% of specimens) [28]. After examining 30 adult human hearts Szostakiewicz-Sawicka described MCA as little convexities and in 3 cases reported their absence. In our material MCA was always present [3].

This considerable variability may explain the relatively limited number of publications devoted to these structures and the difficulties in their classification. For this reason their name is seldom mentioned in literature or referred only as to 'small muscles', 'minor cones' or 'free and direct cords' [35-37]. Considerable individual variation in the arrangement of tricuspid valve papillary muscles has been described, reinforcing that the so-called 'normal' configuration may vary widely among healthy individuals [38].

The anatomical variability of the septal papillary muscles, particularly within the conal region, may also influence the pathophysiology of arrhythmias arising from the RVOT. Knowledge of the individual papillary muscle anatomy is crucial for successful catheter-based treatment, as the complex structure and variability of these muscles directly affect catheter stability, mapping accuracy, and long-term ablation outcomes. Despite continuous technical progress, the long-term success of papillary muscle ablation remains moderate, highlighting the importance of precise anatomical understanding before intervention [39]. Accurate knowledge of this part of the heart is also essential for effective ablation and prevention of iatrogenic injury [10, 13].

Beyond their arrhythmogenic relevance, variations in the septal papillary muscles may affect tricuspid valve mechanics and contribute to leaflet tethering in functional regurgitation or dilated cardiomyopathy. Studies have demonstrated that tethering angles and septal muscle displacement predict recurrence of tricuspid regurgitation after surgical repair [23-24, 40]. Hence, the structural variability described in our study could have direct implications for preoperative imaging and repair strategy. This variability may also be reflected in a slightly less stable attachment of these structures along the septum. While the MCA occupied mainly the 3rd level (from 2 to 4) (Table3), the MPS +/- the corresponding cords were most frequently observed between levels 1 and 7, predominantly in the upper levels (Table 4). Statistical analysis confirmed the variability of the levels of both muscles' location (including tendinous cords) on the septum. Particular muscles constituting a complex of papillary muscles of a relevant group in the RV are called "heads" by Nigri et al. [41]. It seems that these authors included a part of the papillary muscles complex into the posterior papillary muscle group. They also distinguished various types of the muscles' shapes: conical, flat-topped, mammillated, arched. It can be assumed that the authors accommodated different criteria for the muscles' valuation.

The high frequency of poorly developed or absent septal papillary muscles suggests that this structure cannot be regarded as a constant anatomical landmark. This finding contrasts with traditional anatomical descriptions, highlighting a greater degree of variability than previously recognized [31, 42].

Conclusions

In summary, the considerable variability in the morphology and location of septal papillary muscles has important implications for right ventricular geometry, tricuspid valve function, and RVOT arrhythmogenicity. The present correlations between relative muscle length, attachment height, and functional relevance underscore the clinical importance of these structures in imaging, surgery, and electrophysiology.

The frequent finding of poorly developed muscles located on the interventricular septum (or even replacement by tendinous cords) appears particularly noteworthy, as these may represent vestigial remnants of septal papillary muscles. Future studies combining gross anatomy with histological and microstructural analyses would help clarify whether these formations reflect regressed remnants or functionally specialized components, and how their tissue composition may influence subtle variations in right ventricular mechanics.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. von Luschka H. Die Anatomie der Brust des Menschen. Verlag der H. Laupp'schen Buchhandlung; 1863.
2. Loukas M, Shane Tubbs R, Louis RG, Apaydin N, Bartczak A, Huseng V, et al. An endoscopic and anatomical approach to the septal papillary muscle of the conus. Surg Radiol Anat [Internet]. 2009;31(9):701–6. Available from: https://doi.org/10.1007/s00276-009-0510-2
3. Szostakiewicz-Sawicka H. Zastawka przedsionkowo-komorowa prawa u naczelnych. Rozprawa habilitacyjna. Acta Biol Med Soc Sc Gedan. 1967;11:545–89.
4. Wenink AC. The medial papillary complex. Br Heart J [Internet]. 1977 Sep 1;39(9):1012 LP – 1018. Available from: http://heart.bmj.com/content/39/9/1012.abstract
5. Restivo A, Smith A, Wilkinson JL, Anderson RH. The medial papillary muscle complex and its related septomarginal trabeculation. A normal anatomical study on human hearts. J Anat [Internet]. 1989;163:231–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2606775
6. Jeżyk D, Duda B, Jerzemowski J, Grzybiak M. Positions of septal papillary muscles in human hearts. Folia Morphol (Warsz). 2010;69(2):101–6.
7. Anzola J. Right Ventricular Contraction. Am J Physiol Content [Internet]. 1956;184(3):567–71. Available from: https://www.physiology.org/doi/10.1152/ajplegacy.1956.184.3.567
8. Gould SE. Pathology of the heart and blood vessels. (No Title). 1968;
9. Brandt W. The closing mechanism of the tricuspidal valve in the human heart. Cells Tissues Organs. 1957;30(1–4):128–32.
10. HAI JJ, DESIMONE C V., VAIDYA VR, ASIRVATHAM SJ. Endocavitary Structures in the Outflow Tract: Anatomy and Electrophysiology of the Conus Papillary Muscles. J Cardiovasc Electrophysiol [Internet]. 2014;25(1):94–8. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jce.12291
11. Josowitz R, Rogers LS. Double outlet right ventricle–the 50% rule has always been about the conus. Curr Opin Cardiol [Internet]. 2024;39(4):348–55. Available from: https://journals.lww.com/co-cardiology/_layouts/15/oaks.journals/downloadpdf.aspx?an=00001573-202407000-00019
12. Xalxo N, Kaur S, Chauhan M, Sharma E, Sophia L, Agarwal S, et al. Papillary muscles: morphological differences and their clinical correlations. Anat Cell Biol [Internet]. 2025;58(1):44–53. Available from: https://acbjournal.org/journal/view.html?doi=10.5115/acb.24.210
13. Naksuk N, Kapa S, Asirvatham SJ. Spectrum of Ventricular Arrhythmias Arising from Papillary Muscle in the Structurally Normal Heart. Card Electrophysiol Clin [Internet]. 2016;8(3):555–65. Available from: https://doi.org/10.1016/j.ccep.2016.04.005

14. Kaczyńska A, Kosiński A, Kamiński R, Zajączkowski M, Nowicka E, Gleinert-Rożek M. A novel approach to visualization of the right ventricular outflow tract. Eur J Transl Clin Med [Internet]. 2019;1(2):36–40. Available from: https://ejtcm.gumed.edu.pl/articles/22
15. Saremi F, Ho SY, Cabrera JA, Sánchez-Quintana D. Right Ventricular Outflow Tract Imaging With CT and MRI: Part 1, Morphology. Am J Roentgenol [Internet]. 2013;200(1):W39–50. Available from: http://www.ajronline.org/doi/abs/10.2214/AJR.12.9333
16. Mikhaylov SS. Klinicheskaya anatomiya serdtsa [in Russian] (Clinical Anatomy of the Heart). Moscow: Medicina; 1987.
17. PASIC M, VONSEGESSER L, CARREL T, JENNI R, TURINA M. Severe tricuspid regurgitation following blunt chest trauma: indication for emergency surgery. Eur J Cardio-Thoracic Surg [Internet]. 1992;6(8):455–7. Available from: https://academic.oup.com/ejcts/article-lookup/doi/10.1016/1010-7940(92)90073-7
18. Kozłowski D, Dubaniewicz A, Koźluk E, Grzybiak M, Krupa W, Kołodziej P, et al. The morphological conditions of the permanent pacemaker lead extraction. Folia Morphol (Warsz). 2000;59(1):25–9.
19. Kaçar D, Barut Ç. Conus arteriosus: an anatomic and terminologic evaluation. Anatomy [Internet]. 2014;8:40–4. Available from: http://dergipark.gov.tr/doi/10.2399/ana.11.213
20. Crucean A, Spicer DE, Tretter JT, Mohun TJ, Anderson RH. Revisiting the anatomy of the right ventricle in the light of knowledge of its development. J Anat [Internet]. 2024;244(2):297–311. Available from: https://onlinelibrary.wiley.com/doi/10.1111/joa.13960
21. Anderson RH, Spicer DE, Hlavacek AJ, Hill A, Loukas M. Describing the Cardiac Components—Attitudinally Appropriate Nomenclature. J Cardiovasc Transl Res [Internet]. 2013;6(2):118–23. Available from: http://link.springer.com/10.1007/s12265-012-9434-z
22. Musso F, Rodrigues H, Anderle DV, Dalfior Junior L, Marim T. Morphology and blood supply of papillary muscle in the arterial conus. Braz j morphol sci [Internet]. 2000;137–40. Available from: https://pesquisa.bvsalud.org/portal/resource/pt/lil-313971
23. Yamauchi H, Vasilyev N V., Marx GR, Loyola H, Padala M, Yoganathan AP, et al. Right ventricular papillary muscle approximation as a novel technique of valve repair for functional tricuspid regurgitation in an ex vivo porcine model. J Thorac Cardiovasc Surg [Internet]. 2012;144(1):235–42. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022522312000542
24. Couetil J-P, Nappi F, Spadaccio C, Fiore A. Papillary muscle septalization for functional tricuspid regurgitation: Proof of concept and preliminary clinical experience. JTCVS Tech [Internet]. 2021;10:282–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S266625072100657X
25. Huwyler B. Zur Anatomie des Schweineherzens. Anat Anz. 1926;62:49–76.
26. Rouviere H. Anatomia Humana – Descriptiva Y Topografica. 8th ed. Madrit: Casa Editorial Bailly-Bailliere, SA; 1961.
27. Gheorghitescu (Janca) Ruxandra, Toba M, Iliescu DM, Bordei P. Morphological features of papillary muscles in the right ventricle. ARS Medica Tomitana [Internet]. 2016;22(3):135–44. Available from: https://www.sciendo.com/article/10.1515/arsm-2016-0023
28. Saha A, Roy S. Papillary muscles of left ventricle — Morphological variations and it's clinical relevance. Indian Heart J [Internet]. 2018;70(6):894–900. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0019483217305539
29. Sinha MB, Somkuwar SK, Kumar D, Sharma DK. Anatomical variations of papillary muscles in human cadaveric hearts of Chhattisgarh, India. Indian J Clin Anat Physiol [Internet]. 2021;7(4):374–80. Available from: https://ijcap.org/article-details/13034
30. Farzana T, Khalil M, Mannan S, Sultana J, Sumi MS, Sultana R. Length of papillary muscles in both ventricles of different age group on Bangladeshi cadaver. Mymensingh Med J [Internet]. 2015;24(1):52–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25725668
31. Bhadoria P, Bisht K, Singh B, Tiwari V. Cadaveric Study on the Morphology and Morphometry of Heart Papillary Muscles. Cureus [Internet]. 2022; Available from: https://www.cureus.com/articles/83366-cadaveric-study-on-the-morphology-and-morphometry-of-heart-papillary-muscles
32. Varghese G, Setiya M. Morphological and Morphometric Study of Papillary Muscles in Adult Cadaveric Hearts: A Crosssectional Study from Central India. J Clin DIAGNOSTIC Res [Internet]. 2025; Available from: https://www.jcdr.net/article_fulltext.asp?issn=0973-709x&year=2025&month=July&volume=19&issue=7&page=AC01-AC04&id=21242

33. Testut L, Latarjet A. Traité d'anatomie humaine: Angéiologie. Gaston Doin; 1929.
34. Israel CW, Tribunyan S, Yen Ho S, Cabrera JA. Anatomy for right ventricular lead implantation. Herzschrittmachertherapie + Elektrophysiologie [Internet]. 2022;33(3):319–26. Available from: https://link.springer.com/10.1007/s00399-022-00872-w
35. Bargman W, Doerr W. Das Herz des Menschen. Stuttgart: G. Thieme; 1963.
36. Netter FH. Atlas of Human Anatomy. 7th ed. Elsevier Health Sciences; 2019.
37. Schummer A, Nickel R, Seiferle E. Lehrbuch der Anatomie der Haustiere. 3rd ed. Berlin: Parey; 1976.
38. Aktas EO, Govsa F, Kocak A, Boydak B, Yavuz IC. Variations in the papillary muscles of normal tricuspid valve and their clinical relevance in medicolegal autopsies. Saudi Med J [Internet]. 2004;25(9):1176–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15448762
39. Mariani MV, Piro A, Magnocavallo M, Chimenti C, Della Rocca D, Santangeli P, et al. Catheter ablation for papillary muscle arrhythmias: A systematic review. Pacing Clin Electrophysiol [Internet]. 2022;45(4):519–31. Available from: https://onlinelibrary.wiley.com/doi/10.1111/pace.14462
40. Kabasawa M, Kohno H, Ishizaka T, Ishida K, Funabashi N, Kataoka A, et al. Assessment of functional tricuspid regurgitation using 320-detector-row multislice computed tomography: Risk factor analysis for recurrent regurgitation after tricuspid annuloplasty. J Thorac Cardiovasc Surg [Internet]. 2014;147(1):312–20. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022522312013943
41. Nigri GR, Di Dio LJA, Baptista CAC. Papillary muscles and tendinous cords of the right ventricle of the human heart morphological characteristics. Surg Radiol Anat [Internet]. 2001;23(1):45–9. Available from: http://link.springer.com/10.1007/s00276-001-0045-7
42. Wysoczański J, Zaborowski G, Anczyk A, Handzel K, Karaś R, Lepich T, et al. Anatomical and clinical aspects of the papillary muscles. Med Stud [Internet]. 2024;40(3):279–88. Available from: https://www.termedia.pl/doi/10.5114/ms.2024.142945

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META-ANALYSIS

Meta-analysis of cytotoxic T lymphocyte antigen-4 (CTLA-4) genetic polymorphisms and Graves' disease

Iyshwarya Bhaskar Kalarani, Ramakrishnan Veerabathiran

Human Cytogenetics and Genomics Laboratory, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Chennai, India

Abstract

Background: Graves' disease (GD) is an autoimmune disorder specific to the thyroid, characterized by a complex etiology influenced by both genetic and environmental factors. The CTLA-4 gene, known for its involvement in immune regulation, has been scrutinized regarding its potential contribution to GD susceptibility, particularly concerning the rs3087243 polymorphism. We conducted a meta-analysis to explore the association between this genetic variation and the likelihood of GD development. **Methods:** We thoroughly searched the PubMed, Medline, and EMBASE databases, as well as the reference lists of pertinent articles published up to 2024. Studies examining the association between GD and the CTLA-4 CT60 polymorphism were selected for inclusion. Data extraction and statistical analyses were conducted using Review Manager 5.4 software, which assessed multiple genetic models and the publication bias. **Results:** Six studies encompassing 1904 controls and 926 GD cases met the inclusion criteria. Our meta-analysis uncovered a substantial correlation between the CTLA-4 (rs3087243) polymorphism and GD across multiple genetic models (allele, homozygous, dominant, and recessive), suggesting the potential role of this genetic variant in predisposing individuals to GD. An examination of publication bias revealed symmetrical funnel plots. **Conclusion:** Our meta-analysis provides evidence for a significant association between GD and the CTLA-4 (rs3087243) polymorphism, highlighting its potential contribution to GD susceptibility. Additional investigation utilizing larger sample sizes and diverse populations is essential to corroborate these findings and clarify the exact mechanisms governing the association between the CTLA-4 gene and GD.

Keywords: autoimmune thyroid disease · Graves' disease · polymorphism · rs3087243 · heterogeneity

Citation

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Corresponding author:
Ramakrishnan Veerabathiran, Human Cytogenetics and Genomics Laboratory, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Chennai, India
e-mail: rkgenes@gmail.com
Available online: ejtcm.gumed.edu.pl

Introduction

Autoimmune diseases (ADs) encompass more than 100 conditions distinguished by the disrupted regulation of inflammatory mechanisms targeting one or more autoantigens [1]. Autoimmune thyroid disease (AITD) is the most prevalent form of organ-specific AD [2]. AITDs, which impact over 5% of the population, are the most common autoimmune diseases. These include Graves' disease (GD) and Hashimoto's thyroiditis (HT). Additionally, abnormal thyroid function varies across populations, from 1-2% in males to 7-9% in females [3]. The development of autoantibodies and inflammation result in hypothyroidism. On the other hand, GD is distinguished by the generation of antibodies against the thyroid-stimulating hormone receptor (TSHR), which leads to overstimulation and excessive release of thyroid hormone by thyrocytes. Thyroid-specific genes are the genetic components implicated in etiopathogenesis [4].

GD impacts approximately 0.5% of the global population, yet its occurrence has not been explicitly documented within the Indian population [5]. While environmental factors (e.g. infection, stress) play a significant role in triggering GD in susceptible individuals, research involving twins has indicated that genetic factors are the primary influencers, responsible for approximately 80% of the predisposition to GD [6]. Additionally, both linkage and association studies have identified the involvement of genes related to thyroid function and genes responsible for immune regulation in GD pathogenesis [7]. The immune-regulatory genes associated with GD development are also linked to other autoimmune disorders. These genes include CTLA-4, HLA-DR, PTPN22, CD40, thyroglobulin, and the TSH receptor [8]. A co-stimulatory molecule that promotes peripheral self-tolerance and inhibits T-cell activation is encoded by the CTLA-4 gene, which is found on chromosome 2q33. Autoimmune disorders have been associated with modifications in CTLA-4, including the functional polymorphism CT60 (rs30807243). The G allele of CT60 is associated with the reduced expression of soluble CTLA-4 (sCTLA-4) compared to the A allele [9]. When CTLA-4 is dysregulated or genetically altered, it affects the T cell's capacity to inhibit immunological activation, which can result in autoimmunity, including GD [10]. The aim of this study was to evaluate the correlation between the +6230 G/A (rs3087243) single nucleotide polymorphisms (SNPs) and GD using the meta-analysis methodology.

Material and methods

A comprehensive literature search was conducted to identify relevant studies for inclusion in the meta-analysis. To minimize bias and ensure the thoroughness of the review, both authors independently screened articles based on pre-established inclusion and exclusion criteria. Any discrepancies between reviewers were resolved through discussion. Data from the selected studies were extracted in duplicate, with any inconsistencies addressed in the same manner, further ensuring the accuracy and reliability of the results.

Literature search

We searched the literature published up to 2024 using PubMed, Medline and EMBASE. The search terms "Graves' disease", "GD", and "cytotoxic T lymphocyte-associated antigen 4", "CTLA-4", combined with "polymorphism" were used. All research investigating the relationship between GD and the CTLA-4 (rs3087243) polymorphism was carefully chosen and thoroughly examined. In the interim, additional articles were identified by reviewing the references of the studies.

Inclusion and exclusion criteria

All studies that were published in English were taken into account for this meta-analysis and had to fulfill the subsequent inclusion requirements: case-control studies, genotype distribution or allelic frequency data were available for comparison, sufficient data were available to calculate an OR along with a 95% CI. The following were among the exclusion criteria: studies with overlapping data, studies where family members were examined (linkage considerations were the basis for the analysis) and studies where the control population's genotype distribution was outside the Hardy-Weinberg equilibrium (HWE). A PRISMA flowchart illustrating the inclusion and exclusion of studies is provided in Figure 1.

Data extraction

The data collected from each study include information such as the title, the primary author, the year of publication, the place of origin, the study design and research methods, the genotyping strategy used and the specific goals of the study. We did not address or account for potential discrepancies or conflicting results among the articles included in the analysis.

Quality assessment

The Newcastle-Ottawa Scale (NOS) was employed to assess the quality of case-control studies [11]. Using this tool we evaluated each study's comparability, outcomes, and methodological integrity, including sampling techniques, response rates, and representativeness. Studies scoring 7 out of 10 were considered suitable, a threshold determined after reviewing relevant literature-based meta-analyses.

Statistical analysis

We used the Review Manager 5.4 software (The Cochrane Collaboration, London, UK) for data analysis, with the statistical significance set at $p < 0.05$ for all genetic variants. Essential tools and procedures were utilized to conduct comprehensive genetic association meta-analyses, assess the clinical relevance of genetic variations, ensure significance testing in large-scale genetic studies, and maintain statistical power. The heterogeneity assumption from previous research was evaluated using the Chi-square-based Q statistic test and the I2 metric value. Significant data were identified with a p-value of less than 0.1. The preceding research used the random effect model to assess the odds ratio, accompanied by a 95% CI. An analysis of the HWE was conducted using the Chi-square test [12]. A forest plot, incorporating 95% confidence intervals and the overall odds ratio, was generated to evaluate the strength of the association between gene polymorphism and autoimmune thyroid disease. Publication bias in the meta-analysis was assessed through a funnel plot.

Power analysis

Using the GPower 3.1 tool (The G*Power Team, Heinrich-Heine-Universität Düsseldorf, Germany), we computed the statistical power under a 95% confidence interval with an α error probability set at 0.05 [13]. For each selected gene, the power of the case and control study sample sizes was combined and analyzed separately.

CTLA-4 gene interaction network analysis

To better understand the role of CTLA-4 and its potential functional partners in GD, we used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) online server (STRING Consortium, http://string-db.org/) to build a network of CTLA-4 gene interactions [14].

Results

Of the 671 studies on the correlation between the collected data and GD, 6 specifically investigated the relationship with the CTLA-4 (rs3087243) polymorphism and contained data from 926 GD cases and 1904 controls. These articles were then examined to see which ones fulfilled the inclusion criteria for our study and had valuable data. Detailed information regarding each study we included is outlined in Table 1, along with the characteristics of the cases and controls regarding the correlation between GD and the CTLA-4 polymorphism. These 6 articles included patients from diverse ethnic groups [9, 15-19].

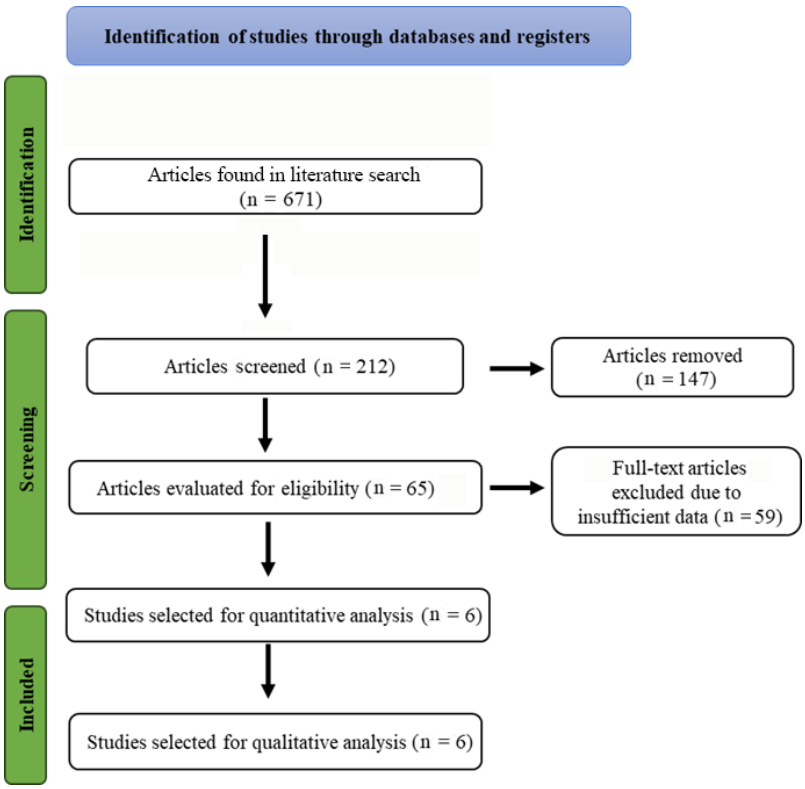

Figure 1. Study selection of the CTLA-4 gene polymorphism and Graves' disease

Publication bias and the analysis of quantitative data

We conducted 6 investigations to assess the relationship between AITD, particularly GD, and the CTLA-4 gene (rs3087243) polymorphism. Our research revealed a strong correlation between GD risk and CTLA-4 polymorphism in various genetic models. The allele model (A vs. G) exhibits an I2 of 65%, an OR of 1.96, a 95% CI of 1.53--2.50, and a p-value < 0.00001. In the homozygous model (AA vs. GG), the I2 is 56%, presenting an OR of 4.48, with a 95% CI of 2.57-7.82 and a p-value of 0.0001. The dominant model (AA + AG vs. GG) showcases an OR of 2.83, a 95% CI of 1.82-4.40, and a p-value of 0.0001. Meanwhile, the recessive model (AA vs. AG+GG) displays an

I2 of 68%, an OR of 2.29, a 95% CI of 1.57-3.34, and a p-value of less than 0.0001 under the random effects model. Fixed effects were preferred when the I2 was 39% in the heterozygote model (AG vs. GG), yielding an OR of 0.58, with a 95% CI of 0.48-0.70 and a p-value of less than 0.0001 (Figure 2). We employed a funnel plot (Figure 3) and Egger's regression test in our investigation to assess publication bias. The funnel plot suggests that the dataset may contain small-study effects or publication bias. The majority of research indicates a positive correlation between GD and CTLA-4 polymorphisms. We used the 3DSNP tool to provide additional information on the relationship between chromosomes and SNPs [20]. As a result, we created Circos plots, which offer an intricate and precise representation of the whole genomic dataset displayed in Figure 4. The genomic landscape of the CTLA-4 gene area on chromosome 2q33 is highlighted by this Circos plot (Figure 4), which displays important SNPs (like rs3087243), gene-gene closeness (like CD28, ICOS), and potential linkage disequilibrium or functional connections. With internal arcs indicating strong genetic interactions that may affect CTLA-4 expression and function, important to autoimmune disorders like GD disease, the clustered red SNPs indicate locations of high polymorphism.

Power analysis

We conducted a power analysis to determine the significance level for each selected SNP. Our results indicated that the sample sizes from the selected literature were sufficient to meet this stringent significance level, ensuring the robustness and reliability of our findings. This comprehensive approach confirms that our meta-analysis can detect significant associations for the SNPs under investigation, as shown in Figure 5.

Gene-gene network and interactions

Our STRING online server analysis indicated that CTLA-4 interacts with several genes. The 10 most significant genes from the network of gene-gene interactions are shown in Figure 6. According to these results, CTLA-4 exhibits high-confidence functional connections with other important immune regulators, such as PD-1, PD-L1, CD28, ICOS, and galectin-9 (LGALS9). Because of these connections, CTLA-4 is positioned as a key participant in immune homeostasis maintenance, and as a crucial target in the modulation of autoimmune diseases.

Discussion

This meta-analysis, which comprised 1904 controls and 926 GD patients, investigated the relationship between the CTLA-4 (rs3087243) polymorphism and GD. Egger's test results offered statistical support for the symmetry of the funnel plot. According to the overall findings, all genetic models showed a strong ($p < 0.01$) association between variant genotypes and GD risk. Although GD has severe clinical significance, its pathomechanism is still unclear. However, stress and infection play a significant role in the susceptibility of specific individuals to GD. SNPs help to analyze genetic changes and the likelihood of developing certain diseases. GD is a thyroid-specific

Table 1. Characteristics of studies included in the meta-analysis

Author and Year	Genotyping Frequency						Allele Frequency				Case	Control	NOS	Patient group	Chi-square X^2	HWE
	Case			Controls			Case		Controls							
	AA	AG	GG	AA	AG	GG	A	G	A	G						
Pawlak--Adamska et al. 2017 [15]	18	80	74	133	187	68	116	228	453	323	172	388	6	Poland	0.02	0.87
Shehjar et al. 2020 [9]	37	70	28	73	69	8	144	126	215	85	135	150	7	Kashmir	2.64	0.1
Hasan et al. 2022 [16]	10	9	11	8	14	8	29	31	30	30	30	30	6	Iraq	0.13	0.71
Fouad et al. 2017 [17]	4	15	21	11	10	9	23	57	32	28	40	30	7	Saudi Arabia	3.27	0.7
Ting et al. 2016 [18]	3	81	205	46	382	630	87	491	474	1642	289	1058	8	Chinese	1.57	0.2
Chen et al. 2018 [19]	20	100	140	43	101	104	140	380	187	309	260	248	7	Han Chinese	4.38	0.03

NOS – Newcastle-Ottawa Scale; HWE – Hardy-Weinberg Equilibrium

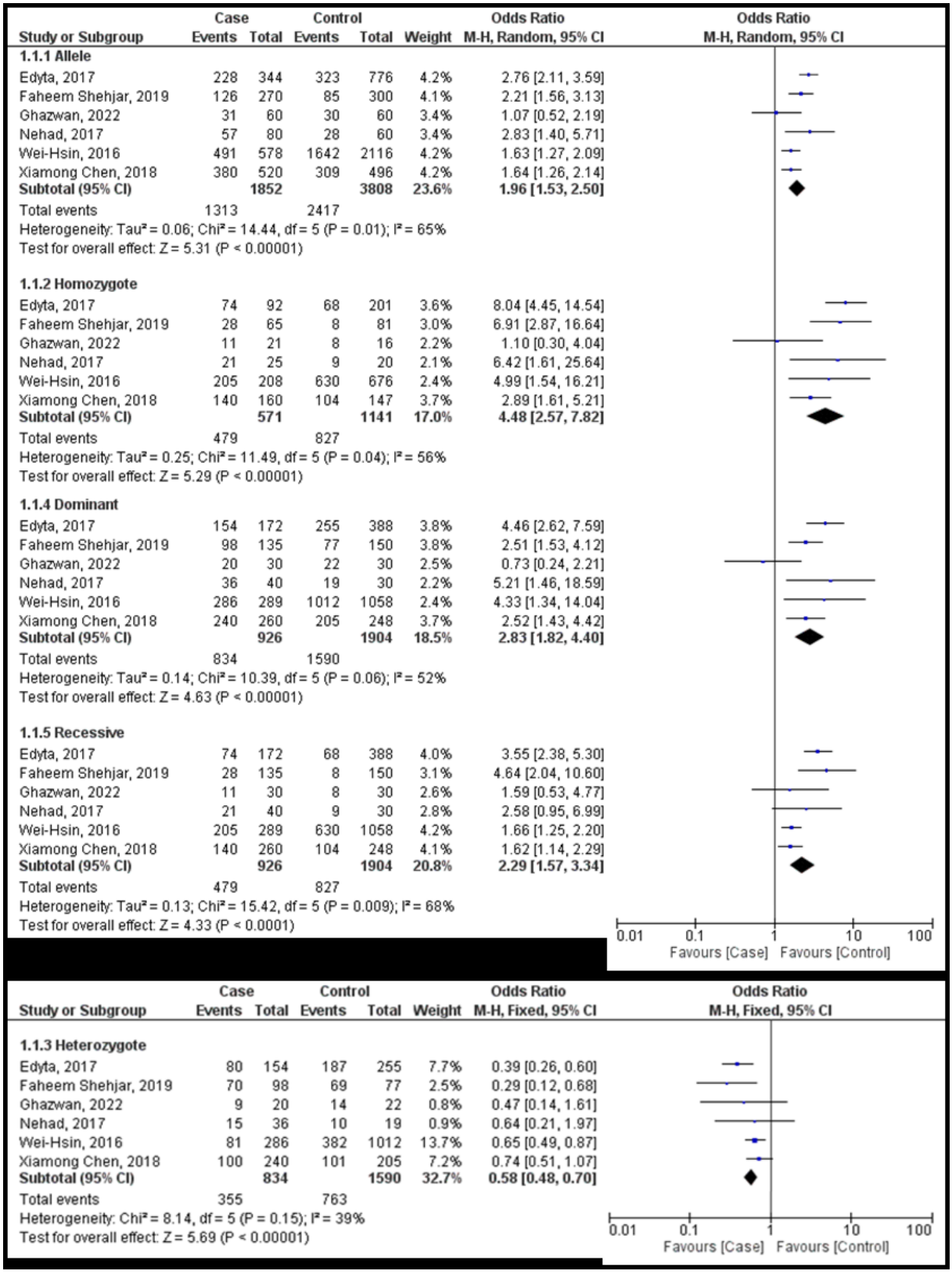

Figure 2. Forest plot showing the association between the CTLA-4 gene polymorphism and Graves' disease in all 5 models

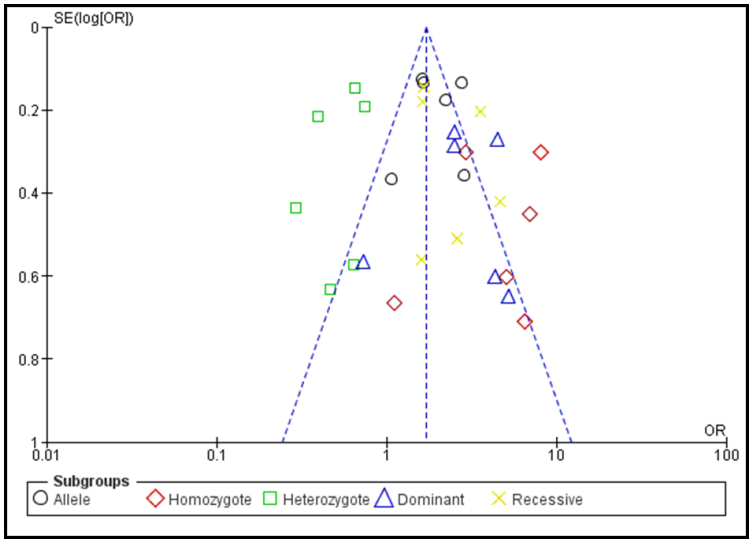

Figure 3. Publication bias in the association between the CTLA-4 gene polymorphism and Graves' disease in all models

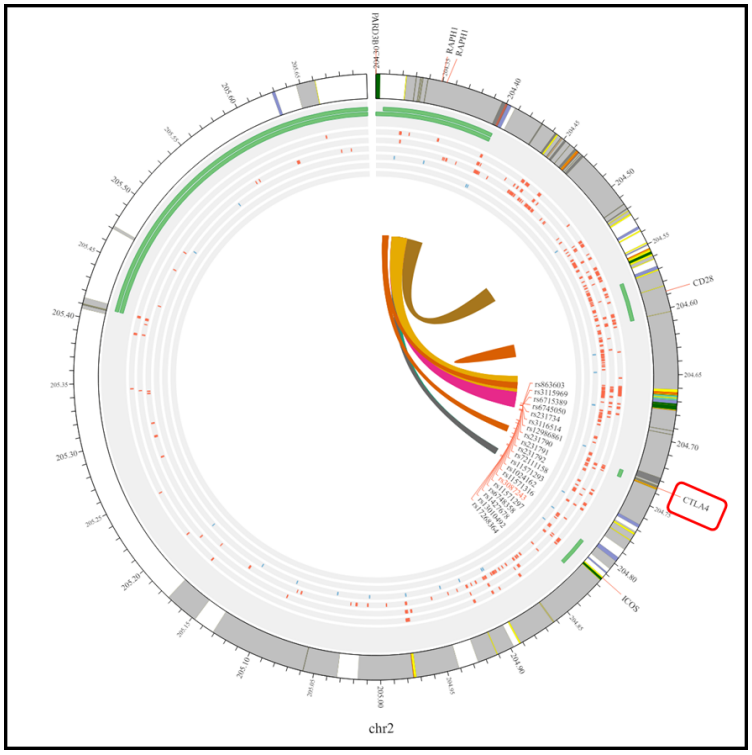

Figure 4. Circos plot illustrating the chromosomal interactions among rs3087243 polymorphisms

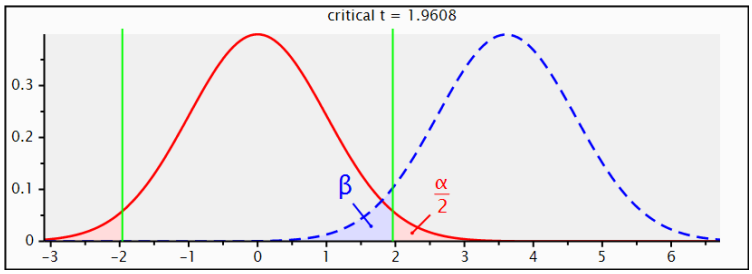

Figure 5. Power analysis result of the CTLA-4 gene

autoimmune disease mediated by antibodies [21]. The CTLA-4 protein is susceptible to autoimmunity and can send an inhibitory signal to T-cells [22]. Si et al. identified the CTLA-4 protein as the gatekeeper of conjugation timing; decreased conjugation may offer protection against extended durations of time that cytotoxic T-cells spend close to autoantigen-defined targets [22]. That finding has received much attention because of its significant contribution to autoimmunity [22]. Our meta-analysis showed a strong ($p < 0.01$) correlation between the CTLA-4 polymorphism and GD. In addition, we report that there may be a relationship between the risk of GD and the CTLA-4 (rs3087243) polymorphism. In conclusion, our meta-analysis demonstrates a strong correlation between increased susceptibility to GD illness and the CTLA-4 (rs3087243) polymorphism, specifically the G allele. These findings emphasize the genetic role of CTLA-4 in GD and the necessity for further investigation into its functional significance and potential as a therapeutic target or biomarker.

While our study provides valuable insights, it has several limitations that need to be considered. First, our sample size was relatively small, which may limit the generalizability of the findings and reduce the statistical power, particularly in subgroup analyses. Additionally, the potential for population stratification could have influenced the results, as genetic variations may differ across populations. Although the funnel plots appeared symmetrical (suggesting no significant publication bias), we acknowledge that this visual inspection method has limitations and a more formal test for publication bias could provide further clarity. Furthermore, due to the small number of studies included, we were unable to perform a meta-regression or a more in-depth exploration of certain sources of heterogeneity. Given these limitations, further research with larger sample sizes and more diverse populations is needed to confirm and expand upon the observed associations between the CTLA-4 gene and GD.

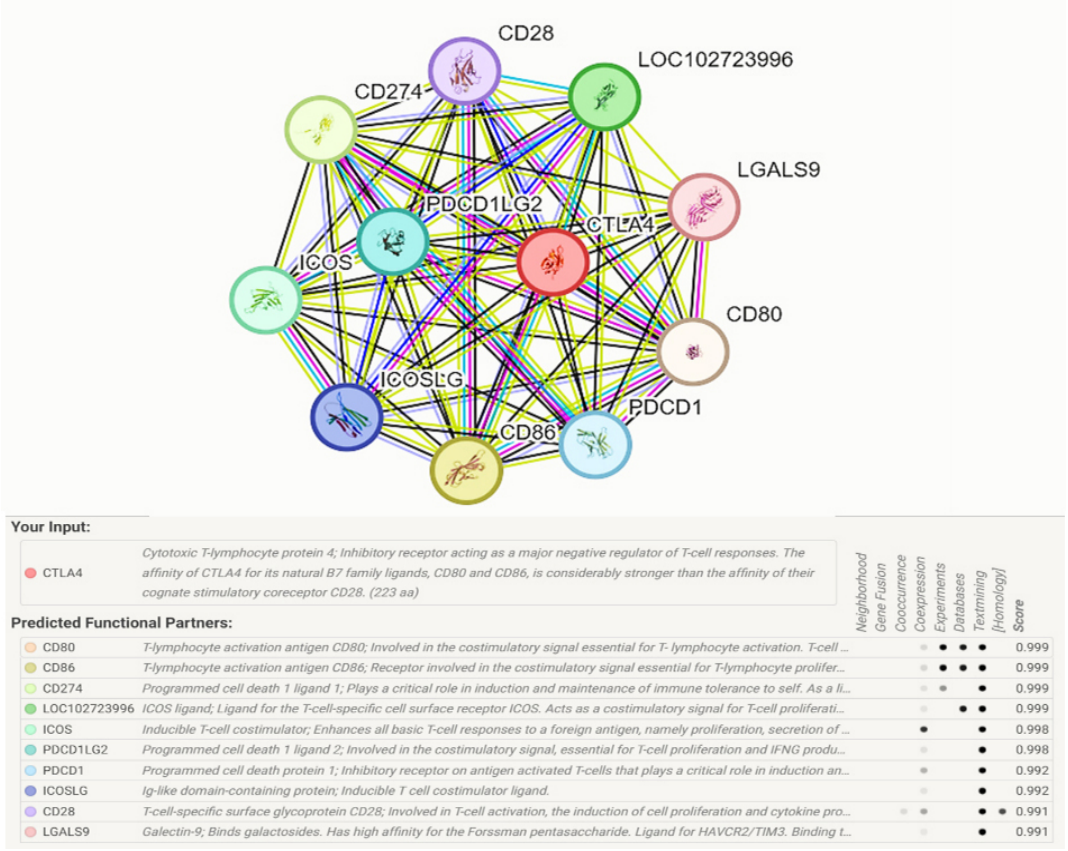

Figure 6. Human CTLA-4 interaction network with other genes obtained from the STRING server

Conclusion

Our meta-analysis investigated the relationship between GD risk and the CTLA-4 (rs3087243) polymorphism, involving 1904 controls and 926 GD patients across six studies. In several genetic models, our research revealed a strong correlation between the CTLA-4 polymorphism and GD, indicating that this genetic variation may play a role in predisposing people to GD. The CTLA-4 gene, critical in regulating T-cell activity, has garnered attention for its involvement in autoimmune diseases, including GD. Despite the strengths of our analysis (e.g. comprehensive literature search and robust statistical methods), it has limitations, including the small sample sizes in some of the included studies. Well-designed research is warranted to elucidate the precise relationship between the CTLA-4 gene and GD susceptibility.

Conflict of interest

None.

Funding

None.

References

1. Bieber K, Hundt JE, Yu X, Ehlers M, Petersen F, Karsten CM, et al. Autoimmune pre-disease. Autoimmun Rev [Internet]. 2023;22(2):103236. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1568997222002063
2. McLeod DSA, Cooper DS. The incidence and prevalence of thyroid autoimmunity. Endocrine [Internet]. 2012;42(2):252–65. Available from: http://link.springer.com/10.1007/s12020-012-9703-2
3. Miteva MZ, Nonchev BI, Orbetzova MM, Stoencheva SD. Vitamin D and Autoimmune Thyroid Diseases - a Review. Folia Med (Plovdiv) [Internet]. 2020;62(2):223–9. Available from: https://foliamedica.bg/article/47794/
4. Ramgopal S, Rathika C, Padma MR, Murali V, Arun K, Kamaludeen MN, et al. Interaction of HLA-DRB1* alleles and CTLA4 (+ 49 AG) gene polymorphism in Autoimmune Thyroid Disease. Gene [Internet]. 2018;642:430–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378111917310223

5. Weetman AP. Graves' Disease. N Engl J Med [Internet]. 2000;343(17):1236–48. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM200010263431707
6. Tomer Y. Mechanisms of Autoimmune Thyroid Diseases: From Genetics to Epigenetics. Annu Rev Pathol Mech Dis [Internet]. 2014;9(1):147–56. Available from: https://www.annualreviews.org/doi/10.1146/annurev-pathol-012513-104713
7. Eschler DC, Hasham A, Tomer Y. Cutting Edge: The Etiology of Autoimmune Thyroid Diseases. Clin Rev Allergy Immunol [Internet]. 2011;41(2):190–7. Available from: http://link.springer.com/10.1007/s12016-010-8245-8
8. Shehjar F, Afroze D, Misgar RA, Malik SA, Laway BA. Association of polymorphic variants of IL-1β and IL-1RN genes in the development of Graves' disease in Kashmiri population (North India). Hum Immunol [Internet]. 2018;79(4):228–32. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0198885918300442
9. Shehjar F, Dil-Afroze, Misgar RA, Malik SA, Laway BA. A significant association of the CTLA4 gene variants with the risk of autoimmune Graves' disease in ethnic Kashmiri population. Cell Immunol [Internet]. 2020;347:103995. Available from: https://linkinghub.elsevier.com/retrieve/pii/S000887491930293X
10. Simone R, Tenca C, Fais F, Luciani M, De Rossi G, Pesce G, et al. A Soluble Form of CTLA-4 Is Present in Paediatric Patients with Acute Lymphoblastic Leukaemia and Correlates with CD1d+ Expression. Moormann AM, editor. PLoS One [Internet]. 2012;7(9):e44654. Available from: https://dx.plos.org/10.1371/journal.pone.0044654
11. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses [Internet]. The Ottawa Hospital. Research Institute. Department of Epidemiology and Community Medicine, University of Ottawa; 2000 [cited 2025 Aug 1]. Available from: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
12. Rodriguez S, Gaunt TR, Day INM. Hardy-Weinberg Equilibrium Testing of Biological Ascertainment for Mendelian Randomization Studies. Am J Epidemiol [Internet]. 2009;169(4):505–14. Available from: https://academic.oup.com/aje/article-lookup/doi/10.1093/aje/kwn359
13. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav Res Methods [Internet]. 2009;41(4):1149–60. Available from: http://link.springer.com/10.3758/BRM.41.4.1149
14. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res [Internet]. 2023;51(D1):D638–46. Available from: https://academic.oup.com/nar/article/51/D1/D638/6825349
15. Pawlak-Adamska E, Frydecka I, Bolanowski M, Tomkiewicz A, Jonkisz A, Karabon L, et al. CD28/CTLA-4/ICOS haplotypes confers susceptibility to Graves' disease and modulates clinical phenotype of disease. Endocrine [Internet]. 2017;55(1):186–99. Available from: http://link.springer.com/10.1007/s12020-016-1096-1
16. Hasan GA, Altamemi IA. CTLA-4 Polymorphism along with Proinflammatory Cytokines in Autoimmune Thyroiditis Disease. Wiadomości Lek [Internet]. 2022;75(3):577–83. Available from: https://wiadlek.pl/wp-content/uploads/archive/2022/03/WLek202203103.pdf
17. Fouad NA, Saeed AM, Mahedy AW. Association of CTLA-4+ 49 A/G and CT60 gene polymorphism with Graves' disease. Egypt J Immunol [Internet]. 2017;24(2):63–70. Available from: https://www.bu.edu.eg/portal/uploads/Medicine/INTERNAL MEDICINE/297/publications/Ahmed Wageh Mahdey _3.pdf
18. Ting W-H, Chien M-N, Lo F-S, Wang C-H, Huang C-Y, Lin C-L, et al. Association of Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4) Gene Polymorphisms with Autoimmune Thyroid Disease in Children and Adults: Case-Control Study. Pietropaolo M, editor. PLoS One [Internet]. 2016;11(4):e0154394. Available from: https://dx.plos.org/10.1371/journal.pone.0154394
19. Chen X, Hu Z, Liu M, Li H, Liang C, Li W, et al. Correlation between CTLA-4 and CD40 gene polymorphisms and their interaction in graves' disease in a Chinese Han population. BMC Med Genet [Internet]. 2018;19(1):171. Available from: https://bmcmedgenet.biomedcentral.com/articles/10.1186/s12881-018-0665-y
20. Lu Y, Quan C, Chen H, Bo X, Zhang C. 3DSNP: a database for linking human noncoding SNPs to their three-dimensional interacting genes. Nucleic Acids Res [Internet]. 2017;45(D1):D643–9. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkw1022
21. Pastuszak-Lewandoska D, Sewerynek E, Domańska D, Gładyś A, Skrzypczak R, Brzeziańska E. CTLA-4 gene polymorphisms and their influence on predisposition to autoimmune thyroid diseases (Graves' disease and Hashimoto's thyroiditis). Arch Med Sci [Internet]. 2012;3:415–21. Available from: http://www.termedia.pl/doi/10.5114/aoms.2012.28593
22. Si X, Zhang X, Tang W, Luo Y. Association between the CTLA-4 +49A/G polymorphism and Graves' disease: A meta-analysis. Exp Ther Med [Internet]. 2012;4(3):538–44. Available from: https://www.spandidos-publications.com/10.3892/etm.2012.618

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REVIEW ARTICLE

Challenges in orthodontic treatment of patients after childhood cancer disease – a literature review

Joanna Maciejewska[1], **Bogna Racka-Pilszak**[2]

[1] Orthodontics Clinic, University Dental Center, Medical University of Gdańsk, Poland
[2] Division of Orthodontics, Medical University of Gdańsk, Poland

Abstract

Nowadays, cancer in children is increasingly common. Thanks to effective treatment, survival rates are continually rising. However, the applied chemotherapy, radiotherapy, or combined treatment leaves a number of side effects. There are disturbances in growth, including within the bones of the craniofacial complex, as well as developmental anomalies in dentition. Among these, the most frequently observed are defects in the structure of tooth roots, tooth agenesis and microdontia. These disorders cause aesthetic and occlusal problems, therefore there is a need to modify the orthodontic treatment plan for patients after cancer therapy. The higher risk of caries in these patients (due to xerostomia and enamel hypoplasia) complicates or even makes it impossible to achieve the intended results of orthodontic treatment. We analysed the available literature in Scopus, PubMed and Google Scholar databases from the years 2010-2022 to understand the challenges orthodontists face when treating patients who experienced cancer in childhood.

Keywords: cancer · orthodontic treatment · carcinoma

Citation

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Corresponding author:
Joanna Maciejewska, Orthodontics Clinic, University Dental Center, Medical University of Gdańsk, Poland
email: joanna.maciejewska@gumed.edu.pl
Available online: ejtcm.gumed.edu.pl

Introduction

Nowadays, an increasing number of children are diagnosed with cancer, most commonly leukaemia, central nervous system (CNS) tumours and lymphomas [1-3]. Treatment includes radiotherapy, chemotherapy, surgical methods, bone marrow transplantation or their combinations [3-8]. Thanks to advances in medicine, the survival rate of patients with childhood cancers increased to about 80% [3-4, 6, 9], hence patients who underwent such treatment in childhood are increasingly met in orthodontic clinics [1, 5, 7, 10-11]. It is estimated that currently 1/900 young adults have successfully undergone oncological treatment in childhood [1, 3]. Oncological therapies cause a series of adverse effects and orthodontists must be aware of the impact of the therapeutic procedures applied on the craniofacial complex and the oral cavity tissues, including the bite and dentition. Orthodontic treatment of these patients presents real challenges not only for the patient and the orthodontist, but also the patient's family [11-12]. Neill et al. demonstrated that 85% of orthodontists did not acquire knowledge on treating children post-cancer treatment during their specialty training and such cases are usually handled by older, more experienced orthodontists [6]. Therefore, regardless of work experience it is crucial for every orthodontist to continually update their knowledge to competently treat such patients [12]. The purpose of this review is to discuss the challenges faced by orthodontists working with patients who have undergone oncological treatment in childhood, with particular emphasis on the limitations of possible orthodontic procedures.

Material and methods

Electronic databases PubMed, Scopus and Google Scholar were searched using the keywords "cancer", "carcinoma", "orthodontic treatment", yielding 17 (Scopus), 182 (PubMed) and 15900 (Google Scholar) results (see Figure 1). The search results were limited to English and Polish language only, which resulted in 15 (Scopus), 179 (PubMed) and 11700 (Google Scholar) items. The results were further narrowed down to publication years 2010-2022, which reduced the number of records to 13 (Scopus), 162 (PubMed) and 11400 (Google Scholar). Articles on adult patients, epidemiology and duplicates were excluded, and a total of 26 articles were selected. Their content was analysed and finally 21 articles that matched the topic and contained valuable information were included in the review (see Table 1).

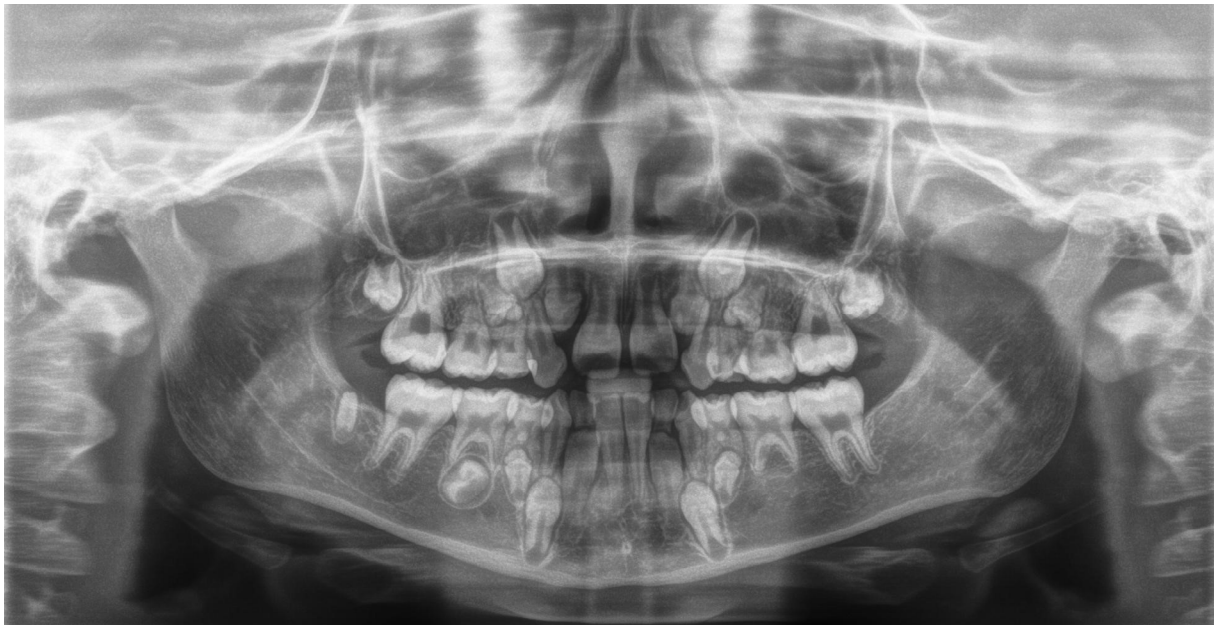

Figure 1. Panoramic radiograph of a 9-year-old girl treated for acute lymphoblastic leukaemia from the age of 15 months with subsequent 2 years of radiotherapy, chemotherapy, immunotherapy and antibiotic therapy, along with allogenic hematopoietic stem cell transplantation and two administrations of mesenchymal stem cells. Visible root shortening of all permanent first molar teeth, V-shaped roots of teeth 16 and 26, narrow roots of teeth 22, 36 and 46, absence of tooth buds 15, 25, 35 and 37, residual bud of tooth 47, microdontic buds of teeth 17, 14, 24, 27, 34 and 44 with disturbed mineralization (irregular crown outlines).

Table 1. List of included articles

	References	Year of publish	Country
1.	Mituś-Kenig et al. [4]	2015	Poland
2.	Boyer et al. [12]	2017	France
3.	Mituś-Kenig et al. [1]	2020	Poland
4.	Michalak et al. [5]	2019	Poland
5.	Radej et al. [2]	2013	Poland
6.	Mishra [13]	2017	India
7.	Neill et al. [6]	2015	USA
8.	Deshpande et al. [7]	2020	India, USA
9.	Radej et al. [10]	2013	Poland
10.	Ritwik [9]	2018	USA
11.	Hassan et al. [20]	2020	India
12.	Ritwik et al. [16]	2020	USA
13.	Dental Management of Pediatric Patients [19]	2018	USA
14.	Krasuska-Sławińska et al. [17]	2016	Poland
15.	Mituś-Kenig et al. [8]	2021	Poland
16.	Mituś-Kenig et al. [3]	2020	Poland
17.	Hernandez et al. [21]	2019	France
18.	Kim et al. [31]	2019	Korea
19.	Nemeth et al. [25]	2013	Hungary
20.	Carrillo et al. [11]	2014	Brazil
21.	Halperson et al. [15]	2022	Israel

Results and discussion

The reviewed articles did not include any statistical data, therefore analysis of factors such as sample size variations, potential biases or effect sizes was not possible. For this reason we were able to conduct a narrative review only. Articles included in this study were rated according to the Scale for the Assessment of Narrative Review Articles (SANRA) (Table 2) [13].

Adverse effects

The adverse effects of oncological therapies conducted in childhood are caused by the cancer itself, the applied treatment (including immunosuppressive therapy), the supportive care or their combinations [5-6]. The severity and extent of adverse effects depend on the patient's age (and thus their stage of development), psychological state, tumour-related factors (location, stage and extent at the time of detection), treatment (type, intensity and duration), as well as genetically conditioned sensitivity [4, 10, 14]. Systemic complications can be immediate or distant and influence the overall growth and development of children, including their hormonal, cardiovascular, respiratory, nervous, skeletal and reproductive systems [4, 15]. The development of the cranium, cervical vertebrae and the entire oral cavity (including teeth and jaws) is also altered [6, 9].

Overall, the younger the patient then the risk of adverse effects within the craniofacial bones is greater, particularly in children treated for cancer before the age of 5 [1, 8, 10]. This increases the risk of altered odontogenesis, which is also affected by exposure to higher doses of chemotherapeutic agents and radiation [1, 11]. Greater susceptibility to adverse effects were found in females [10] and during puberty [5, 10]. Childhood cancers usually respond well to chemotherapy due to the rapid growth of tumour tissue, however these drugs are not selective and also destroy healthy cells [12, 16]. Additionally, multi-drug chemotherapy ± radiotherapy complicate the assessment of the individual agent's influence on the dental pulp and stages of odontogenesis [8, 14-15, 17]. Radiotherapy to the (CNS) results in a reduction in growth hormone and TSH secretion, leading to pituitary and thyroid function disorders [5, 10]. Roman et al. observed that chemotherapy was the only oncologic treatment method that disrupted children's growth and

caused growth hormone deficiency both during and after treatment [18]. Concurrent malnutrition during treatment and additional steroid therapy also impede a child's growth [7]. All of this leads to changes in the onset of puberty and growth delay in the patient. Reduced growth may also be due to early puberty and shortening the duration of the growth spurt [10].

The most significant consequence of radiotherapy is hypovascularization and cytotoxic effects on growth plate chondrocytes [11-12]. Reduced blood supply to the bones leads to osteoradionecrosis, which is rare in childhood [5]. Chemotherapy interferes with bone development, leading to decreased mineral density, which may persist throughout life [2, 10-11]. In addition, it damages the bone remodelling system with a predominance of osteoclast action contributes to increased bone resorption and pathological fractures [11-12]. Radiotherapy to the head and neck area (e.g. during treatment of CNS tumours) during childhood results in various deformities of the craniofacial region (e.g. reduced cranial base), bone and soft tissue hypoplasia (including maxillary hypoplasia) and facial asymmetry [10-11]. Significant reduction in the height of alveolar bone in the anterior and lateral segments has been observed after combined chemo-radiotherapy in children, as well as shortening of all linear measurements in cephalometric analysis [10]. Individuals who have undergone full-body irradiation before bone marrow transplantation are particularly susceptible to growth delay in the temporomandibular joints leading to disorders, e.g. the condylar processes assuming a pathological anterior position [10]. Trismus and temporomandibular joint pain may also occur, affecting nutrition and oral hygiene [7].

Table 2. Articles included in this study rated according to the Scale for the Assessment of Narrative Review Articles (SANRA); 0 – low standard; 2 – high standard.

	Author [Reference number]	Justification of the article's importance for the readership	Statement of concrete aims or formulation of questions	Description of the literature search	Referencing	Scientific reasoning	Appropriate presentation of data	Total
1.	Mituś-Kenig et al. [4]	2	2	2	2	2	1	11
2.	Boyer et al. [12]	2	1	1	1	1	2	8
3.	Mituś-Kenig et al. [1]	2	2	2	2	2	2	12
4.	Michalak et al. [5]	2	1	1	2	2	2	10
5.	Radej et al. [2]	2	2	0	2	2	2	10
6.	Mishra [11]	2	2	0	1	1	1	7
7.	Neill et al. [6]	2	2	2	2	2	2	12
8.	Deshpande et al. [7]	2	2	0	1	2	1	8
9.	Radej et al. [10]	2	2	2	2	2	1	11
10.	Ritwik [9]	2	2	1	2	1	2	10
11.	Hassan et al. [20]	2	0	0	2	1	1	6
12.	Ritwik et al. [16]	2	2	1	2	2	2	11
13.	Dental Management of Pediatric Patients [19]	2	2	1	2	2	2	11
14.	Krasuska-Sławińska et al. [17]	2	2	2	2	2	2	12
15.	Mituś-Kenig et al. [8]	2	2	2	2	2	2	12
16.	Mituś-Kenig et al. [3]	2	2	2	2	2	2	12
17.	Hernandez et al. [21]	2	1	0	1	1	2	7
18.	Kim et al. [31]	2	2	2	2	2	2	12
19.	Nemeth et al. [25]	2	2	2	2	2	2	12
20.	Carrillo et al. [14]	2	1	0	2	2	2	9
21.	Halperson et al. [15]	2	2	2	2	2	2	12

Cancer treatment damages epithelial cells, leading to the thinning of the mucous membrane oral of the oral cavity which becomes very sensitive to, even the slightest, leading to easy irritation, injury and inflammation, resulting in painful ulcers and erosions [8, 12]. Cancer therapy reduces the regenerative abilities of the mucous membrane shows and its adverse effects are exacerbated by the presence of dental caries, dental plaque and other irritating factors (e.g. dental fillings or orthodontic brackets) [12]. Infections within the oral cavity are more likely to occur [5, 12]. Salivary glands produce in lesser quantity and poorer quality saliva (increased density and viscosity), resulting in xerostomia which may persist after completion of therapy and impede chewing and speaking [7-9, 12, 16, 19]. All these factors affect the pH of saliva, dental plaque formation, the composition of the mucosal microflora and the willingness and quality of maintaining oral hygiene [8, 12]. The quantity of cariogenic bacteria (particularly Streptococcus mutans) increases along with susceptibility to periodontal diseases and opportunistic fungal, bacterial and viral infections [5, 7, 9, 16, 20]. The progression of these infections may be atypical due to accompanying neutropenia [9, 16, 20]. Acute oral complications typically arise 5-7 days after the start of chemotherapy, corresponding to changes in the blood count parameters [9].

Long-term adverse effects of cancer treatment also include abnormalities in dental development, manifested by changes in both crowns and roots [5]. Table 3 contains a summary of adverse effects of cancer treatment. Their degree can vary from mild to severe and there is a strong correlation between the dosage and type of cancer treatment, its duration, the patient's age, dental development stage and the frequency and severity of developmental tooth abnormalities [5, 9, 15-17, 19, 21]. The risk of disruptions during odontogenesis increases in children < 5 years of age and with increasing doses of radiation or chemotherapeutic agents [2, 6, 8]. The use of additional medications (e.g. antibiotics, immunosuppressants) also plays a role [21].

Combined radiochemotherapy or radiation targeted at the head and neck area appears to increase the risk of dental anomalies [15]. Developmental tooth defects result from the direct action of chemotherapeutics on odontoblasts, which also delays the development of Hertwig's epithelial root sheath, as well as indirectly through the influence of early chemotherapy complications such as vomiting and mucosal inflammation [17]. Amelogenesis and dentinogenesis may be disrupted during radiotherapy when the radiation beam is directed at the oral cavity or its immediate surroundings, but it has little impact on tooth formation when targeting distant body parts [5]. Radiotherapy affects cells during their mitotic division, disrupting enamel and dentin formation, whereas at very high doses it also damages non-proliferating cells [11].

The first signs of dental developmental disorders can be expected after 1-2 years of cancer treatment and they are visible on X-ray images [15] (Figure 2). Cancer treatment leads to changes in the shape, size of crowns and roots, degree of mineralization, enamel and dentin structure with frequent dental aplasia, therefore [1, 4, 6, 11, 15] hypodontia, microdontia, enamel hypoplasia and developmental root defects are typically observed [1, 4, 6, 11, 15]. Staining, discoloration and grooves on tooth crowns are often present [7, 10]. The tooth eruption process is also affected (often due to root development issues), leading to occlusal disturbances [2, 15]. Delays in primary tooth shedding and in permanent tooth replacement are frequent outcomes [3, 6-8].

All the above-mentioned adverse effects predispose to worsened aesthetics, function, dental misalignments and contribute to the development of malocclusions, which are mostly of skeletal aetiology [3, 6, 8-9, 15-16]. Crowding is observed, resulting from a lack of space in the dental arch as a consequence of maxillary hypoplasia [4, 10]. Malocclusions include crossbites, open bites, class II malocclusions and asymmetries [4, 7]. It's important to note that hypodontia also leads to malocclusion by inhibiting facial skeletal growth [7]. Presence of microdontic teeth and reduced numbers of tooth buds result in unwanted interdental spaces and tooth displacements, leading to changes in tooth alignment and malocclusion development [1, 4].

In general, the systemic adverse effects of oncologic treatment occur in approximately 50% of patients [1-2, 4]. Dahloff and Huggare found that 93% of patients experienced at least 1 adverse effect, with an average of 3.7 adverse effects per person [22]. On the other hand, Geenen et al. stated that it affects nearly 75% of individuals [23]. Ritwik et al. emphasized that up to 60% of children treated for cancer suffer from infertility, heart failure and secondary tumours in the future [16]. Patients described by Radej et al. experienced stunted growth and thyroid dysfunction as complications of their oncologic treatment [2]. As adverse effects are most common after radiotherapy [10], the current standards recommend minimizing its use in favour of chemotherapy and surgery [10, 12].

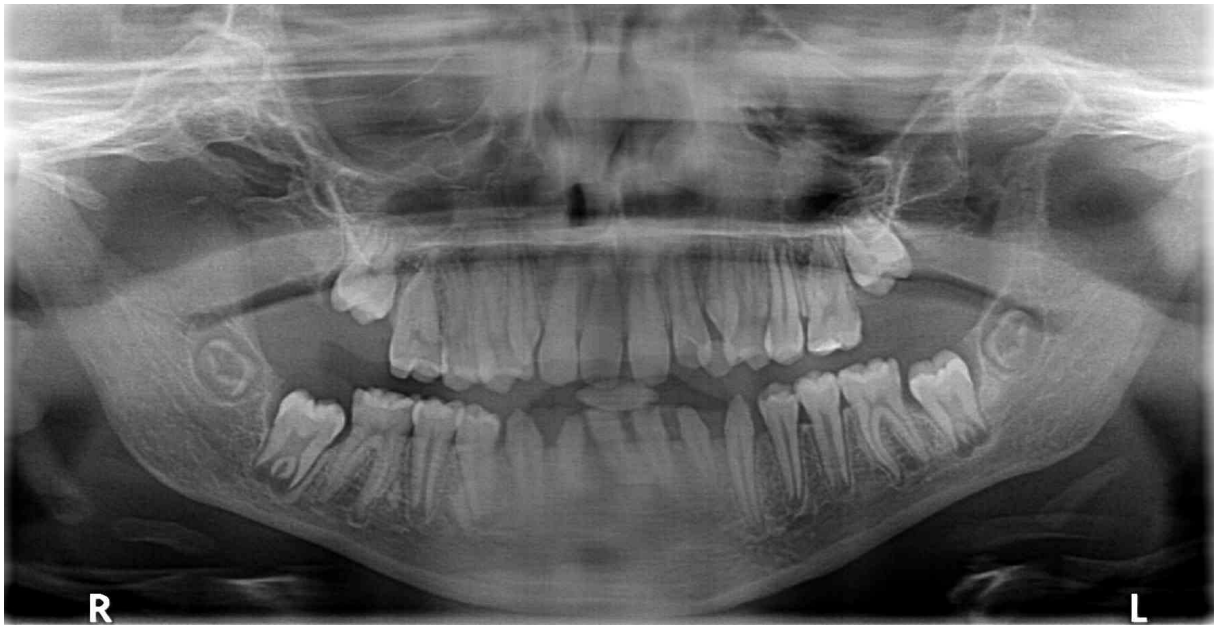

Figure 2. Panoramic radiograph of a 12-year-old girl treated for a malignant eye tumour with chemotherapy from 5 weeks of age to 6 months of age. Visible V-shaped roots of teeth 16 and 26, reduced conical crowns of teeth 32 and 42 and root shortening of the lower incisors.

Inflammation of oral mucosa (mucositis) after radiotherapy, chemotherapy and bone marrow transplantation affects up to 80%, 40% and 75% of paediatric patients, respectively [9]. It is noteworthy that mucositis is perceived by patients as the most painful complication of oncological treatment [16, 20].

Radiotherapy of the craniofacial structure leads to bone growth changes. It is noteworthy that the mandible was reported 4 times more sensitive to radiation compared to the maxilla [10]. There is a particular risk for the development of craniofacial disorders, (e.g. underdeveloped mandible), particularly with simultaneous chemo-radiotherapy. Sonis et al. noted greater retrognathism of the mandible in children irradiated under the age of 5 [24]. Radiotherapy in this age group often results in up to 14.72 times more frequent microdontia and root growth inhibition than in patients treated without it, although this risk decreases between the ages of 5-9 and above 10 [6]. Halperson et al. reported that tooth malformations were more common in patients treated at the age of 6 and younger (56%) compared to those treated between the ages of 6-12 (44%) [15]. The most significant growth occurs under the age of 5 and the adverse effects of chemo and radiotherapy are most apparent during adolescence [11]. Cephalometric measurements have shown a decrease exceeding 5% in the distance between sella-pogonion, sella-nasion and articulare-pogonion points. The most significant impairment in growth concerns the maxillary alveolar bone – its height decreased even by 50% [10].

Caries

Nemeth et al. address the issue of dental caries after cancer treatment. Researchers found that its prevalence is as high as 81.6%, which is 4.6% higher compared to healthy individuals [25]. Notably, post-radiation caries is extensive, has a rapid onset and aggressive progression [15]. The intensity of caries development can also be influenced by changes in oral microflora and a sweeter, claggy diet to compensate for feeding difficulties during cancer treatment [15, 25]. Treatment with chemotherapy only often results in fewer teeth affected by caries, missing teeth and fillings compared to individuals subjected to any amount of radiotherapy [15].

Oncological treatment and odontogenesis

Proc et al. demonstrated that dental abnormalities occur more frequently in cancer survivors (62%) compared to healthy individuals (13%) [26]. Halperson et al. reported that tooth anomalies after exclusive chemotherapy occurred in 43% of individuals and in 60% after radiotherapy in the head and neck region [15]. Teeth present in the irradiated field receive 45% of the applied dose [5]. According to Dahloff et al., a 10 Gy dose is the radiation dose that induces cell changes in

Table 3. Summary of the adverse effects of cancer treatment

Factor type	Affected area	Ailment
Systemic factors	1. Reproductive system	• Reduced fertility and infertility [5, 10, 12, 14]
	2. Hormonal system	• Premature [5, 7] or delayed puberty [2] • Shortened duration of the puberty growth spurt [1, 4] • Hypoparathyroidism [7] • Hypothyroidism [2]
	3. Nervous system	• Neuropathic pain [7, 18] • Alterations in hot/cold sensation [7, 18] • Intellectual disability [10]
	4. Respiratory system	• Pulmonary fibrosis and dysfunction [7, 13] • Frequent infections [7, 13]
	5. Cardiovascular system	• Heart failure [5, 12, 14] • Arrhythmias [7] • Cardiomyopathies [7]
	6. Skeletal system	• Bone atrophy [13] • Osteoradionecrosis [13] • Pathological fractures [13]
	7. Ophthalmological problems	• Cataract [7] • Blindness [10]
	8. Gastrointestinal disorders	• Malnutrition [1, 3] • Loss of appetite [13] • Nausea [13] • Weight loss [13]
	9. Growth	• Short stature [2] • Muscle growth and development disorders [7] • Other growth disorders [5, 7]
	10. Others	• Hearing disorders [7, 10] • Secondary cancers [10, 14, 18]
Local factors	11. Craniofacial	• Soft tissue and bone damage [2, 5, 10, 13] • Disorders, inhibition and asymmetries in craniofacial growth [1-2, 4-5, 7, 10, 12] • Reduction in mandible and maxilla length [10, 18] – retrognathism [2] • Changes in growth rotation of mandible and maxilla [2] • Reduction in height of alveolar processes [1-2, 4, 18] • Osteoradionecrosis [5, 10, 12] • Reduction in anteroposterior and vertical dimensions of face [2, 18] • Reduction in length of cranial base [2, 10, 13] • Decreased mandible angle value [2] • Temporomandibular joint disorders and trismus [1, 4, 9-10, 12]
	12. Oral cavity	• Taste disorders [1, 3, 7, 10, 12-13] • Increased sensitivity to hot/cold sensations [7] • Salivary gland dysfunction and xerostomia [4 ,7-10, 12-14, 18] • Dysphagia [7, 13] • Reduced chewing and speaking capability [7] • Neurotoxicity [12, 18] • Neuropathic pain [18] • Graft-versus-host disease [12] • Periodontal diseases [7, 12, 10] • Caries (increased risk of and exacerbation) [1, 3, 8-10, 13-14] • Poor oral hygiene [8]

Factor type	Affected area	Ailment
Local factors	13. Mucosal membrane	• Mucositis and bleeding [1, 3-4, 10, 12-13] • Ulcers of oral mucosa and throat [1, 4, 8, 12-13] • Secondary infections (bacterial, viral, fungal) [4, 10, 12] • Decreased resistance to irritants and damaging factors [4] • Mucosal atrophy [4] • Cheilitis angular [9, 18]
	14. Anomalies of teeth	• Inhibition/delay in tooth development [1, 4, 11] • Changes in shape and size of teeth crowns [2, 12, 18] • Microdontia [1-6, 8-9, 11-14, 16, 18] • Macrodontia [11] • Reduced number of tooth buds (hypodontia, oligodontia) [1, 3-8, 10-13] • Complete absence of tooth buds (anodontia), tooth agenesis [1-2, 4, 9-10, 14-15, 18] • Supernumerary teeth [11, 16] • Persistent primary teeth [3, 7-8, 12] • Taurodontism and enlarged pulp chambers [10-11, 15-16] • Odontomas [16] • Hypomineralization of hard tooth tissues, enamel hypoplasia [1-4, 6, 8-9, 11-14, 16, 18] • Enamel discoloration [6-8, 10-11] • Inhibited development and shortening/narrowing of roots [1-6, 8, 10-13, 18] • Complete absence of roots [5, 10] • Premature closure of apical foramina [1-2, 4, 6, 8, 10-13, 18] • V-shaped roots [1-2, 4, 10-11, 13,] • Root resorption [1, 4, 16] • Increased tooth mobility [1, 4, 10] • Disruption in tooth eruption [2, 5, 12] including delayed eruption [10] and presence of impacted teeth [11-12]
	15. Occlusion	• Crowding [4, 10] • Crossbites [4, 7, 10] • Open bites [4, 7, 10] • Class II malocclusions (primarily of skeletal origin) [2, 4, 7] • Asymmetries [4, 7] • Misalignment of teeth [3, 6, 8, 11-12] • Incorrect overbite and overjet [7]

developing permanent teeth [27]. Higher doses lead to the death of ameloblasts and odontoblasts, inhibiting further tooth tissue development and partially formed teeth remain in the bone due to root agenesis [5]. Nishimura et al. and Cubukcu et al. did not find any correlation between conventional chemotherapy duration and odontogenic disturbances [28-29]. Although no clear difference in the risk of dental abnormalities based on the child's gender was observed, microdontia was more common in females, while caries was more prevalent in males [15]. Neill et al. reported that individuals treated with chemotherapy had 2.93 times fewer dental complications than those treated with combined therapy [6]. In contrast, treatment with radiotherapy or a combination of therapeutic methods yielded 5.07 times higher risk of root growth inhibition or microdontia. Researchers found that 72% of paediatric patients experienced ≥ 1 complication related to the stomatognathic system, while only 28% had no such complications. The most common complications were misalignment of teeth, root growth inhibition and changes in their development [6]. Halperson et al. stated that dental anomalies occurred in 46% of individuals after cancer therapy [15]. Vincristine is mainly responsible for these complications, although Halperson et al. did not find any specific chemotherapeutic agent to be more associated with dental abnormalities than others [15, 17].

According to Krasuska-Sławinska et al., 46.7% of patients after cancer treatment presented more than one developmental dental defect, most commonly shortening of roots (affecting 60% of patients), mainly in the permanent first molars (21.6%), followed by incisors and premolars (15% each), less frequently second molars (10%) [17]. The occurrence of these defects was not influenced by early chemotherapy complications, but by the age at the beginning of therapy and the administered doses. Tooth agenesis affected 26.67% of patients, while microdontia affected 21.67% [17]. Similarly, Cubukcu et al. noted a more frequent presence of developmental root deformities in 86.4% of patients previously treated for cancer [29]. Michalak et al. observed root problems, e.g. absence of roots, arrest of root development and abnormal shape of the newly formed permanent tooth roots (narrow and V-shaped) [5]. Radej et al. also highlighted the presence of shortened roots, their V-shape and inhibition of further development [2].

Enamel

Krasuska-Sławinska et al. noticed a positive correlation between enamel defects in permanent teeth, the age at the beginning of chemotherapy and its duration [17]. Enamel defects occur significantly more often in children after cancer treatment, including enamel hypoplasia and areas of opacities, affecting 76.7% of children. Vomiting post-chemotherapy was linked to enamel opacities, whereas mucositis was associated with enamel hypoplasia [17].

Microdontia

Krasuska-Sławinska et al. observed tooth size changes in up to 8 teeth of each paediatric patients after cancer treatment [17]. The number of affected teeth increased with the chemotherapy dose and duration, as well as the occurrence of vomiting and mucositis during treatment [17]. Microdontia of premolars and permanent second molars was most common. Other authors have highlighted that microdontia affects 19% of incisors and 45% of permanent second molars [1, 4]. Microdontia and tooth agenesis following chemotherapy before the age of 4 affected 66.7% of individuals, reaching 100% in high-dose cases [17]. Halperson et al. observed that when children began cancer treatment at age 6 or younger, they often experienced microdontia (33%), while those starting treatment later (between 6 and 12 years) had more hypocalcification or enamel hypoplasia (23%) [15]. Radej et al. also noted microdontia of second premolars and second molars in their patients after cancer treatment [2].

Hypodontia

According to Halperson et al. hypodontia is the most serious developmental disorder of dentition after cancer treatment, impacting dental arch symmetry, function and aesthetics [15]. Krasuska-Sławinska et al. indicated that the number of missing teeth increases with the chemotherapy dose and treatment duration, mainly affecting premolars (75% of children), second molars (25%) and lower incisors (12.5%) [17]. Michalak et al. found missing tooth buds of upper and lower second premolars and lower second molars in their studies as an adverse effect of cancer treatment [5]. Radej et al. observed mainly the absence of lower second molar buds in their patients after cancer treatment [2]. A cross-sectional study involving children after radiotherapy in Lyon (France), showed that 83% of them microdontic teeth and premature closure of root apical foramen, whereas facial asymmetries and delayed growth affected 74% [30].

Mental well-being

Hernandez et al. point out that odontogenic disorders also have psychological aspects. Dental abnormalities and malocclusion in children after cancer treatment represent vare stigmas that remind them of traumatic experiences and can impair the quality of life in adulthood [21]. All authors agree that complications after childhood cancer treatment significantly impact the potential for orthodontic treatment later in life. Yet, such patients require orthodontic treatment, which can help boost their self-confidence and enhance their overall self-esteem. Mituś-Kenig et al. demonstrated a positive impact of orthodontic treatment on the quality of life in patients with a cancer history, where the treatment duration had no significant effect [3].

Orthodontic treatment

First of all, a comprehensive orthodontic diagnosis is necessary, recognizing all tooth abnormalities alongside a full health evaluation and a review of the patient's medical history [2, 6-7, 31]. Finding out the cancer diagnosis date and the end date of oncological therapy, along with obtaining written consent from the oncologist, is imperative to commence

orthodontic treatment [7, 10]. Whenever possible, prior test results should be utilized to minimize additional radiation exposure to the patient [4]. The orthodontist must comprehend the underlying disease and assess the risk of complications resulting from cancer treatment [2, 11] Risk factors for orthodontic treatment complications in patients with a cancer history are presented in Table 4 [2, 5, 10].

When formulating an individual orthodontic treatment plan, it is necessary to consider the complications arising from radiotherapy and chemotherapy, the patient's overall health and medical prognosis [2, 4-5, 7, 10-11]. The outcome of the treatment of malocclusion is influenced by the degree of craniofacial growth disorders and the level of dental development following oncological treatment [2]. The orthodontist must anticipate various difficulties during the treatment and assess the options and accept possible compromises in the treatment results [4-5, 11]. Sometimes, it is necessary to develop an alternative treatment plan in case of failure due to shortened growth spurt and slowed growth of the child [2]. If any doubts arise, the orthodontist should contact the oncologist [2, 10, 12]. The reduction in the range of jaw opening, which accompanies temporomandibular joint dysfunctions after chemotherapy, presents an additional difficulty while making dental impressions and attaching the orthodontic appliance [5].

Orthodontic treatment must be not only deferred until the completion of the full cycle of anti-cancer therapy, but it is also recommended to postpone it by at least 24 months after the end of oncological treatment if no symptoms of cancer are present during this time [1-2, 4-5, 10-11]. This is related to bone metabolism disturbances caused by oncological treatment and the risk of cancer recurrence, which is reported in 2.6% - 12.1% of patients [4, 12]. An additional condition is the cessation of immunosuppressive treatment [1-2]. A shorter deferment period lasting a few months post-oncological therapy may be considered in patients treated with chemotherapy only [12]. In cases of exclusively surgical treatment, immediate commencement of orthodontic treatment is permissible without a 24-month latency period, provided the tumour has been completely excised and the lymph nodes are normal [2, 5, 10].

Treatment method and type of orthodontic appliance should be chosen carefully. The orthodontic forces should be low, (20 to 150 g/tooth) and the mechanics used as simple as possible to reduce the risk of root resorption [1-2, 4-5, 7-11, 19]. Points of force application must be carefully considered, anchorage and methods of affixing of the appliance must be closely monitored [2, 7]. Additionally, it is advisable to shorten the duration of orthodontic treatment as much as possible and finish earlier than usual [1-3, 5, 8-11, 19]. Due to the increased risk of osteoradionecrosis, tooth extraction (if required) should be postponed until 2 years after the completion of cancer therapy and performed atraumatically with precise wound management [2, 5, 10-11]. Oncological patients often have short, narrow roots that are particularly susceptible to resorption during movement [11-12]. This increases demands on anchorage and limits possible orthodontic

Table 4. Summary of the risk factors for orthodontic treatment complications in patients with a cancer history [2, 5, 10]

Risk factors	Complications
Time	1. Cancer diagnosed before the age of 8 2. Time up to 2 years since the end of cancer therapy
Cancer	1. Solid tumour at diagnosis 2. Cancer location particularly within the craniofacial region or the central nervous system
Past oncological treatment	1. Whole-body or head and neck radiotherapy 2. Radiation > 2400 cGy 3. Allogenic stem cell transplantation 4. Prolonged immunosuppressive therapy (administered due to graft-versus--host disease) 5. Chemotherapy with bisulphan/cyclophosphamide
Systemic complications	1. Hypopituitarism 2. Hypothyroidism 3. Graft-versus-host disease 4. Relapse of the primary disease
Local complications	1. Anomalies of tooth roots 2. Microdontia 3. Tooth agenesis 4. Gingival overgrowth (after cyclosporine A)

movements [9-10]. Routine X-ray radiographs are necessary every 12-18 months to detect any changes in the tooth crown-to-root length ratio [10]. Additionally, Levander et al. recommend performing a panoramic radiograph after the first 6 months of active orthodontic treatment [32]. Observations suggest that rootless teeth can function in the oral cavity for some time (in most cases correctly), despite increased mobility [10]. Mituś-Kenig highlight the need for a pause in orthodontic treatment when signs of root resorption are observed [4]. There is no need to remove braces and it is recommended to use passive arch wires for 2-3 months [4]. Levander et al. have shown that thanks to a routine 2-3 month break in active orthodontic treatment and the use of passive arch wires after the first 6 months of treatment, the risk of advanced root resorption can be significantly reduced [32].

It is advisable to use protective orthodontic waxes and silicones along with appliances that irritate the mucous membrane as little as possible due to the patients' reduced resistance to infections, decreased saliva secretion and increased sensitivity [2, 4-5, 10-11]. Nickel-containing steel brackets should be avoided due to the possibility of increased generation of free radicals that lead to cytotoxicity [7, 11]. If possible, it is better to choose aligners or ceramic brackets, which also cause significantly fewer artifacts in imaging tests [4, 10]. Before such tests, removable elements of the appliance should be removed and the quality of adhesion of the remaining elements should be checked [4].

Patient should maintain perfect oral hygiene to limit the development of caries in the course of the already reduced saliva production [4-5, 7, 11]. Severe xerostomia may constitute a contraindication to undertaking orthodontic treatment [7]. It is good to eliminate elastic ligatures in favour of metal ones and repeat hygiene instructions [4, 12]. A paedodontist (paediatric dentist) should also concurrently supervise such a patient and implement an individual fluoride prophylaxis plan [4-5, 7, 9, 11].

Radiotherapy of the head and neck region in a growing patient significantly worsens the prognosis of orthodontic treatment [5]. Due to shortened puberty and inhibited mandible growth, orthodontic treatment is suggested only in the upper dental arch, which additionally accelerates the orthodontic therapy [1, 4-5]. Treatment of Class II malocclusions is exceptionally challenging and modification of growth may be ineffective or not even possible [4, 10-11]. During functional orthodontic treatment, growth hormone therapy may be necessary to normalize the patient's craniofacial growth [2, 5]. After completing growth hormone therapy, good effects of functional treatment can no longer be expected [2].

Michalak et al. [5] remind of the potential need for prosthetic reconstruction in patients after cancer treatment, which may be due to the high risk of tooth loss with aplastic roots or the absence of permanent tooth buds. Paediatric dentures used in such cases restore the ability to chew and improve speech and general facial aesthetics. However, prosthesizing conditions may often be unfavourable due to underdevelopment of alveolar bone. After growth cessation, dental implants can be placed [5]. Additionally, Deshpande et al. recommend the use of removable dentures whenever deemed important [7]. Regular check-ups are necessary as they can be a source of potential mucosal irritations. Proper hygiene of both oral cavity dentures must be maintained [7].

Hernandez et al. point out contraindications to orthodontic treatment in case of underdeveloped, too short permanent tooth roots due to a strong risk of their resorption [21]. It is necessary to monitor the eruption of such teeth, assess their mobility and try to keep the present primary teeth in the oral cavity as long as possible. In the case of failure, prosthetic treatment should be applied [21]. Radej et al. note that in the case of shortened, V-shaped roots of lower incisors, their intrusion is ill-advised, therefore, an orthodontic appliance cannot be used to level a deepened Spee's curve [2]. Additionally, in the case of chronic gingivitis and decreased teeth mineralization, it is necessary to refrain from using fixed appliances. If there is a need for distalization of teeth example (e.g. to recreate space in the arch for canines), extractions should be chosen as a simpler treatment method [2]. In the case of treatment with removable appliances, they should be frequently checked, sharp areas should be smoothed and adjusted to current occlusal conditions to minimize the risk of mucosal irritation [2, 7]. Patients should frequently disinfect them by soaking in disinfectant solutions to limit microbial growth and the possibility of infections [19].

Mituś-Kenig et al. point out, that the results of orthodontic treatment in oncological patients do not significantly differ from healthy individuals [1, 4]. They reported no serious complications of orthodontic treatment and in most cases proper occlusion was achieved [1, 4]. However, patients after cancer treatment had mucositis and gingivitis while wearing braces more frequently and had root resorption slightly more often than healthy individuals. They also experienced a higher discomfort during the first 3 months of orthodontic treatment [1]. Mituś-Kenig et al. noted

a significant decline in stability of orthodontic treatment effects among patients post-cancer treatment over a 3-year retention period compared to a healthy group [8]. Therefore, they require intensified observation to maintain the stability of orthodontic treatment effects and should be warned before starting treatment about the increased risk of relapse. It should also be considered that the stability of orthodontic treatment results also depends on factors related to the periodontal tissues and pressure from soft tissues, which were not considered in this study [8]. Retention after orthodontic treatment should be well planned. The retention using well-fitted appliances that do not irritate the mucous membrane (to prevent wounds and ulcers) [11]. The patient must constantly monitor them and maintain precise hygiene in their area. Retention splints can additionally be disinfected in a chlorhexidine solution [12].

Summary of guidelines for orthodontic treatment in patients after childhood cancer treatment is presented in Table 5.

Recurrence of cancer requires an immediate cessation of active orthodontic treatment and removal of fixed appliances, including space maintainers and bands, if cancer therapy may lead to mucositis and when oral hygiene is not adequate [7, 9-11, 19-21]. Removable appliances can be worn as long as they do not irritate mucous membrane and the patient can tolerate them [9, 16, 19-20]. The same approach should be taken if the patient develops cancer for the first time during orthodontic treatment [12]. Patients should be provided with a removable retainer [9-10, 19-20]. Resuming the original orthodontic treatment can be considered after achieving remission lasting at least 2 years [9-11, 16, 19].

The American Academy of Pediatric Dentistry reminds of the possibility of secondary cancers within the head and neck area, therefore, for the orthodontist it is very important to maintain oncological vigilance on the part of [19]. During orthodontic treatment, the orthodontist should pay attention to health status of the oral cavity at each check-up visit and, in case of any suspicious lesions, refer for further diagnostics to a specialist in mucosal diseases and oral surgeons [9, 16, 20].

Orthodontic treatment of individuals who have survived childhood cancer should be planned on an individual basis. The tissue response to the same treatment can vary, consequently various treatment results can be achieved. Additionally, each patient will perceive their new bite and the aesthetics of their teeth differently, just as there are various standards of beauty around the world. The outcome of orthodontic treatment may also be assessed differently by the orthodontists themselves. The stability of orthodontic treatment or the achieved bite is difficult to evaluate, as it requires close cooperation from the patient, who should attend regular check-ups after completing treatment with orthodontic appliances. For this reason, retrospective studies often do not include long-term follow-ups, which is a limitation of this review.

Table 5. Summary of guidelines for orthodontic treatment in patients after childhood cancer treatment

Oncological treatment	Orthodontic treatment	
Radiotherapy	• Initiation of orthodontic treatment should be delayed for at least 24 months after the completion of radiotherapy. • Tooth extractions should be postponed for at least 2 years after the completion of radiotherapy. • Orthodontic treatment should only be performed on the upper dental arch.	• Starting orthodontic treatment after discontinuation of immunosuppressive therapy. • Thorough analysis of the patient's medical history. • Assessment of the current health status. • Comprehensive orthodontic diagnosis and individual orthodontic treatment plan based on precise diagnosis of the dentition. • Application of low orthodontic forces ranging from 20 to 150 g/tooth. • Simple mechanics of orthodontic treatment. • Use of non-irritating orthodontic appliances. • Reduction of the duration of orthodontic treatment. • Periodic x-rays of tooth roots during orthodontic treatment. • Pause and use of passive arch wires for 2-3 months in the event of root resorption during orthodontic treatment. • Proper oral hygiene during orthodontic treatment. • Well-planned retention and periodic stability assessment.
Chemotherapy	• Postponement of the start of orthodontic treatment for a few months after the completion of chemotherapy.	
Surgery	• No need to postpone the start of orthodontic treatment after exclusively surgical oncological treatment.	

An unfortunate limitation of this review is the fact that none of the analyzed articles included information about new orthodontic techniques. Nowadays, with the help of intraoral scanners, it is possible to digitally record teeth and bite. Additionally, access to cone beam computed tomography (CBCT) scans enables a precise understanding of the dimensions and shapes of tooth roots along with the surrounding bone of the alveolar processes. This allows for the digital planning of favourable tooth movements and the installation of appliance components, as well as the prediction of potential adverse effects of orthodontic treatment. Based on this information, templates for appliance mounting can be made using 3D printers. Moreover, the use of skeletal anchorage systems temporary anchorage devices (TADs) or Bollard plates can significantly reduce the negative impact on tooth roots by applying forces directly to the bone, and more effectively modify growth in cases of jaw deformities, such as prognathism of mandible or constricted maxilla, even when there are no developed permanent tooth buds or when there are compromised teeth (e.g. those with shortened roots), making traditional braces unsuitable. In the future, it will likely be possible to treat orthodontic patients even more effectively due to the developments in AI technology, e.g. assisting orthodontists in the digital planning of orthodontic treatment, which may lead to potentially better outcomes with simultaneously fewer adverse effects.

Conclusions

Cancer treatment during childhood contributes to the development of a range of dental and skeletal abnormalities, including those of the craniofacial complex. Risk of their occurrence increases with use of combined chemotherapy and radiotherapy, particularly of the head and neck area, which significantly worsens orthodontic prognosis. Among the most commonly encountered developmental defects of teeth are abnormalities in root structure, lack of tooth buds and microdontia are. Orthodontists must be aware of the patient's full medical history and take it into consideration each time when planning their treatment in order to prevent or at least minimize possible adverse outcomes. Orthodontic treatment should be modified using simple methods and appliances that do not irritate the mucous membrane, omit the mandible, employ lower forces, and ideally shorten the duration of orthodontic treatment. There is a need to introduce education about patients with a cancer history during the course of undergraduate dental education.

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Conflicts of interests

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References

1. Mitus-Kenig M, Derwich M, Czochrowska E, Pawlowska E. Quality of Life in Orthodontic Cancer Survivor Patients – A Prospective Case – Control Study. Int J Environ Res Public Health [Internet]. 2020 Aug 12;17(16):5824. Available from: https://www.mdpi.com/1660-4601/17/16/5824
2. Radej I, Bugała-Musiatowicz B, Szarmach I, Grodzka I. Planning of orthodontic treatment in patients with a history of neoplastic disease – case reports. Orthod Forum [Internet]. 2013;9(40):267–83. Available from: https://publisherspanel.com/api/files/view/31320.pdf
3. Mitus-Kenig M, Derwich M, Czochrowska E, Pawlowska E. Comparison of Oral Health Impact Profile (OHIP-14) Values in Cancer Survivor Patients Treated Orthodontically with Either Rapid or Standard Duration Protocols of Treatment—A Prospective Case–Control Study. Int J Environ Res Public Health [Internet]. 2020 Dec 4;17(23):9068. Available from: https://www.mdpi.com/1660-4601/17/23/9068

4. Mituś-Kenig M, Łoboda M, Marcinkowska-Mituś A, Durka-Zajac M, Pawłowska E. Orthodontic treatment in oncological patients. Przegląd Lek [Internet]. 2015;72(5):243–5. Available from: https://ruj.uj.edu.pl/xmlui/bitstream/handle/item/136066/mitus-kenig_et-al_orthodontic_treatment_in_oncological_2015.pdf?sequence=1&isAllowed=y
5. Michalak I, Kuśmierczyk D, Zadurska M. Radiological imaging and orthodontic treatment in the case of growing patients after oncological treatment: Case reports. Dent Med Probl [Internet]. 2019 May 10;56(2):209–15. Available from: https://dmp.umw.edu.pl/en/article/2019/56/2/209/
6. Neill CC, Migliorati C, Trojan T, Kaste S, Karydis A, Rowland C, et al. Experience and expertise regarding orthodontic management of childhood and adolescent cancer survivors. Am J Orthod Dentofac Orthop [Internet]. 2015 Nov;148(5):765–70. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0889540615008938
7. Deshpande M, Jain D, Mehta S, Bhat D, Gambhir L, Patel R. Orthodontic Care in Pediatric Cancer Survivors: A Review. J Dent Oral Sci [Internet]. 2020 Apr 3;2(3):1–6. Available from: https://maplespub.com/article/Orthodontic-Care-in-Pediatric-Cancer-Survivors-A-Review
8. Mitus-Kenig M, Derwich M, Czochrowska E, Pawlowska E. Cancer survivors present significantly lower long-term stability of orthodontic treatment: a prospective case–control study. Eur J Orthod [Internet]. 2021 Dec 1;43(6):631–8. Available from: https://academic.oup.com/ejo/article/43/6/631/6088062
9. Ritwik P. Dental Care for Patients With Childhood Cancers. Ochsner J [Internet]. 2018 Dec 13;18(4):351–7. Available from: http://www.ochsnerjournal.org/lookup/doi/10.31486/toj.18.0061
10. Radej I, Bugała-Musiatowicz B, Szarmach I, Grycz M. Orthodontic treatment for oncological patients – literature review. J Stoma. 2013;66(4):517–33. Available from: https://www.researchgate.net/publication/273376720_Orthodontic_treatment_for_oncological_patients_-_Literature_review
11. Mishra S. Orthodontic Therapy for Paediatric Cancer Survivors: A Review. J Clin DIAGNOSTIC Res [Internet]. 2017;11(3):ZE01–4. Available from: http://jcdr.net/article_fulltext.asp?issn=0973-709x&year=2017&volume=11&issue=3&page=ZE01&issn=0973-709x&id=9404
12. Boyer É, Robert G, Gandemer V, Bonnaure-Mallet M. Orthodontic strategies in pediatric oncology. J Dentofac Anomalies Orthod [Internet]. 2017 Apr 23;20(1):104. Available from: https://www.jdao-journal.org/10.1051/odfen/2016035
13. Baethge C, Goldbeck-Wood S, Mertens S. SANRA—a scale for the quality assessment of narrative review articles. Res Integr Peer Rev [Internet]. 2019 Dec 26;4(1):5. Available from: https://researchintegrityjournal.biomedcentral.com/articles/10.1186/s41073-019-0064-8
14. Carrillo CM, Corrêa FNP, Lopes NNF, Fava M, Filho VO. Dental anomalies in children submitted to antineoplastic therapy. Clinics [Internet]. 2014 Jun;69(6):433–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1807593222009073
15. Halperson E, Matalon V, Goldstein G, Saieg Spilberg S, Herzog K, Fux-Noy A, et al. The prevalence of dental developmental anomalies among childhood cancer survivors according to types of anticancer treatment. Sci Rep [Internet]. 2022 Mar 16;12(1):4485. Available from: https://www.nature.com/articles/s41598-022-08266-1
16. Ritwik P, Chrisentery-Singleton TE. Oral and dental considerations in pediatric cancers. Cancer Metastasis Rev [Internet]. 2020 Mar 27;39(1):43–53. Available from: http://link.springer.com/10.1007/s10555-020-09842-5
17. Krasuska-Sławińska E, Brożyna A, Dembowska-Bagińska B, Olczak-Kowalczyk D. Antineoplastic chemotherapy and congenital tooth abnormalities in children and adolescents. Współczesna Onkol [Internet]. 2016;5:394–401. Available from: http://www.termedia.pl/doi/10.5114/wo.2016.64602
18. Román J, Villaizán CJ, García-Foncillas J, Salvador J, Sierrasesúmaga L. Growth and growth hormone secretion in children with cancer treated with chemotherapy. J Pediatr [Internet]. 1997 Jul;131(1):105–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S002234769770132X
19. Dental Management of Pediatric Patients Receiving Immunosuppressive Therapy and/or Radiation Therapy. Pediatr Dent [Internet]. 2018 Oct 15;40(6):392–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32074911
20. Ali Hassan S, Bhateja S, Arora G, Prathyusha F. Dental care of pediatric cancer patient. IP Int J Med Paediatr Oncol [Internet]. 2020 Jul 28;6(2):43–7. Available from: https://ijmpo.com/article-details/11697

21. Hernandez M, Pochon C, Chastagner P, Droz D. Long-term Adverse Effects of Acute Myeloid Leukemia Treatment on Odontogenesis in a Child. Int J Clin Pediatr Dent [Internet]. 2019 Jun;12(3):243–6. Available from: https://www.ijcpd.com/doi/10.5005/jp-journals-10005-1614
22. Dahllöf G, Huggare J. Orthodontic considerations in the pediatric cancer patient: A review. Semin Orthod [Internet]. 2004 Dec;10(4):266–76. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1073874604000520
23. Geenen MM, Cardous-Ubbink MC, Kremer LCM, van den Bos C, van der Pal HJH, Heinen RC, et al. Medical Assessment of Adverse Health Outcomes in Long-term Survivors of Childhood Cancer. JAMA [Internet]. 2007 Jun 27;297(24):2705. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.297.24.2705
24. Sonis AL, Tarbell N, Valachovic RW, Gelber R, Schwenn M, Sallan S. Dentofacial development in long-term survivors of acute lymphoblastic leukemia: A comparison of three treatment modalities. Cancer [Internet]. 1990 Dec 15;66(12):2645–52. Available from: https://onlinelibrary.wiley.com/doi/10.1002/1097-0142(19901215)66:12%3C2645::AID-CNCR2820661230%3E3.0.CO;2-S
25. Nemeth O, Hermann P, Kivovics P, Garami M. Long-term Effects of Chemotherapy on Dental Status of Children Cancer Survivors. Pediatr Hematol Oncol [Internet]. 2013 Mar 13;30(3):208–15. Available from: http://www.tandfonline.com/doi/full/10.3109/08880018.2013.763391
26. Proc P, Szczepańska J, Skiba A, Zubowska M, Fendler W, Młynarski W. Dental Anomalies as Late Adverse Effect among Young Children Treated for Cancer. Cancer Res Treat [Internet]. 2016 Apr 15;48(2):658–67. Available from: http://e-crt.org/journal/view.php?doi=10.4143/crt.2015.193
27. Dahllöf G, Rozell B, Forsberg C-M, Borgström B. Histologic changes in dental morphology induced by high dose chemotherapy and total body irradiation. Oral Surgery, Oral Med Oral Pathol [Internet]. 1994 Jan;77(1):56–60. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0030422006801076
28. Nishimura S, Inada H, Sawa Y, Ishikawa H. Risk factors to cause tooth formation anomalies in chemotherapy of paediatric cancers. Eur J Cancer Care (Engl) [Internet]. 2013 May 21;22(3):353–60. Available from: https://onlinelibrary.wiley.com/doi/10.1111/ecc.12038
29. Cubukcu CE, Sevinir B, Ercan İ. Disturbed dental development of permanent teeth in children with solid tumors and lymphomas. Pediatr Blood Cancer [Internet]. 2012 Jan 19;58(1):80–4. Available from: https://onlinelibrary.wiley.com/doi/10.1002/pbc.22902
30. Akharzouz C, Chauty S, Bodard A-G. Enfants ayant reçu une irradiation de la région cranio-cervico-faciale : évaluation du besoin de traitement orthodontique. L'Orthodontie Française [Internet]. 2013 Jun 30;84(2):157–68. Available from: https://www.jle.com/10.1051/orthodfr/2013047
31. Kim J-H, Jih M. An orthodontic approach for Class III malocclusion in a pediatric cancer patient: A case report. Oral Biol Res [Internet]. 2019 Mar 31;43(1):103–9. Available from: http://www.chosunobr.org/journal/view.html?doi=10.21851/obr.43.01.201903.103
32. Levander E, Malmgren O, Eliasson S. Evaluation of root resorption in relation to two orthodontic treatment regimes. A clinical experimental study. Eur J Orthod [Internet]. 1994 Jun 1;16(3):223–8. Available from: https://academic.oup.com/ejo/article-lookup/doi/10.1093/ejo/16.3.223

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REVIEW ARTICLE

The milestone – role of miRNAs as predictors of diabetes complications: a literature review

Karol Bigosiński[1], Michał Granat[2], Klaudia Salamon[2], Joanna Góra[2], Marcin Caliński[2], Hubert Bednarz[2], Maciej Dubaj[1], Dominik Porada[3]

[1] Doctoral School, Medical University of Lublin, Poland
[2] Section of Diabetology of the Student Scientific Association, Department of Endocrinology, Diabetology and Metabolic Diseases, Medical University of Lublin, Poland
[3] Department of Endocrinology, Diabetology and Metabolic Diseases, Medical University of Lublin, Poland

Abstract

Diabetes mellitus (DM) is one of the biggest health problems of the 21st century. The complications of DM are macrovascular (ischemic diseases of the heart and brain) and microvascular (retinopathy, nephropathy, neuropathy). The aim of this review was to describe the role of micro ribonucleic acids (miRNAs) as markers for the development of distant complications of diabetes. A search was conducted in the PubMed, Scopus, Google Scholar and EMBASE databases for articles published in 2014-2024. Fifty-three original articles, systematic reviews and meta-analyses were included in the review. MiRNAs are involved in metabolic pathways responsible for the onset and development of DM and their altered expression may serve as a non-invasive biomarker for the development of distant DM complications. Among the most significant is miR-21, responsible for normal angiogenesis, whose expression was higher in patients with macroangiopathy and retino-, nephro- and neuropathy. Certain miRNAs may have potential as potential therapeutic targets, e.g. miR-203 (ischemic heart disease), miR-181c (retinopathy) and miR-184-5p (neuropathy). MiRNAs are potential biomarkers for the development of distant complications of diabetes and may serve as therapeutic targets for their reduction.

Keywords: microRNAs · diabetes mellitus · complications · predictors

Corresponding author:
Maciej Dubaj, Doctoral School, Medical University of Lublin, Poland
e-mail: maciej.dubaj99@gmail.com
Available online: ejtcm.gumed.edu.pl

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Introduction

Diabetes mellitus (DM) is a growing global health and economic challenge, with 828 million cases reported in 2022 and projections reaching 1.31 billion by 2050, mainly in low- and middle-income countries [1-3]. Furthermore, in 2021 approximately 11.8% of deaths globally in the working-age population (< 60 years) were estimated to be caused by DM or its complications [1-3].

Chronic complications of DM lead to poor outcomes (increased morbidity, mortality, and healthcare costs) and are traditionally classified as microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (coronary artery disease [CAD], stroke, peripheral artery disease [PAD]) [4-5]. However, Yu et al. suggested a revision of this classification, in accordance with the affected tissues: vascular, parenchymal and hybrid (both vascular and parenchymal) [5].

Microribonucleic acids (miRNAs) are small non-coding RNAs (20-25 nucleotides) that regulate gene expression post-transcriptionally [6]. Circulating miRNAs are stable in body fluids (due to protection in exosomes or protein complexes) and show potential as non-invasive diagnostic and prognostic biomarkers. In DM, miRNAs modulate key pathogenic mechanisms, including beta-cell responses to metabolic, genetic, and inflammatory stimulation [6-7]. Specific miRNAs, such as miR-34 and miR-146, are of particular interest [7]. Moreover, miRNAs offer the potential for the early detection of a particular type of DM and its specific complications [8-9]. Given the urgent need for effective biomarkers of DM, in this article we present a comprehensive review of the available data on the involvement of miRNAs in predicting its complications [10].

Material and methods

A literature search was conducted independently by 3 authors using the PubMed, Scopus, Google Scholar, and EMBASE databases. The results were then compared. The following keywords were used: 'diabetes,' 'complications,' 'miRNAs,' 'miRNAs,' 'markers,' 'retinopathy,' 'neuropathy,' 'nephropathy,' and the 'AND' operator. The articles were searched sequentially in the given databases, and the results were combined by the same 3 authors. The inclusion criteria were as follows: article published in 2014-2024, article format (original/research article, systematic review and meta-analysis), article about the role of miRNAs in DM, article describing distant complications of DM, study included patients > 18 years of age or animal models. The exclusion criteria were: articles published before 2014, inappropriate format (editorial, letter to the Editor, abstract-only, poster, case report, non-systematic review), articles describing acute complications of DM, and articles on DM in children. The discrepancies between the searches were minor. After analysis, articles that did not meet the inclusion criteria were rejected, and a combined list of articles for the final review was developed. Initially, 476 studies were identified. After excluding duplicate articles and screening the titles and abstracts, 70 articles remained. After a substantive analysis, 53 articles were included in the review.

Results and discussion

Macroangiopathy

Vascular disorders are characteristic complications of DM and lead to high risk of death. Morrison et al. demonstrated that patients with vascular complications of DM have elevated levels of HDL-related miRNAs. MiR-181c-5p may underlie proangiogenic processes, with 14-fold higher activity in subjects with PAD (1454 ± 1346%) than in the healthy group (100 ± 121%) and patients without PAD (82 ± 77%). This could also be applied to miRNA-181c-5p in plasma, however the difference was not statistically significant. In contrast, HDL-bound miRNA-27b-3p showed 10-fold higher activity in patients with PAD (260 ± 232%) than in those without it (27 ± 23%) [11].

The adverse effect of DM on the prognosis of patients with heart failure (HF) and CAD has been known for a long time. The correlation between miR-1 and miR-21 expression and these conditions was studied by Al-Hayali et al.

in a group of 35 patients with insulin-dependent DM. The results showed significantly (0.22-fold) lower serum levels of miR-1 in patients with HF than in a group with type 2 DM alone, as well as in a group with CAD ($p < 0.001$). Moreover, miR-21 expression was higher in patients with HF (1.7 times) and CAD (1.37 times) than in patients without these conditions. Additionally, they demonstrated positive correlations between miR-21 and NT-proBNP and galactin-3. When miR-21 levels were > 1.695 and NT-proBNP > 4747 pg/mol, the probability of DM and HF co-occurring was 95.2%. In contrast, in cases where miR-21 levels were < 1.695 and NT-proBNP levels were < 4747 pg/mol, the probability of DM alone was 66.7%. In cases where the miR-21 value was > 1.695 and the galactin-3 value was > 9.25 ng/mol, the probability was as high as 74.4% [12].

Other researchers observed decreased miR-130a and miR-130b levels in patients with DM and CAD compared to those with DM alone and in the control group [13]. In their studies of mice, Dai et al. reported an unknown role for miR-21 in alleviating the symptoms and progression of diabetic cardiomyopathy by affecting gelsolin. A notable decrease in miR-21 expression was observed in the hearts with diastolic dysfunction. The supply of exogenous miR-21 effectively protected the heart from the early onset of impaired diastolic function in the reduced emission of reactive oxygen species, increased bioavailability of nitric oxide, and mitigation of cardiomyocyte hypertrophy in diabetic mice [14].

Lopes et al. tested miRNA values from the left ventricle of diabetic rodents to elucidate their role in diabetic cardiomyopathy. They noted decreased expression of rno-miR-877, rno-miR-320 and rno-miR-214 in the study group, together with increased expression of rno-miR-17, rno-miR-187, rno-miR-34a, rno-miR-322, rno-miR-188, rno-miR-532 and rno-miR-21. These results demonstrate the potential usefulness of the aforementioned miRNAs in the early detection of diabetic heart complications [15].

Chen et al. measured miR-30c levels in the myocardium of rodents with diabetic cardiomyopathy and healthy controls. A notable reduction in miR-30c was observed in the myocardium of subjects with cardiomyopathy compared to controls. Moreover, miR-30c overexpression reduced diabetic myocardial dysfunction. In addition, the data showed that overexpression of miR-30c silences the autophagy-initiating protein BECN1, thereby protecting myocardial function in diabetic rodents. However, the reduction of miR-30c expression increased the expression of BECN1 protein and through it, autophagy and progression of pathology within the myocardium [16]. In addition, Chen et al. observed a relationship between decreased levels of miR-133 in cardiac muscle and increased levels of fibrosis biomarkers in a mouse model of diabetic cardiomyopathy. Overexpression of miR-133 can even reverse DM-induced cardiac remodeling by attenuating these biomarkers [17].

Liu et al. were the first to report the role of miR-222 in the protective effect of physical exercise on the cardiovascular system. Mice exposed to swimming showed higher expression of miR-222 in cardiomyocytes, which stimulated cell proliferation and growth. In contrast, miR-222 inhibition led to apoptosis of cardiomyocytes, indicating its potential therapeutic relevance [18]. Other researchers have found that increased levels of circulating miR-1 and miR-133 are correlated with a higher probability of CAD in patients with type 2 DM [19]. Similar conclusions were published in a study of miR-126, the expression of which was significantly reduced in the type 2 DM and CAD groups compared to the reference group [20]. Jansen et al. noted that DM alters vascular endothelial miRNA expression in circulating endothelial microvesicles. MiR-126 and miR-26a expression levels were lower in the DM group than in the controls. The group with reduced expression of the molecules mentioned above was more likely to have concomitant CAD [21]. Xubin et al. noted that increased expression of miR-203 could act as a cardio-protective factor in diabetic cardiomyopathy by inhibiting the PI3K/Akt path [22]. Deng et al. investigated that the level of circulating miR-24 was significantly reduced in the peripheral blood of patients with type 2 DM and CAD compared to controls [23]. The summary is presented in Table 1.

Retinopathy

Hyperglycemia causes vascular wall dysfunction and that is why diabetic retinopathy (DR) is one of the most common complications of DM and the most common cause of vision loss. One study showed that miR-21 expression was increased in patients with non-proliferative DR (n = 73) compared to controls (n = 115) and increased in patients with proliferative DR (n = 51) than in those with non-proliferative DR. Increased miR-21 levels were associated with the development of DR and can be used as an indicator of its severity [24]. Moreover, Qing et al. proved that the merger of miR-21, miR-181c and miR-1179 is useful in distinguishing the proliferative DR from the non-proliferative form. They noted that miR-21 was strongly correlated with angiogenesis in hyperglycemia. Simultaneously, miR-181c expression was higher in endothelial cells in a DM-like environment, indicating that it is related to vascular proliferation at high glucose concentrations [25]. McAuley et al. noted that the A allele of the miR-126 polymorphism is associated with sight-threatening DR compared to those without DR or early-stage disease. It acts as a vascular endothelial growth factor (VEGF) and boosts the probability of progression of the DR, thus it could become a potential therapeutic target [26]. Other scholars have identified

Table 1. Role of miRNAs as potential biomarkers in diabetic macroangiopathy [11-23]

Authors	Study group (n)*	Study material	miRNA	Change in expression	Targets
Morrison et al. 2019 [11]	27	Plasma endothelial cell cultures	miR-181c-5p	↑	FOXO1 COX-2 BCL2 LIF HIF1A
Al-Hayali et al. 2019 [12]	135	plasma	miR-1	↓	XPO6 Irx5 HAND2 KLF4
			miR-21	↑	Spry1, PTEN
Yuan et al. 2019 [13]	201	plasma	miR-130	↓	PPAR-γ
Dai et al. 2018 [14]	-	mice cell lines	miR-21	↓	gelsolin
Lopes et al. 2017 [15]	-	rats	rno-miR-877 rno-miR-320 rno-miR-214	↓	Pla2g2a
			rno-miR-17, rno-miR-187, rno-miR-34a, rno-miR-322, rno-miR-188, rno-miR-532 rno-miR-21	↑	Hk2
Chen et al. 2017 [16]	91	mice plasma	miR-30c	↓	BECN1
Chen et al. 2014 [17]	-	mice	miR-133a	↓	TGFB1 COL4A1 FN1 ERK1/2 SMAD-2
Liu et al. 2015 [18]	28	mice plasma	miR-222	↑	HIPK1 HMBOX1
Al-Muhtaresh et al. 2019 [19]	60	whole blood	miR-1	↑	HDAC4
			miR-133	↑	SRF
Al-Kafaji et al. 2017 [20]	135	plasma	miR-126	↓	SPRED1 VEGF PIK3R2
Jansen et al. 2016 [21]	135	plasma	miR-26a miR-126	↓	TRPC6 BMP/SMAD1 CXCL12
Yang et al. 2019 [22]	-	mice	miR-203	↑	PI3K/Akt
Deng et al. 2017 [23]	94	plasma	miR-24	↑	YKL-40

* Not all articles mentioned the exact number of people and animals participating in the descbed study.

miR-1281 as a sensitive biomarker for the early detection of DR. It had the most elevated expression in patients with non-proliferative DR compared to healthy controls, and its expression was increased in retinal cell cultures in high-glucose environments [27]. The authors observed an overexpression of miR-423-5p in DR with enhanced hyperglycemia-induced apoptosis in retinal pigment epithelial cells [28]. Moreover, Santovito et al. found that DR was correlated with higher circulating miR-25-3p and miR-320b and lower miR-495-3p levels compared to the subjects without DR and healthy controls [29]. Blum et al. showed a reduced expression of miR-423 in a group with DR compared to controls [30]. Moreover, García de la Torre et al. observed that the level of miR-126 did not differ between the groups with and without DR, whereas the level of miRNA-221 was increased [31]. Gomaa et al. noticed that miR-200b expression was approximately 5 times higher in vitreous body samples collected from patients with proliferative DR [32]. In another study, miR-15a, miR-320a, miR-320b, miR-93, miR-29a, and miR-423-5p were significantly elevated in patients with proliferative DR [33]. Li et al. investigated the role of miR-200b in the evolution of DR. They found that its increased expression could reduce the expression of VEGFA,mitigating DR progression [34]. According to Liang et al., miR-28-3p, miR-151a-5p, and miR-148a-3p were correlated with the progression of DR and can serve as non-invasive diagnostic markers [35]. Liu et al. discovered that miR-211 may also serve as a new marker with high sensitivity and specificty for DR by affecting Sirtuin 1 [36]. Murray et al. showed overexpression of miR-200b in Akita mice retinas (experimental benchmark of insulin-dependent DM). This miRNA reduces expression of Oxr1. These results suggest that downregulating miRNA-200b expression while enabling Oxr1 expression may have a protective role against DR progression [37]. Rezk et al. found that the group with DR had a lower miR-126 level compared to the group without it [38]. Zampetaki et al., in a group of patients with type 1 DM (n = 300), identified high expression of miR-320a and miR-27b as potential biomarkers of DR progression [39]. Moreover, Zou et al. identified increased miR-93 as a new promising diagnostic biomarker of DR progression [40]. Pastukh et al. showed that miR-122 expression increased in subjects with severe DR compared to healthy controls. However, when the illness progressed to proliferative DR, miR-122 expression decreased [41]. Moreover, Huihui et al. identified elevated expression of both miR-3197 and miR-2116-5p as potential diagnostic biomarkers for DR [42]. The above data are summarized in Table 2. The role of miRNAs as diagnostic and prognostic markers in DR is shown in Figure 1.

Nephropathy

Diabetic nephropathy (DN) manifests in approximately one-third of diabetic patients and is associated with multiple complications and premature mortality. The disease progresses as the number of podocytes is reduced, the mesangial matrix expands, the glomerular basement membrane thickens, and the glomeruli undergo sclerosis. Initially, DN is often asymptomatic and the patient can be unaware of its progression. Zang et al. reported significantly higher urinary miR-21-5p (2.13-fold) and lower miR-30b-5p (0.82-fold) in DN patients versus subjects without kidney injury, supporting their use in early detection [43]. Beltrami et al. examined the profile of 754 miRNAs and noted increases in miR-126 (2.8-fold), miR-155 (1.8-fold), and miR-29b (4.6-fold) levels in urine from a group with confirmed DN (n = 20) compared to controls (n = 20). Concurrently, comparing patients without DN and the control group was statistically significant for miR-126 (3.1-fold increase) and miR-155 (1.6-fold increase), with a trend toward increased miR-29b (4.1-fold increase). Histopathological analysis revealed that the detection of miRNA-126 and miR-29b was significantly higher in podocyte cells and endothelial cells from glomeruli, while miR-155 was higher in proximal tubule epithelial cells, which could also be used as a valuable biomarker of DN progression [44]. Moreover, An et al. proved that increased urinary miRNA-196a expression is correlated with the level of kidney injury and may constitute a valuable biomarker of progressive fibrotic lesions in DN. Urinary miR-196a levels correlated positively with proteinuria (rho = 0.385), duration of DM (rho = 0.255) and systolic blood pressure (rho = 0.267). Additionally, miR-196a was associated with glomerular sclerosis and renal interstitial fibrosis in patients with DN [45]. Zhao et al. discovered that miR-142-3p is weakly expressed in renal tubular epithelial cells stimulated by hyperglycemia. Increasing its expression can attenuate apoptosis and oxidative stress in chronic hyper-

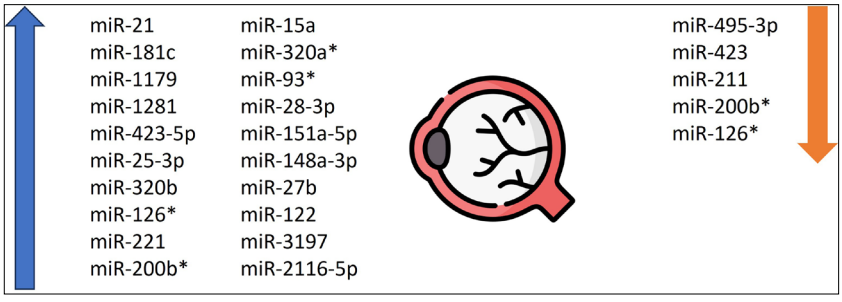

Figure 1. The role of miRNAs as diagnostic and prognostic markers in diabetic retinopathy

* molecules appearing in 2 or more articles

Table 2. Role of miRNAs as potential biomarkers in diabetic retinopathy [24-42]

Authors	Study group (n)*	Study material	miRNA	Change in expression	Targets
Jiang et al. 2017 [24]	189	plasma	miR-21	↑	TMEM49
Qing et al. 2014 [25]	200	serum	miR-21 miR-181c miR-1179	↑	PTEN Akt Erk1/2 VEGF
McAuley et al. 2015 [26]	531	plasma and urine	miR-126	SNP rs4636297-A	VEGF EGFL7
Greco et al. 2016 [27]	60	serum	miR-1281	↑	VEGFA HIF1AN
Xiao et al. 2017 [28]	30	plasma	miR-423-5p	↑	TFF1 NF-κB NFE2
Santovito et al. 2019 [29]	30	plasma	miR-25-3p miR-320b	↑	CDH1 PTEN NOTCH1
			miR-495-3p	↓	
Blum et al. 2019 [30]	69	serum	miR-423	↓	VEGF
García de la Torre et al. 2015 [31]	114	serum	miR-126 miR-221	↑	Spred-1 PI3K/Akt/eNOS Ras/ERK/VEGF MEK/ERK PAK1
Gomaa et al. 2017 [32]	59	vitreous	miR-200b	↑	Ets1 VEGFR2
Hirota et al. 2015 [33]	8	human vitreous and human whole blood	hsa-miR-15a hsa-miR320a hsa-miR-320b hsa-miR-93 hsa-miR-29a hsa-miR-423-5p	↑	-
Li et al. 2017 [34]	508	human plasma	miR-200b	↓	VEGFA PEDF
	70	rat retinal cells			
Liang et al. 2018 [35]	129	serum	miR-28-3p miR-151a-5p miR-148a-3p	↑	TGF-β MAPK
Liu et al. 2018 [36]	-	human vitreous cell lines	miR-211	↓	Sirt1
Murray et al. 2014 [37]	-	Mice cell lines	miR-200b	↑	Oxr1
Rezk et al. 2016 [38]	286	serum	miR-126	↓	VEGF EGFL7
Zampetaki et al. 2016 [39]	300	serum	miR-27b miR-320a	↑	TSP-1
Zou et al. 2017 [40]	267	plasma	miR-93	↑	TNF-α VEGF
Pastukh et al. 2019 [41]	40	serum	miR-122	↑	TIMP3
Huihui et al. 2020 [42]	90	serum cell lines	miR-3197 miR-2116-5p	↑	NOTCH2

* Not all articles mentioned the exact number of people and animals participating in the described study.

glycemia [46]. Moreover, miR-31 expression was reduced in the group with type 2 DM and coexisting DN was reduced relative to that in the group without complications. Interestingly, this difference was more prominent in the group with macroalbuminuria than in that with microalbuminuria [47]. Assmann et al. showed increased expression of miR-21-3p and miR-378-3p in a group with DN. In contrast, miR-16-5p and miR-29a-3p showed decreased expression compared with a group of patients with type 1 DM and moderate DN [48]. Furthermore, Delić et al. concluded that urinary miR-320c levels were elevated in DN patients compared to diabetic patients without complications and healthy controls [49]. Huang et al. found that increased level of miR-155 and miR-146a in subjects with DM and animal models leads to inflammation-induced glomerular endothelial damage, inducing the progression of DN [50]. Other researchers have observed that miR-155 deficiency attenuates DN during chronic exposure to hyperglycemia [51]. Liu et al. noted that miR-25 is downregulated in patients and animals with DN and the administration of miR-25 to mice inhibited the progression of renal damage [52]. Zanchi et al. concluded in their DN rat model with tubulointerstitial fibrosis, there was an 18-fold higher expression of miR-184 than in controls [53]. Another molecule with decreased expression in advanced DN was miR-98, and its overexpression inhibits the disease progression [54]. The data on DN are presented in Table 3. The role of miRNAs as diagnostic and prognostic markers in DN is shown in Figure 2.

Neuropathy

Diabetic neuropathy is a significant complication of DM and it occurs due to chronic exposure to elevated blood glucose levels. Recent studies and observations have shown that miRNAs are crucial for the onset of neuropathic pain. The contribution of miR-190a-5p to the overall pathomechanism is therefore significant. In an experiment on mice with induced DM and neuropathy, significantly reduced miR-190a-5p expression was identified after sampling the dorsal horns of the lumbar spine. Regarding the positive effects of expression-enhancing therapy and even the regression of some neuropathic lesions, this offers great hope for a novel treatment strategy for this disease [55]. Moreover, a study by Wang et al. provided reliable evidence for the role of miR-146a in the initiation of apoptosis of dorsal root ganglion neurons in an environment of chronic hyperglycemia and they found that DM reduced miR-146a expression in the mice model [56]. Another study identified miR-184--5p and miR-190a-5p as valuable therapeutic targets for patients with diabetic neuropathy [57]. Ciccacci et al. demonstrated increased levels of miR-128a in patients with diabetic polyneuropathy, whereas miR-155 and miR-499a levels were decreased in the patient group [58]. The same authors in another study noted that the T allele SNP in miR-128a was associated with a higher risk, while the C allele SNP in miR-146a had a lower risk of developing diabetic polyneuropathy. Moreover, the SNP in miR-27a was correlated with the probability of initial cardiovascular autonomic neuropathy, while the SNP in miR-146a proved to have a risk-reducing role [59]. The same group demonstrated that miR-499a may be involved in the development of diabetic neuropathy, specifically showing a higher risk of developing severe cardiovascular autonomic neuropathy [60]. Furthermore, Feng et al. noted decreased miR-146a

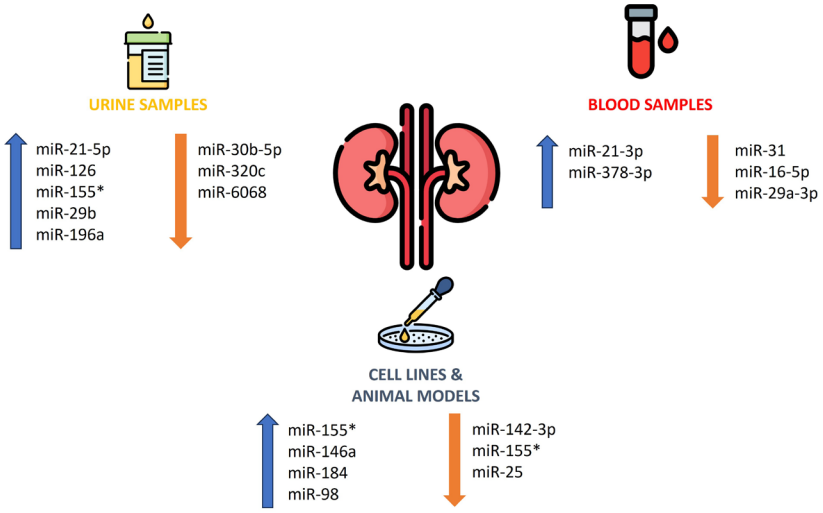

Figure 2. The role of miRNAs as diagnostic and prognostic markers in diabetic nepropathy
* molecules appearing in 2 or more articles

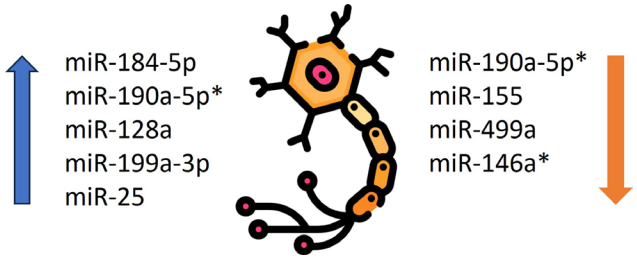

Figure 3. The role of miRNAs as diagnostic and prognostic markers in diabetic neuropathy
* molecules appearing in 2 or more articles

Table 3. Role of miRNAs as potential biomarkers in diabetic nephropathy [43-54]

Authors	Study group (n)*	Study material	miRNA	Change in expression	Targets
Zang et al. 2020 [43]	84	urine	miR-21-5p	↑	PTEN TGF-β EMT
			miR-30b-5p	↓	
Beltrami et al. 2018 [44]	192	urine	miR-126 miR-155 miR-29b	↑	TNF-α TGF-β
An et al. 2020 [45]	209	urine	miR-196a	↑	TGF-β Bram1
Zhao et al. 2021 [46]	-	HG-stimulated HK-2 cells	miR-142-3p	↓	BOD1
Rovira-Llopis et al. 2018 [47]	31	serum	miR-31	↓	Satb2 MAPK2
Assmann et al. 2019 [48]	58	serum	miR-21-3p miR-378-3p	↑	TGF-β1 Pi3K/Akt AGE-RAGE
			miR-16-5p miR-29a-3p	↓	
Delić et al. 2016 [49]	24	urine	miR-320c miR-6068	↓	TGF-β TSP-1
Huang et al. 2014 [50]	6	renal tissue cell lines	miR-155 miR-146a	↑	TGF-β1 TNF-α NF-κB
Lin et al. 2015 [51]	-	mice	miR-155	↓	WT-1 IL17A SOCS1
Liu et al. 2017 [52]	-	mice serum	miR-25	↓	CDC42
Zanchi et al. 2017 [53]	-	rats	miR-184	↑	LPP3 NF-κB
Zhu et al. 2019 [54]	-	Mouse mesangial cells and HEK-293T cells	miR-98	↓	HMGA2 Nedd4L TGF-β/ Smad2/3

* Not all articles mentioned the exact number of people and animals participating in the described study.

Table 4. Role of miRNAs as potential biomarkers in diabetic neuropathy [55-63]

Authors	Study group (n)*	Study material	miRNA	Change in expression	Targets
Yang et al. 2017 [55]	-	mice	miR-190a-5p	↓	SLC17A6
Wang et al. 2014 [56]	-	mice	miR-146a	↓	IRAK1 TRAF6
Gong et al. 2015 [57]	-	mice	miR-184-5p miR-190a-5p	↑	-
Ciccacci et al. 2020 [58]	49	serum	miR-128a	↑	PTEN
			miR-155 miR-499a	↓	
Ciccacci et al. 2014 [59]	260	serum	miR-128a miR-146a miR-27a	SNP	-
Ciccacci et al. 2018 [60]	150	serum	miR-499a	SNP	-
Feng et al. 2018 [61]	-	rats	miR-146a	↓	TNF-α IL-1β NF-κB
Li et al. 2017 [62]	110	plasma skin tissue	miR-199a-3p	↑	SerpinE2
Zhang et al. 2018 [63]	-	mice	miR-25	↑	AGE-RAGE Nox4

* Not all articles mentioned the exact number of people and animals participating in the described study.
SNP – single nucleotide polymorphism

expression in rats with diabetic neuropathy compared to those without it [61]. Li et al. proved that expression of miR-199a-3p was higher in the diabetic neuropathy group compared to patients without this complication [62]. Other researchers have pointed out that miR-25 plays a protective role against diabetic neuropathy, while the use of drugs that inhibit miR-25 may contribute to the development of this complication [63]. The data are presented in Table 4. The role of miRNAs as diagnostic and prognostic markers in diabetic neuropathy is shown in Figure 3.

Limitations of the study

We used a narrative rather than a systematic review method. No quality or risk of bias assessment was conducted. Due to the relatively small number of studies in human groups, data from both human groups and animal models were included in our review.

Conclusions

MiRNAs play an essential function in metabolic pathways and the pathogenesis of DM. MiRNAs are potential biomarkers for the evolution of distant consequences of DM and may serve as their therapeutic targets. Randomized trials on large groups of human populations are still lacking. More research is needed to understand the role and potential future diagnostic use of miRNAs.

Conflict of interest

None.

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References

1. Ong KL, Stafford LK, McLaughlin SA, Boyko EJ, Vollset SE, Smith AE, et al. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet [Internet]. 2023;402(10397):203–34. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673623013016
2. The Lancet. Diabetes: a defining disease of the 21st century. Lancet [Internet]. 2023;401(10394):2087. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673623012965
3. Zhou B, Rayner AW, Gregg EW, Sheffer KE, Carrillo-Larco RM, Bennett JE, et al. Worldwide trends in diabetes prevalence and treatment from 1990 to 2022: a pooled analysis of 1108 population-representative studies with 141 million participants. Lancet [Internet]. 2024;404(10467):2077–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673624023171
4. Papatheodorou K, Banach M, Bekiari E, Rizzo M, Edmonds M. Complications of Diabetes 2017. J Diabetes Res [Internet]. 2018;2018:1–4. Available from: https://www.hindawi.com/journals/jdr/2018/3086167/
5. Yu MG, Gordin D, Fu J, Park K, Li Q, King GL. Protective Factors and the Pathogenesis of Complications in Diabetes. Endocr Rev [Internet]. 2024;45(2):227–52. Available from: https://academic.oup.com/edrv/article/45/2/227/7252742
6. Ho PTB, Clark IM, Le LTT. MicroRNA-Based Diagnosis and Therapy. Int J Mol Sci [Internet]. 2022;23(13):7167. Available from: https://www.mdpi.com/1422-0067/23/13/7167
7. Li X, Dai A, Tran R, Wang J. Text mining-based identification of promising miRNA biomarkers for diabetes mellitus. Front Endocrinol (Lausanne) [Internet]. 2023;14. Available from: https://www.frontiersin.org/articles/10.3389/fendo.2023.1195145/full
8. Kraczkowska W, Stachowiak L, Pławski A, Jagodziński PP. Circulating miRNA as potential biomarkers for diabetes mellitus type 2: should we focus on searching for sex differences? J Appl Genet [Internet]. 2022;63(2):293–303. Available from: https://doi.org/10.1007/s13353-021-00678-5
9. Dinesen S, El-Faitarouni A, Frisk NLS, Sørensen AE, Dalgaard LT. Circulating microRNA as Biomarkers for Gestational Diabetes Mellitus—A Systematic Review and Meta-Analysis. Int J Mol Sci [Internet]. 2023;24(7):6186. Available from: https://www.mdpi.com/1422-0067/24/7/6186
10. He X, Kuang G, Wu Y, Ou C. Emerging roles of exosomal miRNAs in diabetes mellitus. Clin Transl Med [Internet]. 2021;11(6). Available from: https://onlinelibrary.wiley.com/doi/10.1002/ctm2.468
11. Morrison KR, Solly EL, Shemesh T, Psaltis PJ, Nicholls SJ, Brown A, et al. Elevated HDL-bound miR-181c-5p level is associated with diabetic vascular complications in Australian Aboriginal people. Diabetologia [Internet]. 2021;64(6):1402–11. Available from: https://link.springer.com/10.1007/s00125-021-05414-6
12. Al-Hayali MA, Sozer V, Durmus S, Erdenen F, Altunoglu E, Gelisgen R, et al. Clinical Value of Circulating Microribonucleic Acids miR-1 and miR-21 in Evaluating the Diagnosis of Acute Heart Failure in Asymptomatic Type 2 Diabetic Patients. Biomolecules [Internet]. 2019;9(5):193. Available from: https://www.mdpi.com/2218-273X/9/5/193
13. Yuan Y, Peng W, Liu Y, Xu Z. Circulating miR-130 and its target PPAR-γ may be potential biomarkers in patients of coronary artery disease with type 2 diabetes mellitus. Mol Genet Genomic Med [Internet]. 2019;7(9). Available from: https://onlinelibrary.wiley.com/doi/10.1002/mgg3.909
14. Dai B, Li H, Fan J, Zhao Y, Yin Z, Nie X, et al. MiR-21 protected against diabetic cardiomyopathy induced diastolic dysfunction by targeting gelsolin. Cardiovasc Diabetol [Internet]. 2018;17(1):123. Available from: https://cardiab.biomedcentral.com/articles/10.1186/s12933-018-0767-z
15. Luchessi AD. mRNA-miRNA integrative analysis of diabetes-induced cardiomyopathy in rats. Front Biosci [Internet]. 2017;9(2):483. Available from: https://imrpress.com/journal/FBS/9/2/10.2741/S483
16. Chen C, Yang S, Li H, Yin Z, Fan J, Zhao Y, et al. Mir30c Is Involved in Diabetic Cardiomyopathy through Regulation of Cardiac Autophagy via BECN1. Mol Ther - Nucleic Acids [Internet]. 2017;7:127–39. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2162253117301452
17. Chen S, Puthanveetil P, Feng B, Matkovich SJ, Dorn GW, Chakrabarti S. Cardiac miR-133a overexpression prevents early cardiac fibrosis in diabetes. J Cell Mol Med [Internet]. 2014;18(3):415–21. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jcmm.12218
18. Liu X, Xiao J, Zhu H, Wei X, Platt C, Damilano F, et al. miR-222 Is Necessary for Exercise-Induced Cardiac Growth and Protects against Pathological Cardiac Remodeling. Cell Metab [Internet]. 2015;21(4):584–95. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1550413115000662

19. Al-Muhtaresh HA, Salem AH, Al-Kafaji G. Upregulation of Circulating Cardiomyocyte-Enriched miR-1 and miR-133 Associate with the Risk of Coronary Artery Disease in Type 2 Diabetes Patients and Serve as Potential Biomarkers. J Cardiovasc Transl Res [Internet]. 2019;12(4):347–57. Available from: http://link.springer.com/10.1007/s12265-018-9857-2
20. Al-Kafaji G, Al-Mahroos G, Abdulla Al-Muhtaresh H, Sabry MA, Abdul Razzak R, Salem AH. Circulating endothelium-enriched microRNA-126 as a potential biomarker for coronary artery disease in type 2 diabetes mellitus patients. Biomarkers [Internet]. 2017;22(3–4):268–78. Available from: https://www.spandidos-publications.com/10.3892/etm.2016.3395
21. Jansen F, Wang H, Przybilla D, Franklin BS, Dolf A, Pfeifer P, et al. Vascular endothelial microparticles-incorporated microRNAs are altered in patients with diabetes mellitus. Cardiovasc Diabetol [Internet]. 2016;15(1):49. Available from: http://cardiab.biomedcentral.com/articles/10.1186/s12933-016-0367-8
22. Yang X, Li X, Lin Q, Xu Q. Up-regulation of microRNA-203 inhibits myocardial fibrosis and oxidative stress in mice with diabetic cardiomyopathy through the inhibition of PI3K/Akt signaling pathway via PIK3CA. Gene [Internet]. 2019;715:143995. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378111919306547
23. Deng X, Liu Y, Luo M, Wu J, Ma R, Wan Q, et al. Circulating miRNA-24 and its target YKL-40 as potential biomarkers in patients with coronary heart disease and type 2 diabetes mellitus. Oncotarget [Internet]. 2017;8(38):63038–46. Available from: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.18593
24. Jiang Q, Lyu X-M, Yuan Y, Wang L. Plasma miR-21 expression: an indicator for the severity of Type 2 diabetes with diabetic retinopathy. Biosci Rep [Internet]. 2017;37(2). Available from: https://portlandpress.com/bioscirep/article/37/2/BSR20160589/83197/Plasma-miR-21-expression-an-indicator-for-the
25. Qing S, Yuan S, Yun C, Hui H, Mao P, Wen F, et al. Serum MiRNA Biomarkers serve as a Fingerprint for Proliferative Diabetic Retinopathy. Cell Physiol Biochem [Internet]. 2014;34(5):1733–40. Available from: https://karger.com/article/doi/10.1159/000366374
26. McAuley AK, Dirani M, Wang JJ, Connell PP, Lamoureux EL, Hewitt AW. A genetic variant regulating miR-126 is associated with sight threatening diabetic retinopathy. Diabetes Vasc Dis Res [Internet]. 2015;12(2):133–8. Available from: https://journals.sagepub.com/doi/10.1177/1479164114560160
27. Greco M, Chiefari E, Accattato F, Corigliano DM, Arcidiacono B, Mirabelli M, et al. MicroRNA-1281 as a Novel Circulating Biomarker in Patients With Diabetic Retinopathy. Front Endocrinol (Lausanne) [Internet]. 2020;11. Available from: https://www.frontiersin.org/article/10.3389/fendo.2020.00528/full
28. Xiao Q, Zhao Y, Xu J, Li W-J, Chen Y, Sun H-J. NFE2/miR-423-5p/TFF1 axis regulates high glucose-induced apoptosis in retinal pigment epithelial cells. BMC Mol cell Biol [Internet]. 2019;20(1):39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31455213
29. Santovito D, Toto L, De Nardis V, Marcantonio P, D'Aloisio R, Mastropasqua A, et al. Plasma microRNA signature associated with retinopathy in patients with type 2 diabetes. Sci Rep [Internet]. 2021;11(1):4136. Available from: https://www.nature.com/articles/s41598-021-83047-w
30. Blum A, Meerson A, Rohana H, Jabaly H, Nahul N, Celesh D, et al. MicroRNA-423 may regulate diabetic vasculopathy. Clin Exp Med [Internet]. 2019;19(4):469–77. Available from: http://link.springer.com/10.1007/s10238-019-00573-8
31. García de la Torre N, Fernández-Durango R, Gómez R, Fuentes M, Roldán-Pallarés M, Donate J, et al. Expression of Angiogenic MicroRNAs in Endothelial Progenitor Cells From Type 1 Diabetic Patients With and Without Diabetic Retinopathy. Investig Opthalmology Vis Sci [Internet]. 2015;56(6):4090. Available from: http://iovs.arvojournals.org/article.aspx?doi=10.1167/iovs.15-16498
32. Gomaa AR, Elsayed ET, Moftah RF. MicroRNA-200b Expression in the Vitreous Humor of Patients with Proliferative Diabetic Retinopathy. Ophthalmic Res [Internet]. 2017;58(3):168–75. Available from: https://karger.com/article/doi/10.1159/000475671
33. Hirota K, Keino H, Inoue M, Ishida H, Hirakata A. Comparisons of microRNA expression profiles in vitreous humor between eyes with macular hole and eyes with proliferative diabetic retinopathy. Graefe's Arch Clin Exp Ophthalmol [Internet]. 2015;253(3):335–42. Available from: http://link.springer.com/10.1007/s00417-014-2692-5
34. Li E-H, Huang Q-Z, Li G-C, Xiang Z-Y, Zhang X. Effects of miRNA-200b on the development of diabetic retinopathy by targeting VEGFA gene. Biosci Rep [Internet]. 2017;37(2). Available from: https://portlandpress.com/bioscirep/article/37/2/BSR20160572/83287/Effects-of-miRNA-200b-on-the-development-of
35. Liang Z, Gao KP, Wang YX, Liu ZC, Tian L, Yang XZ, et al. RNA sequencing identified specific circulating miRNA biomarkers for early detection of diabetes retinopathy. Am J Physiol Metab [Internet]. 2018;315(3):E374–85. Available from: https://www.physiology.org/doi/10.1152/ajpendo.00021.2018

36. Liu H-N, Cao N-J, Li X, Qian W, Chen X-L. Serum microRNA-211 as a biomarker for diabetic retinopathy via modulating Sirtuin 1. Biochem Biophys Res Commun [Internet]. 2018;505(4):1236–43. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0006291X18321983
37. Murray AR, Chen Q, Takahashi Y, Zhou KK, Park K, Ma J. MicroRNA-200b Downregulates Oxidation Resistance 1 (Oxr1) Expression in the Retina of Type 1 Diabetes Model. Investig Opthalmology Vis Sci [Internet]. 2013;54(3):1689. Available from: http://iovs.arvojournals.org/article.aspx?doi=10.1167/iovs.12-10921
38. Rezk NA, Sabbah NA, Saad MSS. Role of MicroRNA 126 in screening, diagnosis, and prognosis of diabetic patients in Egypt. IUBMB Life [Internet]. 2016;68(6):452–8. Available from: https://iubmb.onlinelibrary.wiley.com/doi/10.1002/iub.1502
39. Zampetaki A, Willeit P, Burr S, Yin X, Langley SR, Kiechl S, et al. Angiogenic microRNAs Linked to Incidence and Progression of Diabetic Retinopathy in Type 1 Diabetes. Diabetes [Internet]. 2016;65(1):216–27. Available from: https://diabetesjournals.org/diabetes/article/65/1/216/34936/Angiogenic-microRNAs-Linked-to-Incidence-and
40. Zou H-L, Wang Y, Gang Q, Zhang Y, Sun Y. Plasma level of miR-93 is associated with higher risk to develop type 2 diabetic retinopathy. Graefe's Arch Clin Exp Ophthalmol [Internet]. 2017;255(6):1159–66. Available from: http://link.springer.com/10.1007/s00417-017-3638-5
41. Pastukh N, Meerson A, Kalish D, Jabaly H, Blum A. Serum miR-122 levels correlate with diabetic retinopathy. Clin Exp Med [Internet]. 2019;19(2):255–60. Available from: http://link.springer.com/10.1007/s10238-019-00546-x
42. Ji H, Yi Q, Chen L, Wong L, Liu Y, Xu G, et al. Circulating miR-3197 and miR-2116-5p as novel biomarkers for diabetic retinopathy. Clin Chim Acta [Internet]. 2020;501:147–53. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0009898119321011
43. Zang J, Maxwell AP, Simpson DA, McKay GJ. Differential Expression of Urinary Exosomal MicroRNAs miR-21-5p and miR-30b--5p in Individuals with Diabetic Kidney Disease. Sci Rep [Internet]. 2019;9(1):10900. Available from: https://www.nature.com/articles/s41598-019-47504-x
44. Beltrami C, Simpson K, Jesky M, Wonnacott A, Carrington C, Holmans P, et al. Association of Elevated Urinary miR-126, miR-155, and miR-29b with Diabetic Kidney Disease. Am J Pathol [Internet]. 2018;188(9):1982–92. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002944017311446
45. An Y, Zhang C, Xu F, Li W, Zeng C, Xie L, et al. Increased urinary miR-196a level predicts the progression of renal injury in patients with diabetic nephropathy. Nephrol Dial Transplant [Internet]. 2020;35(6):1009–16. Available from: https://academic.oup.com/ndt/article/35/6/1009/5231923
46. Zhao N, Luo Q, Lin R, Li Q, Ma P. MiR-142-3p ameliorates high glucose-induced renal tubular epithelial cell injury by targeting BOD1. Clin Exp Nephrol [Internet]. 2021;25(11):1182–92. Available from: https://link.springer.com/10.1007/s10157-021-02102-y
47. Rovira-Llopis S, Escribano-Lopez I, Diaz-Morales N, Iannantuoni F, Lopez-Domenech S, Andújar I, et al. Downregulation of miR-31 in Diabetic Nephropathy and its Relationship with Inflammation. Cell Physiol Biochem [Internet]. 2018;50(3):1005–14. Available from: https://karger.com/article/doi/10.1159/000494485
48. Assmann TS, Recamonde-Mendoza M, Costa AR, Puñales M, Tschiedel B, Canani LH, et al. Circulating miRNAs in diabetic kidney disease: case–control study and in silico analyses. Acta Diabetol [Internet]. 2019;56(1):55–65. Available from: http://link.springer.com/10.1007/s00592-018-1216-x
49. Delić D, Eisele C, Schmid R, Baum P, Wiech F, Gerl M, et al. Urinary Exosomal miRNA Signature in Type II Diabetic Nephropathy Patients. Jeyaseelan K, editor. PLoS One [Internet]. 2016;11(3):e0150154. Available from: https://dx.plos.org/10.1371/journal.pone.0150154
50. Huang Y, Liu Y, Li L, Su B, Yang L, Fan W, et al. Involvement of inflammation-related miR-155 and miR-146a in diabetic nephropathy: implications for glomerular endothelial injury. BMC Nephrol [Internet]. 2014;15(1):142. Available from: https://bmcnephrol.biomedcentral.com/articles/10.1186/1471-2369-15-142
51. Lin X, You Y, Wang J, Qin Y, Huang P, Yang F. MicroRNA-155 Deficiency Promotes Nephrin Acetylation and Attenuates Renal Damage in Hyperglycemia-Induced Nephropathy. Inflammation [Internet]. 2015;38(2):546–54. Available from: http://link.springer.com/10.1007/s10753-014-9961-7
52. Liu Y, Li H, Liu J, Han P, Li X, Bai H, et al. Variations in MicroRNA-25 Expression Influence the Severity of Diabetic Kidney Disease. J Am Soc Nephrol [Internet]. 2017;28(12):3627–38. Available from: https://journals.lww.com/00001751-201712000-00022
53. Zanchi C, Macconi D, Trionfini P, Tomasoni S, Rottoli D, Locatelli M, et al. MicroRNA-184 is a downstream effector of albuminuria driving renal fibrosis in rats with diabetic nephropathy. Diabetologia [Internet]. 2017;60(6):1114–25. Available from: http://link.springer.com/10.1007/s00125-017-4248-9

54. Zhu Y, Xu J, Liang W, Li J, Feng L, Zheng P, et al. miR-98-5p Alleviated Epithelial-to-Mesenchymal Transition and Renal Fibrosis via Targeting Hmga2 in Diabetic Nephropathy. Int J Endocrinol [Internet]. 2019(1):4946181. Available from: https://doi.org/10.1155/2019/4946181
55. Yang D, Yang Q, Wei X, Liu Y, Ma D, Li J, et al. The role of miR-190a-5p contributes to diabetic neuropathic pain via targeting SLC17A6. J Pain Res [Internet]. 2017;Volume 10:2395–403. Available from: https://www.dovepress.com/the-role-of-mir--190a-5p-contributes-to-diabetic-neuropathic-pain-via-t-peer-reviewed-article-JPR
56. Wang L, Chopp M, Szalad A, Zhang Y, Wang X, Zhang RL, et al. The role of miR-146a in dorsal root ganglia neurons of experimental diabetic peripheral neuropathy. Neuroscience [Internet]. 2014;259:155–63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S030645221301004X
57. Gong Q, Lu Z, Huang Q, Ruan L, Chen J, Liang Y, et al. Altered microRNAs expression profiling in mice with diabetic neuropathic pain. Biochem Biophys Res Commun [Internet]. 2015;456(2):615–20. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0006291X1402169X
58. Ciccacci C, Latini A, Colantuono A, Politi C, D'Amato C, Greco C, et al. Expression Study of Candidate miRNAs And Evaluation of their Potential use as Biomarkers of Diabetic Neuropathy. Epigenomics [Internet]. 2020;12(7):575–85. Available from: https://www.tandfonline.com/doi/full/10.2217/epi-2019-0242
59. Ciccacci C, Morganti R, Di Fusco D, D'Amato C, Cacciotti L, Greco C, et al. Common polymorphisms in MIR146a, MIR128a and MIR27a genes contribute to neuropathy susceptibility in type 2 diabetes. Acta Diabetol [Internet]. 2014;51(4):663–71. Available from: http://link.springer.com/10.1007/s00592-014-0582-2
60. Ciccacci C, Latini A, Greco C, Politi C, D'Amato C, Lauro D, et al. Association between a MIR499A polymorphism and diabetic neuropathy in type 2 diabetes. J Diabetes Complications [Internet]. 2018;32(1):11–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1056872717303215
61. Feng Y, Chen L, Luo Q, Wu M, Chen Y, Shi X. Involvement of microRNA-146a in diabetic peripheral neuropathy through the regulation of inflammation. Drug Des Devel Ther [Internet]. 2018;Volume 12:171–7. Available from: https://www.dovepress.com/involvement-of-microrna-146a-in-diabetic-peripheral-neuropathy-through-peer-reviewed-article-DDDT
62. Li Y-B, Wu Q, Liu J, Fan Y-Z, Yu K-F, Cai Y. miR-199a-3p is involved in the pathogenesis and progression of diabetic neuropathy through downregulation of SerpinE2. Mol Med Rep [Internet]. 2017;16(3):2417–24. Available from: https://www.spandidos-publications.com/10.3892/mmr.2017.6874
63. Zhang Y, Song C, Liu J, Bi Y, Li H. Inhibition of miR-25 aggravates diabetic peripheral neuropathy. Neuroreport [Internet]. 2018;29(11):945–53. Available from: https://journals.lww.com/00001756-201808010-00011

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REVIEW ARTICLE

The potential of CRISPR-Cas technology in medicine: a current overview of advancing therapeutics

Anastazja Murawska[1], Marta Modrzyk[1], Klaudia Maria Kufel[1], Daniel Łukasz Czyż[1], Michał Braczkowski[2]

[1] Student Scientific Society of Physiology, Department of Physiology, Institute of Medical Sciences, University of Opole, Poland
[2] Department of Physiology, Institute of Medical Sciences, University of Opole, Poland

Abstract

he revolutionary CRISPR-Cas9 system, originating from bacterial defense mechanisms, has swiftly reshaped biomedical research, demonstrating broad applicability in addressing diverse human diseases, including hereditary disorders, non-communicable diseases, neurological illnesses, and viral infections. While the immense potential of CRISPR-Cas9 is evident, critical challenges (e.g. off-targe effects, immune responses, and the ethical considerations of germline editing) persist, underscoring the crucial need for ongoing clinical trials and advancements in delivery methods to ensure its efficacy and long-term safety.

Keywords: CRISPR-Cas9 technology in medicine · gene editing · therapeutic applications · off-target effects

Citation

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Corresponding author:
Klaudia Maria Kufel, Student Scientific Society of Physiology, Department of Physiology, Institute of Medical Sciences, University of Opole, Poland
e-mail: klaudiakufel@interia.pl
Available online: ejtcm.gumed.edu.pl

Introduction

In 1987, Yoshizumi Ishino discovered clustered regularly interspaced short palindromic repeats (CRISPRs) while searching the *E. coli* genome. This moment became a landmark in the research of distinct prokaryotic organisms' genomes, and it contributed to the development of CRISPR-Cas9 technology by Emmanuelle Charpentier and Jennifer Doudna. In 2020, the two scientists were awarded the Nobel Prize in Chemistry [1]. This discovery has revolutionized the field of genome editing and opened the door to novel therapies previously unavailable.

Nowadays, the CRISPR-Cas9 technology is being used in a greater number of therapeutic concepts thanks to our comprehensive knowledge of the human genome. It enables the correction of harmful base mutations or the disruption of disease-causing genes with great precision and efficiency, providing a permanent treatment [2]. The initial ideas were focused on hereditary diseases and based on the concept of correcting mutated genes. However, as time passed and additional research was conducted, the potential targets for its application have extended. Over the past decade, research into CRISPR-Cas9 usage in the treatment of various acquired diseases, including cancers, haemolytic and cardiovascular diseases, immunodeficiency and visual or even neurodegenerative disorders, has expanded significantly, with positive outcomes [3]. This technique has a dramatic impact on the field of gene modification, providing a huge foundation for the development of the research field. However, off-target effects and delivery challenges persist.

There is a long-standing ethical debate about using CRISPR-Cas9 for germline editing due to its ability to modify both germ and somatic cells. These modifications could be passed on to future generations, with unpredictable consequences [4].

In this review, we illustrated the evolution of CRISPR-Cas9 from a fundamental biological tool to clinical use and efficacy in treating various diseases, including hereditary conditions, cancers and viral infections. We outlined specific ways of using CRISPR-Cas9 in exact therapy models and the latest treatment methods.

To provide the latest information on the implementation of CRISPR-Cas9 in biomedical research, we reviewed the most relevant and recent studies. Furthermore, covered the current status of CRISPR clinical trials and delivery methods, as well as the related challenges and opportunities.

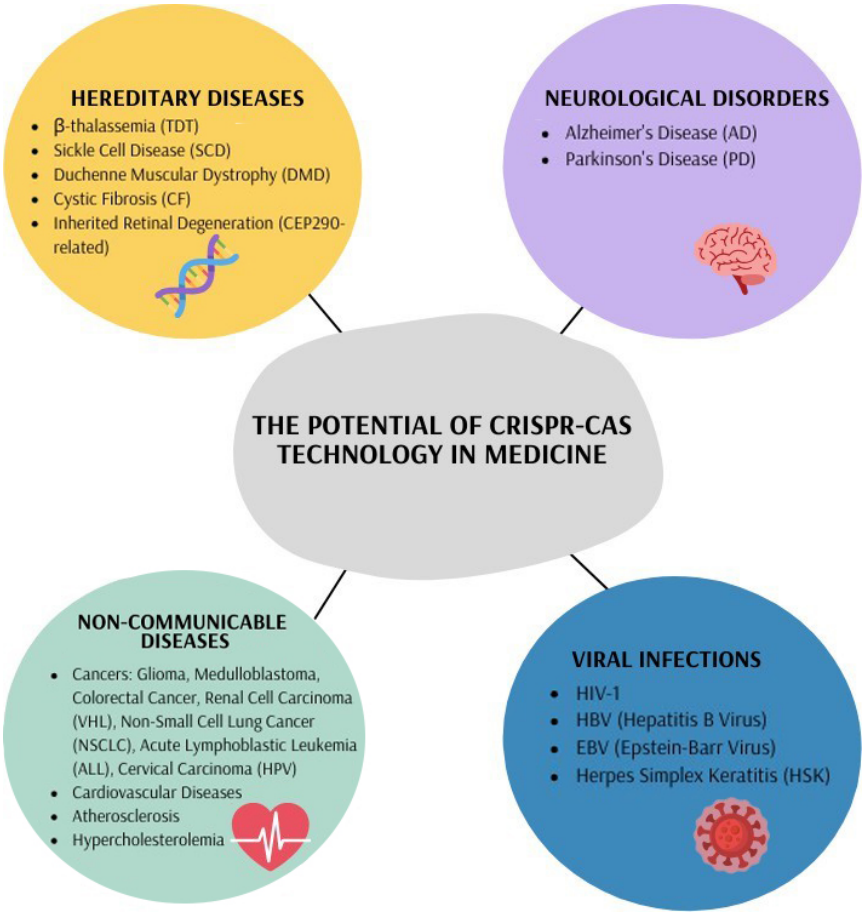

Figure 1. The potential of CRISPR-Cas technology in medicine

An overview of CRISPR-Cas systems

The immune defense of prokaryotes against phage infections, plasmid transfer and foreign substances is determined by the cooperation of CRISPR and an endonuclease called Cas9 protein [5]. Isolated from a prokaryotic cell, the two-component system of single guide RNA (sgRNA) and Cas9, enables a three-step gene modification process in eukaryotic cells: recognition, cleavage and repair. It involves the detection of complementary recipient's DNA (deoxyribonucleic acid) strands and their cleavage, followed by repair of the target sequence by the mechanisms of eukaryotic cells, such as non-homologous end joining or homology-directed repair [6]. The main

delivery strategies for introducing CRISPR-Cas9 systems into cells are physical, viral vector and non-viral vector methods with their distinct advantages and limitations. Electroporation, microinjection and hydrodynamic tail-vein injection are primarily used in vitro studies and are classified as physical methods. Otherwise, in in vivo therapeutic trials, pivotal roles are played by lentivirus (LV) vectors, adeno-associated virus (AAV) vectors, and adenovirus (AV) vectors, as well as polymer and lipid nanoparticles or other inorganic carriers categorized as non-viral tools [7]. Gene therapy utilizes two distinct strategies for delivering therapeutic genetic material – in vivo and ex vivo.

They differ from each other in several ways. In vivo therapy does not involve removing, modifying and reintroducing the patient's cells, which reduces costs. It is mainly used to treat monogenic diseases that affect organs such as the eyes, lungs, liver, and muscles [1, 8].

Ex vivo therapy involves extracting cells from a patient, correcting mutations, and then transplanting the modified cells back into the patient. It frequently targets stem cells because the corrected cells can quickly replace those with faulty genes [8]. Additionally, ex vivo modification of T cells for chimeric antigen receptor (CAR)-T cell therapies carries a lower risk of disrupting normal gene regulation by insertional events near oncogenes or tumor suppressor genes, which could potentially lead to malignant transformation. This approach can be used for specific cell types that are easy to access, isolate, modify genetically and reintroduce into the patient.

However, the number of cells that can be modified is limited by several factors. These include how efficiently the cells can be collected. They also include how viable and expandable the modified cells are and also include how successful the reinfusion of the engrafted cells is [1].

Fewer off-target effects and enhanced editing outcomes while using CRISPR-Cas9 technology play a superior role among other alternative genome editing tools, such as zinc finger nucleases (ZFNs) and TALENs, by providing high efficiency and accuracy [5]. CRISPR gene editing relies on RNA-guided nucleases that introduce double-stranded (DSB) or single-stranded (SSB) breaks in DNA or RNA, and CRISPR genome engineering uses a variety of these nucleases. Each one is distinguished by its target specificity and mechanism of action. For instance, Cas9 generates double-stranded DNA breaks at NGG PAM sites, whereas Cas12 enzymes recognise a protospacer adjacent motif sequence containing mostly thymine bases (T-rich PAMs). They create staggered cuts that facilitate multiplex editing. Cas13 proteins exclusively target single-stranded RNA, enabling precise transcript modulation. The ultra- compact Cas14 family targets single-stranded DNA. This offers advantages for therapeutic delivery where vector size is limited [9-11].

Laboratory examinations indicate that Cas9 can overlook several base-pair mismatches, which could lead to cleavage at off-target sites or slight mismatches in the sequence of the sgRNA [12]. To minimise off-target effects, it is crucial to conduct trials using high-fidelity Cas9 and optimised guide RNA design. Computational algorithms and bioinformatics tools can assist researchers in predicting potential off-target sites [3]. The outcomes of CRISPR-Cas gene editing in humans could be significantly impacted by immune system cascade responses triggered by viral vectors, which could have detrimental consequences for overall wellbeing [3, 13]. Owing to this, pre-existing immune responses to CRISPR effectors and viral vectors (as a form of a cytokine storm) should be considered in personalised treatment strategies. This also applies in terms of exposure to the source bacteria or cross--reactivity with similar epitopes in order to minimise the risk of adverse immune reactions [14]. Another key limitation to the utilisation of CRISPR-Cas9 proteins for genome editing is the requirement that a protospacer adjacent motif (PAM) be present at the target site. The Cas9 target site must contain a protospacer PAM sequence to support recognition by this endonuclease. The requirement for a specific PAM sequence reduces the number of possible target sites available for gene knockouts, knock-ins, or highly precise edits, since Cas9 can only operate where an appropriate PAM is present [15-16]. Particularly, it is problematic in disease-related applications, where pathogenic mutations occur at fixed genomic positions that may lack a suitable PAM nearby, making therapeutic editing difficult or even impossible [16]. PAM scarcity also complicates the investigation of regulatory regions and non-coding elements, which often have low PAM density and cannot be easily targeted [17].

BE (Base Editing) is the latest evolution of CRISPR-Cas systems, allowing the direct introduction of point mutations into cellular DNA without the induction of double-strand breaks (DSBs). Two classes of DNA base editors have been identified: cytosine base editors (CBE) and adenine base editors (ABE). The gene editing toolkit has recently been expanded by prime editing (PE), which allows the introduction of all twelve possible transition and transversion mutations, as well as small insertions or deletions [18].

Material and methods

We searched through PubMed and ScienceDirect databases for clinical trials published between January 2015 and March 2025, which focused on CRISPR-Cas9 applications in medicine. Keywords used in the search included "CRISPR-Cas9 in medicine", "genetic disorders and CRISPR-Cas9 technology", "non-communicable diseases and CRISPR-Cas9 method implementation", "CRISPR-Cas9 applications in neurology",

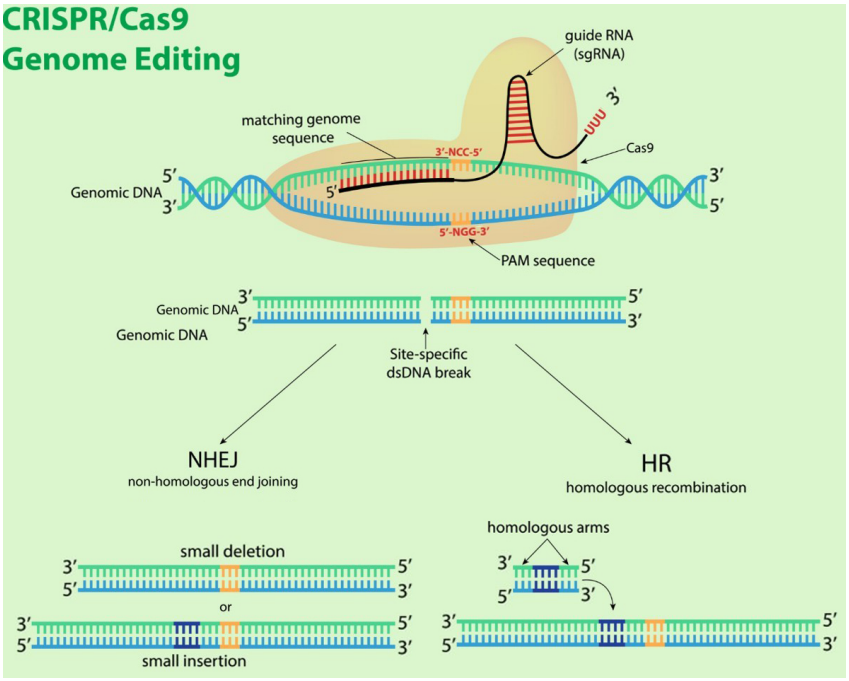

Figure 2. Schematic diagram of CRISPR-Cas9 complex
(Source: AI Generation- Gemini 2.5 Flash Model, 2025)

and "genome modification by CRISPR-Cas9 in viral infections". Studies not written in English were excluded, and the references of included studies were screened for additional relevant publications.

Results and discussion

Hereditary diseases

CRISPR-Cas tools are widely used in correcting genetic mutations for the treatment of hereditary monogenic disorders such as transfusion-dependent thalassemia (TDT) and sickle cell disease (SCD) [19]. Both are caused by mutations in the haemoglobin subunit gene (HBB). CRISPR-Cas9 technology allows the reduction of BCL11A expression, a key regulator of β-globin locus expression, by targeting the erythroid-specific enhancer region of BCL11A in haematopoietic stem and progenitor cells (HSPCs). By restoring γ-globin synthesis and enhancing fetal haemoglobin production, morbidity and mortality measures in patients with TDT and SCD improve [20].

The primary clinical trial for β-thalassaemia, CLIMB-THAL-111, was analysed in the 2024 interim analysis. The study showed that 91% of the 52 TDT patients achieved transfusion independence following the infusion of CD34+ HSPCs that had been edited using CRISPR-Cas9. Haemoglobin levels stabilised at a mean of 13.1 g/dL total haemoglobin (Hb) and 11.9 g/dL fetal haemoglobin (HbF), with a pancellular distribution of ≥ 94% of red blood cells. No deaths or cancers were reported [21].

The normal structure of the retina, kidney, brain, and a wide range of other bodily organs is dependent on primary cilium formation, which is based on the expression of the gene encoding centrosomal protein 290 (CEP290). Inherited retinal degeneration associated with CEP290 (historically known as Leber congenital amaurosis) is a common cause of visual impairment in the first decade of life. This results from the progressive disorganisation of the outer segments of the rod and cone photoreceptors, as well as the early loss of rod cells in the mid-peripheral retina. In vivo CRISPR-Cas9 therapy involves the permanent removal of the CEP290 IVS26 variant using an AAV vector containing the Staphylococcus aureus (SaCas9) nuclease under the photoreceptor-specific GRK1 promoter [22].

Duchenne muscular dystrophy (DMD) is a fatal X-linked recessive disease that is induced by DMD gene mutations, causing muscle fiber damage during contractions [23]. The significant length of the DMD gene has led to the identification of over 7000 mutations associated with DMD. These often trigger frameshifts and premature stop codons, which subsequently result in dystrophin deficiency [24]. CRISPR-mediated gene editing targets either exon 23 or the mutational hotspot in the DMD gene, causing the removal of the nonsense mutation. The major functional improvements, such as increased contractile force and the restoration of the dystrophin protein, are significant, bearing in mind that complete gene correction was not required [25].

Genetic lesions located on human chromosome 7 (7q31.2) form the basis of cystic fibrosis (CF), a systemic disease that affects multiple organs. The disruption of the crucial cAMP-activated anion channel and CF transmembrane conductance regulator (CFTR) protein production can be caused by over 2000 mutations. These contribute to clinical manifestations that vary in severity, presenting a considerable challenge in the development of targeted treatments [26]. Correction of the F508 mutation by the CRISPR-based gene approach results in the restoration of CFTR channel function [27]. Other studies show that CFTR gene mutations in induced

pluripotent stem cells (iPSCs) are achievable via CRISPR-Cas9 technology [28].

Non-communicable diseases

CRISPR-Cas9 technology has become established in cancer research, with applications including: oncogene inactivation, immune checkpoint modulation; genetic mutation correction; and targeted delivery of cancer-killing payloads. The combination of this technique with viability assays and molecular analyses is a valuable approach for identifying drug targets. CRISPR-Cas9 reveals the molecular processes of cancer cells as a result of gene alteration disruption. This enables the selective elimination of cancer cells while sparing healthy ones [29]. The recognition of neoplastic cells and their elimination capabilities by the immune system can be improved in adoptive cell transfer therapies through CRISPR-Cas9-mediated genomic manipulations. Upregulation of gene expression that encodes strategic checkpoint proteins boosts the activation and proliferation of cytotoxic T lymphocytes and other effectors, subsequently improving their ability to eliminate cancer cells [30]. The advantages of CRISPR may help address key challenges associated with chimeric antigen receptor T (CAR-T) cell therapy.

In CAR-T therapy, a patient's (or donor's) T lymphocytes express a chimeric antigen receptor (CAR). It recognises a specific antigen on the patient's cancer cells. Co-delivery of the Cas9-sgRNA complex into primary T cells can either knock out endogenous genes (e.g. TCR and certain inhibitors) or introduce a CAR gene [31]. The use of CRISPR-Cas9 allows the insertion of the CAR gene at a defined genomic locus in a donor's lymphocyte's DNA template. template ('knock-in' strategy). On the other hand, the 'knock-out strategy' disrupts endogenous genes that may limit T-cell performance, cause immune rejection (in the case of donor cells) or reduce persistence. That way, when T cells are reinfused into the patient, they can target and kill cells that express the relevant antigen (e.g. B-cell malignancies) [32].

According to a systematic review and meta-analysis of preclinical studies (i.e. on animal models), CRISPR-Cas9 enhances the therapeutic effect of CAR-T cells. This is achieved through a significant reduction in tumor volume and an improvement in overall survival in almost every model. These findings were consistent across multiple tumor types and multiple CRISPR targets [33]. CRISPR-Cas9 is used to create allogeneic 'universal' CAR-T cells by knocking out the endogenous T-cell receptor (TCR) and/ or eliminating HLA Class I expression, which reduces the risk of graft-versus-host disease (GVHD) and host rejection [34].

CRISPR-Cas9 technology can specifically target genes in non-small-cell lung cancer or, combined with CAR-T, is a promising treatment for relapsed/refractory acute lymphoblastic leukemia [35-36] High-fidelity gene editing, manageable toxicity and an 83.3% complete-remission rate were shown by CRISPR-Cas9-engineered universal CD19/CD22 CAR-T cells (CTA101) in a first-in-human phase I trial for r/r ALL. These results demonstrate that dual-target CRISPR-CAR T-cell therapy is clinically feasible and could eliminate the delays and antigen-escape relapse associated with autologous single-target CAR T-cell therapy [36].

CRISPR-Cas9 has the potential to become a cure for medulloblastoma and glioma, due to targeting genes like Ptch1, Nf1, Pten, and Trp53. There is also ongoing research for orthotopic organoid transplantation to repair Trp53 and APC - tumor suppressor genes in epithelial cells, or the Von Hippel Lindau (VHL) gene in renal cells. This could be meaningful for treating colorectal cancer and renal cell carcinoma [38]. Targeting and disrupting specific viral genes within the human genome, such as the E6 or E7 of human papillomavirus (HPV), offers the advantage of suppressing oncogenesis, which can prevent cervical carcinoma [37-38] Compiling the data, CRISPR-Cas9 can be used as an alternative to epigenetic drugs, causing less delivery problems and minimalizing adverse effects [39]. The emergence of CRISPR genome editing has started a new age of research on treating cardiovascular diseases. It has been a useful tool in generating cell lines and mouse models to study genetic cardiomyopathies caused by site-specific mutations, such as long-QT syndrome, Duchenne muscular dystrophy (DMD), Barth syndrome, and hypertrophic cardiomyopathy (HCM) [40-41]. Lowering blood lipids in hypercholesterolaemia, by using CRISPR-Cas9 can be achieved in following ways: reducing the activity of the lipoprotein and endothelial lipase inhibitor angiopoietin-like 3 (ANGPTL3) or targeting hepato-cyte-derived apolipoprotein C3 (APOC3) [42]. Through the application of the CRISPR-Cas9 system, numerous studies have established disease models of atherosclerosis and revealed potential molecular targets relevant to its pathogenesis. In a Phase 1, first-in-human trial, CTX310 (CRISPR-Cas9 targeting ANGPTL3) was found to significantly lower LDL cholesterol (by approximately 50%) and triglycerides (around 55%) in 15 individuals with severe or refractory lipid disorders such as familial hypercholesterolemia, mixed dyslipidaemia, or severe hypertriglyceridaemia. Phase 2 (or larger-scale) trials have not yet been published. The long-term safety and effectiveness of the treatment, and its impact on atherosclerotic outcomes such as plaque regression and cardiovascular events, are still unknown [43].

Neurological disorders

Alzheimer's disease (AD) is a neurodegenerative disorder caused by the accumulation of beta-amyloid plaques in the extracellular space and the formation of intracellular neurofibrillary tangles composed of tau proteins [44]. There are two

types of AD: sporadic and familial. The familial form is rare, but there is strong evidence which gene variants can disturb the β-amyloid (Aβ) metabolism. Mutations arise in: APP (Amyloid-precursor protein), presenilin-1 (PSEN1), and presenilin-2 (PSEN2). Studies reported that using CRISPR-Cas9 technology the expression of Aβ protein decreases when pathological APP alleles are deleted, and they also identified possible protective deletion mutations in the 3'-UTR of the APP gene, leading to a massive reduction of Aβ accumulation when part of the gene was removed. PSEN1 and PSEN2 are essential components in the regulation of γ-secretase, an enzyme responsible for modulating Aβ levels. These specific genes are additional targets for the CRISPR- Cas9 system [45]. Contrary to intuition, the sporadic form of AD also has genetic factors, such as the Apolipoprotein E gene variant APOE4, which can promote neuroinflammatory processes [46]. Recent studies showed that using the CRISPR-Cas9 method to correct the APOE4 allele to E3, it is possible to reduce neuronal susceptibility to ionomycin-induced cytotoxicity, decrease tau phosphorylation, and affect Aβ metabolism [45]. Parkinson's disease (PD) is a health condition characterized by the degeneration of dopaminergic neurons in the substantia nigra in the area of the basal ganglia. Research has identified several genetic factors that contribute to PD susceptibility, notably mutations in the alpha-synuclein (SNCA), where the mutation results in an abnormal α-synuclein protein that aggregates and accumulates due to impaired proteostasis (UPS – Ubiquitin proteasome pathway and ALP – Autphagy-lysosomal pathway) and PINK1 (PTEN-induced putative kinase), PRKN (Parkin RBR E3 Ubiquitin Protein Ligase), DJ-1 (Daisuke-Junko-1), LRRK2 (Leucine rich repeat kinase 2), and PGC-1α (Pparg coactivator 1 alpha) mutations, which contribute to mitochondrial dysfunction, triggering of neuroinflammatory pathways. These listed genes are also targets for CRISPR-based therapies [47-48].

Viral infections

CRISPR-Cas9 editing offers a potential strategy to target and inactivate integrated viral genomes, particularly in the context of HIV infection [49]. Evidence shows that CRISPR-Cas9 is capable of inducing mutations or excisions in the proviral genome in cells that were latently infected [50]. CRISPR-based HIV-1 strategies include removing the Δ32 to CCR5 (Chemokine C-C motif Receptor 5) mutation in haematopoietic stem cells (HSPCs) and T cells, rendering them resistant to HIV-1 infection [51]. It is also possible to use the CRISPR-Cas9 to modulate HBV's cccDNA and inactivate the expression of HBV core antigen (HBcAg). Moreover, CRISPR-Cas9 can be applied to suppress EBV's LMP1, the viral gene that is present in nasopharyngeal carcinoma (NPC) cell lines. By using this method, the tumor growth was inhibited [52]. As results have shown, CRISPR-Cas9/gRNA technology can be seen as a potential treatment for herpes simplex keratitis (HSK). Using vectors, CRISPR-Cas9/gRNA allows to stop the viral replication, destroying HSV reservoirs inside the trigeminal ganglion neurons [53].

Table 1. Summary of key studies

Disease / study target	Significant findings	Study authors
β-Thalassemia & Sickle Cell Disease (edition of BCL11A enhancer in HSPCs)	Editing the BCL11A enhancer using CRISPR-Cas9 increased HbF expression, with 91% of patients achieving transfusion independence.	Locatelli et al. [21]
CEP290-associated inherited retinal degeneration	In vivo AAV-SaCas9 removal of CEP290 IVS26 mutation has shown improved photoreceptor function in early trials.	Pierce et al. [22]
Duchenne Muscular Dystrophy (DMD gene modification)	Exon 23 or hotspot deletion restored dystrophin expression and improved muscle contraction in preclinical models.	Erkut & Yokota [25]
Cystic fibrosis (CFTR F508del correction)	Correcting the ΔF508 mutation restored the function of the CFTR channel in both airway epithelial cells and in induced pluripotent stem cells (iPSCs) derived from patients.	Maule et al. [27]

Table 1. Summary of key studies (continued)

Disease / study target	Significant findings	Study authors
CAR-T engineering: TCR knockout & CAR knock-in	CRISPR/Cas9 enhanced CAR-T cell therapy, reducing the risk of GVHD and improving treatment safety.	Razeghian et al. [34]
Universal CAR-T for r/r ALL (CTA101 trial)	An 83.3% complete remission rate of the disease was achieved by dual-target CD19/CD22 CAR-T cells engineered by CRISPR/Cas9.	Hu et.al. [36]
HPV associated cervical cancer (E6/E7 disruption)	CRISPR/Cas9 targets E6/E7, suppressing oncogenesis and reducing tumor growth.	Rabaan et al. [37]
Cardiovascular disease – ANGPTL3 gene knockout (CTX310)	CTX310 editing led to a 50% reduction in LDL and a 55% reduction in triglycerides in Phase 1 of the trial.	Laffin et al. [43]
Alzheimer's disease – APP, PSEN1/2 and APOE4 gene editing	The deletion of pathogenic APP alleles reduces Aβ accumulation. Targeting PSEN genes modulates γ-secretase activity. Decreasing tau protein phosphorylation involves APOE4 gene editing.	De Plano et al. [45]
Parkinson's disease (SNCA, PINK1, PRKN, LRRK2)	Editing PD-related genes reduces the accumulation of α-synuclein and neuroinflammation.	Thapar et al. [47], Pinjala et al. [48]
CCR5 Δ32 mimicry for HIV resistance	Editing using CRISPR/Cas9 in HSPCs and T cells conferred resistance to HIV-1 entry.	Zhang et al. [51]
HBV (cccDNA targeting)	Targeting cccDNA with CRISPR/Cas9 reduced HBcAg expression and viral replication.	Najafi et al. [52]
EBV – LMP1 knockout (NPC models)	Suppression of tumor growth by inactivating LMP1 in EBV-positive cell lines.	Najafi et al. [52]
HSV – herpes simplex keratitis	Inhibiting HSV replication and eradicating viral reservoirs in the trigeminal ganglia.	Wang et al. [26]

Conclusion

The biomedical research has been revolutionized by CRISPR-Cas9 technology, which has significantly transformed our perspective even on inherited, previously incurable disorders. Novel treatments for various diseases, including hereditary, cardiovascular and neurological conditions, as well as certain tumors, particularly those associated with viral infections, are reachable by precise and efficient manipulation of the human genome using this technique. The recurring issue of adverse effects highlights the need for further research into high-fidelity Cas9 variants and the optimized guide RNAs. Nevertheless, the ongoing discussion surrounding the ethics of germline manipulation has yet to yield any definitive conclusions.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Gostimskaya I. CRISPR–Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. Biochem [Internet]. 2022;87(8):777–88. Available from: https://link.springer.com/10.1134/S0006297922080090
2. Huang J, Zhou Y, Li J, Lu A, Liang C. CRISPR/Cas systems: Delivery and application in gene therapy. Front Bioeng Biotechnol [Internet]. 2022;10:942325. Available from: http://www.ncbi.nlm.nih.gov/pubmed/36483767
3. Aljabali AAA, El-Tanani M, Tambuwala MM. Principles of CRISPR-Cas9 technology: Advancements in genome editing and emerging trends in drug delivery. J Drug Deliv Sci Technol [Internet]. 2024;92:105338. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1773224724000066
4. Alsmani S. Ethical issues concerned with CRISPR-cas9 system. 2023.
5. Li T, Yang Y, Qi H, Cui W, Zhang L, Fu X, et al. CRISPR/Cas9 therapeutics: progress and prospects. Signal Transduct Target Ther [Internet]. 2023;8(1):36. Available from: https://www.nature.com/articles/s41392-023-01309-7
6. Asmamaw M, Zawdie B. Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. Biologics [Internet]. 2021;15:353–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/34456559
7. Du Y, Liu Y, Hu J, Peng X, Liu Z. CRISPR/Cas9 systems: Delivery technologies and biomedical applications. Asian J Pharm Sci [Internet]. 2023;18(6):100854. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1818087623000818
8. Volodina O, Smirnikhina S. The Future of Gene Therapy: A Review of In Vivo and Ex Vivo Delivery Methods for Genome Editing-Based Therapies. Mol Biotechnol [Internet]. 2025;67(2):425–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38363528
9. Hillary VE, Ceasar SA. A Review on the Mechanism and Applications of CRISPR/Cas9/Cas12/Cas13/Cas14 Proteins Utilized for Genome Engineering. Mol Biotechnol [Internet]. 2023;65(3):311–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/36163606
10. Kleinstiver BP, Sousa AA, Walton RT, Tak YE, Hsu JY, Clement K, et al. Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. Nat Biotechnol [Internet]. 2019;37(3):276–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30742127
11. Swarts DC, Jinek M. Mechanistic Insights into the cis- and trans-Acting DNase Activities of Cas12a. Mol Cell [Internet]. 2019;73(3):589-600.e4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30639240
12. Chen Q, Chuai G, Zhang H, Tang J, Duan L, Guan H, et al. Genome-wide CRISPR off-target prediction and optimization using RNA-DNA interaction fingerprints. Nat Commun [Internet]. 2023;14(1):7521. Available from: https://www.nature.com/articles/s41467-023-42695-4
13. Roy S. Immune responses to CRISPR-Cas protein. Prog Mol Biol Transl Sci [Internet]. 2021;178:213–29. Available from: http://www.ncbi.nlm.nih.gov/pubmed/33685598
14. Ewaisha R, Anderson KS. Immunogenicity of CRISPR therapeutics—Critical considerations for clinical translation. Front Bioeng Biotechnol [Internet]. 2023;11. Available from: https://www.frontiersin.org/articles/10.3389/fbioe.2023.1138596/full
15. Kleinstiver BP, Prew MS, Tsai SQ, Topkar V V., Nguyen NT, Zheng Z, et al. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature [Internet]. 2015;523(7561):481–5. Available from: https://www.nature.com/articles/nature14592
16. Yang Y, Xu C, Shen Z, Yan C. Crop Quality Improvement Through Genome Editing Strategy. Front genome Ed [Internet]. 2021;3:819687. Available from: http://www.ncbi.nlm.nih.gov/pubmed/35174353
17. Karimi MA, Paryan M, Behrouzian Fard G, Sadeghian H, Zarrinfar H, Hosseini Bafghi M. Challenges and Opportunities in the Application of CRISPR-Cas9: A Review on Genomic Editing and Therapeutic Potentials. Med Princ Pract [Internet]. 2025;1–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/40675140
18. Kantor A, McClements ME, MacLaren RE. CRISPR-Cas9 DNA Base-Editing and Prime-Editing. Int J Mol Sci [Internet]. 2020;21(17). Available from: http://www.ncbi.nlm.nih.gov/pubmed/32872311
19. Liu W, Li L, Jiang J, Wu M, Lin P. Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics. Precis Clin Med [Internet]. 2021;4(3):179–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/34541453
20. Frangoul H, Altshuler D, Cappellini MD, Chen Y-S, Domm J, Eustace BK, et al. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N Engl J Med [Internet]. 2021;384(3):252–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/33283989
21. Locatelli F, Lang P, Wall D, Meisel R, Corbacioglu S, Li AM, et al. Exagamglogene Autotemcel for Transfusion-Dependent β-Thalassemia. N Engl J Med [Internet]. 2024;390(18):1663–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38657265
22. Pierce EA, Aleman TS, Jayasundera KT, Ashimatey BS, Kim K, Rashid A, et al. Gene Editing for CEP290-Associated Retinal Degeneration. N Engl J Med [Internet]. 2024;390(21):1972–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38709228

23. Happi Mbakam C, Lamothe G, Tremblay G, Tremblay JP. CRISPR-Cas9 Gene Therapy for Duchenne Muscular Dystrophy. Neurotherapeutics [Internet]. 2022;19(3):931–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/35165856
24. Chemello F, Olson EN, Bassel-Duby R. CRISPR-Editing Therapy for Duchenne Muscular Dystrophy. Hum Gene Ther [Internet]. 2023;34(9–10):379–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/37060194
25. Erkut E, Yokota T. CRISPR Therapeutics for Duchenne Muscular Dystrophy. Int J Mol Sci [Internet]. 2022;23(3):1832. Available from: https://www.mdpi.com/1422-0067/23/3/1832
26. Wang G. Genome Editing for Cystic Fibrosis. Cells [Internet]. 2023;12(12):1555. Available from: https://www.mdpi.com/2073-4409/12/12/1555
27. Maule G, Arosio D, Cereseto A. Gene Therapy for Cystic Fibrosis: Progress and Challenges of Genome Editing. Int J Mol Sci [Internet]. 2020;21(11):3903. Available from: https://www.mdpi.com/1422-0067/21/11/3903
28. Da Silva Sanchez A, Paunovska K, Cristian A, Dahlman JE. Treating Cystic Fibrosis with mRNA and CRISPR. Hum Gene Ther [Internet]. 2020;31(17–18):940–55. Available from: https://www.liebertpub.com/doi/10.1089/hum.2020.137
29. Chehelgerdi M, Chehelgerdi M, Khorramian-Ghahfarokhi M, Shafieizadeh M, Mahmoudi E, Eskandari F, et al. Comprehensive review of CRISPR-based gene editing: mechanisms, challenges, and applications in cancer therapy. Mol Cancer [Internet]. 2024;23(1):9. Available from: https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01925-5
30. Vimal S, Madar IH, Thirumani L, Thangavelu L, Sivalingam AM. CRISPR/Cas9: Role of genome editing in cancer immunotherapy. Oral Oncol Reports [Internet]. 2024;10:100251. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2772906024000979
31. Dimitri A, Herbst F, Fraietta JA. Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing. Mol Cancer [Internet]. 2022;21(1):78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/35303871
32. Song P, Zhang Q, Xu Z, Shi Y, Jing R, Luo D. CRISPR/Cas-based CAR-T cells: production and application. Biomark Res [Internet]. 2024;12(1):54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38816881
33. Maganti HB, Kirkham AM, Bailey AJM, Shorr R, Kekre N, Pineault N, et al. Use of CRISPR/Cas9 gene editing to improve chimeric antigen-receptor T cell therapy: A systematic review and meta-analysis of preclinical studies. Cytotherapy [Internet]. 2022;24(4):405–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/35039239
34. Razeghian E, Nasution MKM, Rahman HS, Gardanova ZR, Abdelbasset WK, Aravindhan S, et al. A deep insight into CRISPR/Cas9 application in CAR-T cell-based tumor immunotherapies. Stem Cell Res Ther [Internet]. 2021;12(1):428. Available from: http://www.ncbi.nlm.nih.gov/pubmed/34321099
35. Jiang C, Lin X, Zhao Z. Applications of CRISPR/Cas9 Technology in the Treatment of Lung Cancer. Trends Mol Med [Internet]. 2019;25(11):1039–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31422862
36. Hu Y, Zhou Y, Zhang M, Ge W, Li Y, Yang L, et al. CRISPR/Cas9-Engineered Universal CD19/CD22 Dual-Targeted CAR-T Cell Therapy for Relapsed/Refractory B-cell Acute Lymphoblastic Leukemia. Clin Cancer Res [Internet]. 2021;27(10):2764–72. Available from: https://aacrjournals.org/clincancerres/article/27/10/2764/665638/CRISPR-Cas9-Engineered-Universal-CD-19-CD22-Dual
37. Rabaan AA, AlSaihati H, Bukhamsin R, Bakhrebah MA, Nassar MS, Alsaleh AA, et al. Application of CRISPR/Cas9 Technology in Cancer Treatment: A Future Direction. Curr Oncol [Internet]. 2023;30(2):1954–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/36826113
38. Solanki D, Murjani K, Singh V. CRISPR-Cas based genome editing for eradication of human viruses. In 2024. p. 43–58. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1877117324001613
39. Ansari I, Chaturvedi A, Chitkara D, Singh S. CRISPR/Cas mediated epigenome editing for cancer therapy. Semin Cancer Biol [Internet]. 2022;83:570–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/33421620
40. Kyriakopoulou E, Monnikhof T, van Rooij E. Gene editing innovations and their applications in cardiomyopathy research. Dis Model Mech [Internet]. 2023;16(5). Available from: http://www.ncbi.nlm.nih.gov/pubmed/37222281
41. Saeed S, Khan SU, Khan WU, Abdel-Maksoud MA, Mubarak AS, Aufy M, et al. Genome Editing Technology: A New Frontier for the Treatment and Prevention of Cardiovascular Diseases. Curr Probl Cardiol [Internet]. 2023;48(7):101692. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0146280623001093
42. Hoekstra M, Van Eck M. Gene Editing for the Treatment of Hypercholesterolemia. Curr Atheroscler Rep [Internet]. 2024;26(5):139–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38498115
43. Laffin LJ, Nicholls SJ, Scott RS, Clifton PM, Baker J, Sarraju A, et al. Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3. N Engl J Med [Internet]. 2025;393(21):2119–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/41211945

44. Bhardwaj S, Kesari KK, Rachamalla M, Mani S, Ashraf GM, Jha SK, et al. CRISPR/Cas9 gene editing: New hope for Alzheimer's disease therapeutics. J Adv Res [Internet]. 2022;40:207–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2090123221001351
45. De Plano LM, Calabrese G, Conoci S, Guglielmino SPP, Oddo S, Caccamo A. Applications of CRISPR-Cas9 in Alzheimer's Disease and Related Disorders. Int J Mol Sci [Internet]. 2022;23(15). Available from: http://www.ncbi.nlm.nih.gov/pubmed/35955847
46. Hanafy AS, Schoch S, Lamprecht A. CRISPR/Cas9 Delivery Potentials in Alzheimer's Disease Management: A Mini Review. Pharmaceutics [Internet]. 2020;12(9). Available from: http://www.ncbi.nlm.nih.gov/pubmed/32854251
47. Thapar N, Eid MAF, Raj N, Kantas T, Billing HS, Sadhu D. Application of CRISPR/Cas9 in the management of Alzheimer's disease and Parkinson's disease: a review. Ann Med Surg [Internet]. 2024;86(1):329–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38222734
48. Pinjala P, Tryphena KP, Prasad R, Khatri DK, Sun W, Singh SB, et al. CRISPR/Cas9 assisted stem cell therapy in Parkinson's disease. Biomater Res [Internet]. 2023;27(1):46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/37194005
49. Hussein M, Molina MA, Berkhout B, Herrera-Carrillo E. A CRISPR-Cas Cure for HIV/AIDS. Int J Mol Sci [Internet]. 2023;24(2). Available from: http://www.ncbi.nlm.nih.gov/pubmed/36675077
50. Bhowmik R, Chaubey B. CRISPR/Cas9: a tool to eradicate HIV-1. AIDS Res Ther [Internet]. 2022;19(1):58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/36457057
51. Zhang Z, Hou W, Chen S. Updates on CRISPR-based gene editing in HIV-1/AIDS therapy. Virol Sin [Internet]. 2022;37(1):1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/35234622
52. Najafi S, Tan SC, Aghamiri S, Raee P, Ebrahimi Z, Jahromi ZK, et al. Therapeutic potentials of CRISPR-Cas genome editing technology in human viral infections. Biomed Pharmacother [Internet]. 2022;148:112743. Available from: http://www.ncbi.nlm.nih.gov/pubmed/35228065
53. Ying M, Wang H, Liu T, Han Z, Lin K, Shi Q, et al. CLEAR Strategy Inhibited HSV Proliferation Using Viral Vectors Delivered CRISPR-Cas9. Pathog (Basel, Switzerland) [Internet]. 2023;12(6). Available from: http://www.ncbi.nlm.nih.gov/pubmed/37375504

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REVIEW ARTICLE

Ivonescimab – the future of cancer therapy

Wiktoria Ficoń[1], Maksymilian Dobosz[1], Beata Całyniuk[2]

[1] Student Scientific Association by the Department of Human Nutrition, Medical University of Silesia in Katowice, Poland
[2] Department of Human Nutrition, Medical University of Silesia in Katowice, Poland

Abstract

Ivonescimab is an innovative bispecific antibody targeting both programmed cell death protein 1 (PD-1) and vascular endothelial growth factor (VEGF-A). Through its dual mechanism (immunomodulatory and anti-angiogenic) it can effectively enhance the body's immune response to cancer and inhibit the formation of new blood vessels, thereby slowing tumor growth. Clinical trials have demonstrated high efficacy of ivonescimab in treating non-small cell lung cancer (NSCLC). When combined with chemotherapy, ivonescimab significantly prolongs progression-free survival compared to pembrolizumab-based therapy. A higher response rate was also observed, with an acceptable safety profile. Results from the HARMONi-A and HARMONi-2 studies suggest that ivonescimab may become the proposed therapeutic regimen for first-line treatment. Further studies are underway to evaluate the broader potential of ivonescimab, as it may represent a promising option pending approval. The globalization of ivonescimab research increases the likelihood of its registration as the first anti-PD-1/anti-VEGF-A bispecific antibody, thus potentially revolutionizing cancer therapy. This article provides a concise summary of the mechanism of action and key clinical evidence supporting the use of Ivonescimab in NSCLC.

Keywords: ivonescimab · carcinoma · non-small cell lung cancer · clinical trials

Citation

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Corresponding author:
Wiktoria Ficoń, Student Scientific Association by the Department of Human Nutrition, Medical University of Silesia in Katowice, Poland
e-mail: wiktoriaficon@gmail.com
Available online: ejtcm.gumed.edu.pl

Introduction

Ivonescimab (AK112 or SMT112) is the first humanized bispecific anti-programmed cell death protein 1 (anti-PD-1) and anti-vascular endothelial growth factor (anti-VEGF antibody) with a heterotetrameric structure composed of two IgG1 heavy chains and two kappa light chains, linked by disulfide bonds (-S-S-) [1-4]. The PD-1-targeting fragment is attached to the C-terminus of each anti-VEGF heavy chain (Figure 1). Modification of the PD-1 Fc region eliminated complement-dependent cytotoxicity. This drug's dual activity enables simultaneous immune system activation (by enhancing recognition and destruction of cancer cells) and inhibition of angiogenesis, potentially slowing or halting tumor growth [5]. The approach based on simultaneous targeting of 2 molecular pathways offers the possibility of overcoming the resistance mechanisms typically observed in single-target therapies, providing a more integrated and effective treatment strategy for patients with non-small cell lung cancer (NSCLC) [6]. VEGF-A and PD-1 expression are strongly correlated in the tumor microenvironment. Blocking both mechanisms simultaneously results in synergistic target binding and enhanced antitumor activity with a better safety profile than separate anti-PD-1 and anti-VEGF therapies [7]. Originally developed by the Chinese biotechnology company Akeso Biopharma, ivonescimab was later licensed globally by Summit Therapeutics, paving the way for international development and commercialization [8]. In this paper we present the mechanism of action and key scientific evidence regarding the use of ivonescimab in NSCLC.

Material and methods

We searched scientific databases such as PubMed, Google Scholar and ScienceDirect to find articles on ivonescimab, its structure, mechanism of action and clinical trials. We also included information from its manufacturer's (Summit Therapeutics) website and the clinicaltrials.gov database. The inclusion criteria were as follows: full text review or original articles describing clinical trials or randomised controlled trials, published in peer-reviewed journals in 2024 and 2025.

Results

A total of 133 abstracts containing the keyword 'ivonescimab' were found as a result of the literature search. After selection, 42 abstracts were included, while 91 were excluded due to lack of open access to the full text, low quality or unclear methodology, and a different subject area than that covered in this review. The full texts of the remaining 42 articles were then searched and reviewed. Ultimately, 10 articles available in scientific databases and 5 articles published on the websites of the drug manufacturer and an international clinical trial database were included in this review.

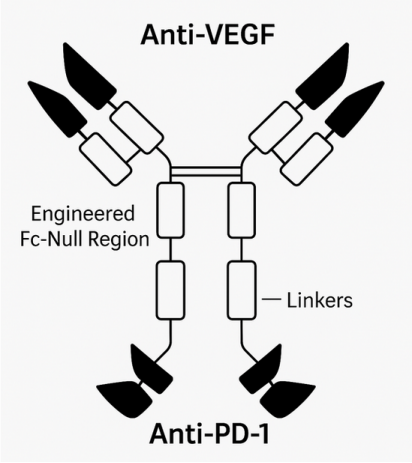

Figure 1. The structure of Ivonescimab
Based on figure published by Zhang et al. in their poster [9]

Clinical trials

Phase 186I, II, and III clinical trials evaluating ivonescimab are currently underway. Phase I trials assess safety and determine the maximum tolerated dose, which is 20 mg/kg every 14 days. Ivonescimab is administered intravenously (IV) every 2 weeks in various concentrations, ranging from 0.3 mg/kg to 30 mg/kg, to patients who have advanced or metastatic solid tumors that are resistant to treatment, have relapsed after standard therapies, and for whom no effective standard therapy is available [1]. The maximum tolerated dose was determined based on drug tolerance and reported adverse events. This dose ensures high PD-1 receptor saturation and a significant reduction in free VEGF. Adverse effects such as skin rash, joint pain, and increased blood pressure (typical for ivonescimab's mechanism of action) were reported by 27.5% of the trial participants [1]. In phase II trials the researchers evaluate the response to treatment (drug efficacy), selection of the optimal dose and the incidence of adverse effects in the wider population. In phase III trials, on the other hand, the aim is to compare the efficacy of the new drug with the current standard of therapy, while monitoring long-term treatment effects and complications. The results of these trials are key to the decision on marketing authorisation.

Phase II clinical trials

The first phase II trial evaluating ivonescimab was conducted among patients with advanced NSCLC in combina-

tion with chemotherapy [10]. All participants had stage III or IV NSCLC and were divided into 3 cohorts. The first included patients with advanced NSCLC who did not have epidermal growth factor receptor (EGFR). Participants in the second group had advanced NSCLC with EGFR-sensitive mutations, but previous EGFR-tyrosine kinase inhibitor (TKI) targeted therapy had failed. The final group consisted of patients with advanced-stage NSCLC who had not received a full course of platinum-based chemotherapy or anti-PD-1 therapy. Adverse effects were reported by all patients, mainly leukopenia (18.1%), neutropenia (16.9%) and thrombocytopenia (12%) [10]. The authors concluded that the combination of chemotherapy with concomitant administration of ivonescimab is promising due to a reduction in tumour volume irrespective of histological nature in the majority of subjects and relatively good tolerability [10].

Another phase II trial evaluated ivonescimab's efficacy against squamous cell carcinoma (SCC) NSCLC and non-squamous cell carcinoma (non-SCC) NSCLC without EGFR/ALK mutations in combination with chemotherapy. The results of this study were evaluated based on the analyzed endpoints, which were safety and the objective response rate (ORR) in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines. The high efficacy of the undertaken treatment was demonstrated irrespective of the NSCLC subtype, thus allowing a wide use of ivonescimab. In addition, adverse effects were observed in 10% of patients, most commonly nasal bleeding, proteinuria and rash, all of which are typical in immune therapy and chemotherapy [9].

Phase III clinical trials

The HARMONi-A phase III study analysed the efficacy and safety of ivonescimab combination therapy with chemotherapy in patients with EGFR-mutated advanced NSCLC who were previously treated with EGFR tyrosine kinase inhibitors (including third-generation EGFR-TKIs). That study involved 322 patients randomly allocated to 2 treatment groups: the first received ivonescimab in combination with chemotherapy (pemetrexed and carboplatin), while the second received placebo with the same chemotherapy regimen. After completion of the full 4 cycles of induction treatment, maintenance therapy was continued with ivonescimab or placebo in combination with pemetrexed. The median disease progression-free survival (PFS) time was 7.1 months in the ivonescimab group, a significant improvement compared to 4.8 months in the placebo group. The ORR was also higher in the ivonescimab group at 50.6% (95% confidence interval (CI), 42.6% - 58.6%), compared to 35.4% (95% CI, 28.0% - 43.3%) in the control group. The most common adverse effects reported in the HARMONi-A trial were related to chemotherapy [11].

The HARMONi-2 study was conducted in 55 hospitals across China. Patients over 18 years of age diagnosed with advanced or metastatic non-small cell lung cancer were divided into two equal groups. The first received 20 mg/kg ivonescimab (IV) every 3 weeks, while the second had 200 mg pembrolizumab administered (IV) every 3 weeks. Adverse effects resulting from ivonescimab did not lead to treatment discontinuation, as was the case in the 5 patients using pembrolizumab. Furthermore, the compared drugs had a similar safety profile (acceptable). The HARMONi-2 study showed that ivonescimab significantly prolongs PFS by approximately 11 months, compared to nearly 6 months in the pembrolizumab group. Ivonescimab also showed a higher response rate of 50% (95% CI 43-57) compared to 39% (95% CI 32-46) for pembrolizumab. Moreover, the therapeutic advantage was evident in different patient subgroups, regardless of PD-L1 expression level, histological type or the presence of liver and brain metastases. Based on the results of the HARMONi-2 study, it can be concluded that ivonescimab is a promising alternative to pembrolizumab for first-line treatment in patients with PD-L1-positive NSCLC, providing more favourable clinical outcomes [12].

Recruitment is currently underway for the HARMONi-3 trial, which aims to compare the use of ivonescimab with chemotherapy and pembrolizumab (also together with chemotherapy) as first-line treatment in patients with metastatic NSCLC of both SCC and non-SCC types [13]. Also, there is an open enrolment in the HARMONi-7 trial to compare the use of ivonescimab monotherapy with pembrolizumab as first-line treatment in patients with metastatic NSCLC with high PD-L1 expression (TPS ≥ 50%) [14].

Limitations and future directions

Despite the promising performance of ivonescimab in clinical trials, it is important to highlight a number of significant facts that limitat the interpretation of the available data. First of all, most of the published articles describe the results of phase I and II studies (focusing mainly on safety, tolerance and preliminary efficacy), resulting in a lack of data from large, international, randomised phase III studies. In addition, the number of participants in the published clinical trials is relatively small, limiting the possibility of generalising the results to a wider patient population. Due to the relatively recent development of ivonescimab, there is no information on the long-term effects of its use. Another significant limitation is the lack of registration and reimbursement of ivonescimab in Poland, which limits its availability outside of clinical trials. Ivonescimab was initially approved for marketing in China in May 2024, and its widespread use in that country began in April 2025. Ivonescimab received Fast Track designation from the US Food & Drug Administration (FDA) for the HARMONi

clinical trial [15]. Currently, there are no PD-1-based bispecific antibodies approved by the FDA or the European Medicines Agency (EMA). An important aspect of future studies will also be the evaluation of long-term clinical outcomes such as overall survival, durability of response and quality of life of patients. Translational research should focus on identifying ivonescimab's mechanisms of action, including the tumor microenvironment and the regulation of the immunomodulatory response, which will contribute to a better understanding of this drug's clinical efficacy.

Conclusion

Approved for treatment in China, ivonescimab shows promising results in the treatment of advanced non-small cell lung cancer (NSCLC). Clinical trials are a key component in the development of effective cancer therapies. Ongoing trials are evaluating the potential for broader clinical use of ivonescimab, including its effectiveness in combination with other therapies and its prospective for use in other types of cancer. Extending the research to other countries will allow the evaluation of ivonescimab in patient groups other than Chinese and strengthen the credibility of the results obtained so far.

Conflict of interests

The authors declare no financial relationships with any commercial entity that could have influenced the outcomes or interpretations presented in this manuscript and report no conflicts of interest.

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References

1. Frentzas S, Austria Mislang AR, Lemech C, Nagrial A, Underhill C, Wang W, et al. Phase 1a dose escalation study of ivonescimab (AK112/SMT112), an anti-PD-1/VEGF-A bispecific antibody, in patients with advanced solid tumors. J Immunother cancer [Internet]. 2024;12(4). Available from: http://www.ncbi.nlm.nih.gov/pubmed/38642937
2. Zhou C, Chen J, Wu L, Wang L, Liu B, Yao J, et al. PL02.04 Phase 3 Study of Ivonescimab (AK112) vs. Pembrolizumab as First-line Treatment for PD-L1-positive Advanced NSCLC: Primary Analysis of HARMONi-2. J Thorac Oncol [Internet]. 2024;19(10):S1. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1556086424008864
3. Chen Z, Wu L, Wang Q, Yu Y, Liu X, Ma R, et al. Brief Report: Ivonescimab Combined With Etoposide Plus Carboplatin as First--Line Treatment for Extensive-Stage SCLC: Results of a Phase 1b Clinical Trial. J Thorac Oncol [Internet]. 2025;20(2):233–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1556086424024213
4. Ivonescimab Overview [Internet]. Summit Therapeutics Inc. 2025 [cited 2025 Jul 18]. Available from: https://www.smmttx.com/ivonescimab-smt112/
5. Wang L, Luo Y, Ren S, Zhang Z, Xiong A, Su C, et al. A Phase 1b Study of Ivonescimab, a Programmed Cell Death Protein-1 and Vascular Endothelial Growth Factor Bispecific Antibody, as First- or Second-Line Therapy for Advanced or Metastatic Immunotherapy-Naive NSCLC. J Thorac Oncol [Internet]. 2024;19(3):465–75. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1556086423023122
6. Al Matairi A, Hammadeh BM, Aldalati AY, Qtaishat FA, Nashwan AJ, Alzibdeh A. Efficacy and Safety of Ivonescimab in the Treatment of Advanced Non-small Cell Lung Cancer (NSCLC): A Systematic Review. Cureus [Internet]. 2025;17(1):e77381. Available from: http://www.ncbi.nlm.nih.gov/pubmed/39944427
7. Coward J, Mislang ARA, Frentzas S, Lemech CR, Nagrial A, Jin X, et al. Safety and efficacy of AK112, an anti-PD-1/VEGF-A bispecific antibody, in patients with advanced solid tumors in a phase I dose escalation study. J Clin Oncol [Internet]. 2021;39(15_suppl):2515–2515. Available from: https://ascopubs.org/doi/10.1200/JCO.2021.39.15_suppl.2515
8. Our Strategic Partnership with Akeso with an Aligned Mission [Internet]. Summit Therapeutics Inc. 2025 [cited 2025 Jul 18]. Available from: https://smmttx.com/ivonescimab-smt112/akeso-partnership/default.aspx
9. Zhang L, Fang W, Zhao Y, Yang Y, Zhou N, Chen L, et al. Phase II results of ivonescimab (AK112/ SMT112), a novel PD-1/VEGF bispecific, in combination with chemotherapy for first line treatment of advanced or metastatic non-small cell lung cancer (NSCLC) without actionable genomic alterations (AGA) in EGFR/ALK. J Clin Oncol [Internet]. 2023;41(16_suppl):9087–9087. Available from: https://ascopubs.org/doi/10.1200/JCO.2023.41.16_suppl.9087

10. Zhao Y, Chen G, Chen J, Zhuang L, Du Y, Yu Q, et al. AK112, a novel PD-1/VEGF bispecific antibody, in combination with chemotherapy in patients with advanced non-small cell lung cancer (NSCLC): an open-label, multicenter, phase II trial. EClinicalMedicine [Internet]. 2023;62:102106. Available from: http://www.ncbi.nlm.nih.gov/pubmed/37593227
11. Fang W, Zhao Y, Luo Y, Yang R, Huang Y, He Z, et al. Ivonescimab Plus Chemotherapy in Non-Small Cell Lung Cancer With EGFR Variant: A Randomized Clinical Trial. JAMA [Internet]. 2024;332(7):561–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38820549
12. Xiong A, Wang L, Chen J, Wu L, Liu B, Yao J, et al. Ivonescimab versus pembrolizumab for PD-L1-positive non-small cell lung cancer (HARMONi-2): a randomised, double-blind, phase 3 study in China. Lancet (London, England) [Internet]. 2025;405(10481):839–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/40057343
13. Clinical Study of Ivonescimab for First-line Treatment of Metastatic NSCLC Patients [Internet]. ClinicalTrials.gov 2025 [cited 2025 Jul 18]. Available from: https://clinicaltrials.gov/study/NCT05899608
14. Clinical Study of Ivonescimab for First-line Treatment of Metastatic NSCLC Patients With High PD-L1 (HARMONi-7) [Internet]. ClinicalTrials.gov. 2025 [cited 2025 Jul 18]. Available from: https://clinicaltrials.gov/study/NCT06767514
15. Ivonescimab Plus Chemotherapy Demonstrates Statistically Significant and Clinically Meaningful Improvement in Progression-Free Survival in Patients with EGFR-Mutant Non-Small Cell Lung Cancer after EGFR TKI Therapy in Global Study [Internet]. Smmttx.com. 2025 [cited 2025 Sep 6]. Available from: https://www.smmttx.com/pressrelease/https-www-smmttx-com-wp-content-uploads-2025-05-2025_pr_0530-_-harmoni-data-_-final-docx-pdf/

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REVIEW ARTICLE

The impact of type II diabetes on the risk of atrial fibrillation – literature review

Aleksandra Mazurek[1], Damian Machaj[2], Dominik Machaj[3]

[1] Poznań University of Medical Science, Poznań, Poland
[2] Wrocław Medical University, Wrocław, Poland
[3] Medical University of Lublin, Lublin, Poland

Abstract

Atrial fibrillation (AF) is the most common type of supraventricular arrhythmia, affecting an estimated 40 million people worldwide. Type 2 diabetes mellitus (T2DM) is a common disease among cardiac patients, and hyperglycemia has a significant impact on cardiovascular function. Oxidative stress causes microvascular damage, while the increased production of vascular endothelial growth factor (VEGF) affects the endothelium, leading to its dysfunction. These factors, along with diabetic cardiomyopathy, contribute to hyperglycemia-induced changes in the vessels, and myocardium, thereby predisposing patients to the development of AF. The aim of this review was to discuss and analyse the factors linking AF with T2DM.

Keywords: atrial fibrillation · diabetes mellitus type 2 · hyperglycemia

Citation

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Introduction

In patients with atrial fibrillation (AF), we observe irregular stimulation of the atria of the heart, leading to abnormal blood flow from the atria to the ventricles. The factors that predispose a patient to both T2DM and AF overlap and include primarily obesity and hypertension. These factors lead to pathologies in the vascular system and cardiac muscle. Hyperglycemia increases the activity of many metabolic pathways (e.g. beta oxidation of fatty acids), thereby increasing the production of toxic metabolites, which increase oxidative stress, leading to inflammation and accelerated aging of cells, including heart muscle cells. Hypertension and atherosclerosis, which are often co-occurring conditions in patients

Corresponding author:
Aleksandra Mazurek, Poznań University of Medical Science, Poznań, Poland
e-mail: aleksandramazurek7@gmail.com
Available online: ejtcm.gumed.edu.pl

with T2DM, may contribute to the development of AF. The remodeling of the atrial wall muscles and the increase in their stiffness in the course of diabetic cardiomyopathy contribute to the risk of AF.

Material and methods

Analysis of risk factors for AF in patients with T2DM is presented based on the literature available in PubMed and Google Scholar. The following keywords were used during the literature search: "atrial fibrillation", "type 2 diabetes mellitus", "oxidative stress", "endothelial dysfunction", "VEGF", "cardiomyopathy" and "metformin". The search was limited to scientific articles published in years 2000-2025, written in English or Polish. Inclusion criteria were: original research and review articles adressing the relation between AF and T2DM in adult humans. Articles on type 1 DM, animal-only models and non-peer-reviewed sources were excluded. Due to data heterogeneity, a narrative literature review was conducted, following general systematic review principles without strict adherence to PRISMA guidelines.

Results

A total of 214 abstracts were identified in the initial search. After title and abstract screening, 137 were excluded due to lack of relevance to the topic or failure to meet the inclusion criteria. Full text analysis was conducted for 77 articles, of which 49 were removed because of insufficient data linking T2DM with AF mechanisms, duplication of findings or unclear methodology. Finally, 18 full-text articles were included in the final review. These articles provided detailed information on the role of oxidative stress, inflammation, VEGF signaling, and myocardial remodeling in the development of AF among patients with T2DM.

Discussion

Changes occurring in the atria as a result of chronic exposure to cardiovascular risk factors, including T2DM, can be divided into 3 main types: structural remodeling, functional remodeling, and electrical remodeling [1-4]. Structural remodeling is a process involving the remodeling of the myocardial structure (including the interstitium) and the loss of cardiomyocytes, which manifests itself through increased atrial mass and volume [1]. In patients with AF one can observe an increase in the intercellular space due to the loss of cardiomyocytes and subsequent fibrosis, which reduces the atrial bipolar potential [2]. The decrease in cardiomyocyte number is compensated by hypertrophy of non-degenerating cells. Fibrosis, accompanied by the accumulation of collagen fibers within the interstitial and perivascular spaces, leads to conduction abnormalities and differences in the efficiency of electrical conduction [2]. Structural remodeling is closely related to electrical remodeling, which creates the so-called AF substrate, i.e. the structural and electrophysiological basis that sustains arrhythmias. The creation of conditions favorable to the stabilization of AF is influenced by a shortened refractory period and a reduced sodium current, which translate into slower conduction [1]. The term autonomic remodeling refers to altered sympathovagal activity and excessive atrial innervation [1]. The imbalance between sympathetic and parasympathetic activity promotes the development of complex reentrant circuits, which are associated with sustained AF. Chronic inflammation, oxidative stress, endothelial dysfunction, increased sympathetic nervous system activity, epicardial fat accumulation, and metabolic disorders within myocytes further enhance the atrial remodeling in patients with T2DM [3]. Obstructive sleep apnea, episodes of myocardial ischemia, hypertension, and increased sinus node cell apoptosis are other factors contributing to the complex pathological process of AF development [3-4]. The most beneficial treatment for patients includes glucose control and treatment of comorbidities [4].

Glucose intolerance and insulin resistance increase the risk of AF in both men and women by 40% and 60%, respectively [5]. Chronic hyperglycemia contributes to glycation of the insulin receptors, which leads to insulin resistance. Poor glycemic control and late diagnosis of DM also have an influence on the potential occurrence of AF. T2DM is characterized by the body's inability to use carbohydrates as an energy source. To meet its energy needs, the body begins to use fatty acids to produce the appropriate amount of adenosine triphosphate (ATP). The consequences of increased fatty acid metabolism are the activation of nuclear peroxisome proliferator-activated receptor-alpha (PPAR-α) and increased β-oxidation, which are not only a source of many toxic metabolites but also of reactive oxygen and nitrogen species that intensify oxidative stress [5]. Furthermore, oxidative stress is a factor that intensifies β-oxidation of fatty acids. These processes trigger each other, leading to further damage to the cardiovascular system. The previously mentioned insulin resistance impairs the ability of vessels to respond to nitric oxide (NO). In addition to the insulin receptor substrate, other proteins are also subject to glycation. As a result of this process, free radicals accumulate as by-products. Abnormalities in the processes occurring inside mitochondria and oxidative stress influence the processes responsible for the adaptation of the heart muscle. Oxidative stress leads to the activation of inflammatory pathways in the cells, which translates into increased C-reactive protein (CRP) levels in patients with

persistent AF. Human mitochondrial DNA is poorly protected against damage associated with hydroxyl radicals, which leads to premature cell aging (including muscle cells) [6].

The aggravating effect of T2DM on patients with AF is supported by the positive effect of hypoglycemic drugs on atrial remodeling [5]. In 2014, Chang et al. published a study that aimed to determine whether metformin, a first-line drug in the treatment of DM, reduces the risk of AF among patients [7]. For this purpose, they analyzed 645710 patients from a subset of the Taiwan National Health Insurance Research Database who were newly diagnosed DM in the years 1999-2010. The patients were divided into a group with metformin as part of their treatment plan (user group) and a non-user group. After 13 years of follow-up it was noticed that the incidence of AF was significantly lower in the group treated with metformin, (hazard ratio of 0.81, 95% confidence interval (CI) 0.76-0.86, $p < 0.001$). The authors concluded that metformin had a protective effect against oxidative stress and myocyte remodeling, thereby reducing the risk of AF among diabetic patients [7]. The beneficial effects of including metformin in the treatment plan likely stem from its influence on adenosine monophosphate (AMP)-activated protein kinase (AMPK) activation and peroxisome proliferator-activated receptor gamma (PPARγ) modulation, which translates into reduced oxidative stress. By inhibiting transforming growth factor beta (TGF-B) signaling, Metformin limits collagen deposition and reduces atrial stiffness. Calcium homeostasis is particularly important for proper cardiomyocyte function. Metformin increases sarcoplasmic/endoplasmic reticulum Ca2+-ATPase isoform 2a (SERCA2a) expression, which prevents unfavorable Ca2+ accumulation in the cytosol and, consequently, the development of early and delayed after depolarizations [8].

Chronic hyperglycemia induces oxidative stress, accompanied by increased concentrations of inflammatory markers, e.g. CRP, fibrinogen and interleukin-6 [9]. Inflammation in the body leads to hypoxia and the release of cytokines, vascular endothelial growth factor (VEGF), factors affecting vascular tone. Oxidative stress induces lipid oxidation, resulting in the formation of OX-LDL, which by acting on macrophages and monocytes, increases the concentration of VEGF in the serum [10]. VEGF-releasing cells include endothelial cells, monocytes/macrophages, platelets, neutrophils, and fibroblasts. In addition to its effects on the cardiovascular system, VEGF also controls hematopoiesis, injury scar formation, and bone formation [11]. Angiogenesis (i.e. the formation of new blood vessels) has a negative impact on the body function, particularly by leading to cardiac hypoxia. VEGF is one of the main pro-angiogenic factors, which additionally participates in the remodeling of muscle tissue at the site of damage.

Zhang et al. indicated a correlation between higher plasma VEGF concentration and hyperglycemia [12]. A platinum EGF-A enzyme-linked immunosorbent assay kit (eBioscience, Vienna, Austria) was used to check the VEGF concentration in plasma collected from a blood sample. The authors concluded that there is a close interrelation between hyperglycemia, inflammation and VEGF in patients with T2DM, indicating that increased VEGF levels may be the cause of microangiopathy in these patients [12]. The results discussed in this review are consistent with and complement the findings of Zhang et al., who demonstrated that plasma VEGF concentrations were significantly higher in patients with poor glycemic control compared to those with normal glucose levels [12]. The comparison of VEGF levels in patients before and after improvement of glycemic control over a 4-month period confirmed that maintaining stable blood glucose reduces the VEGF concentration. This observation supports the conclusion that hyperglicemia directly stimulates VEGF synthesis, leading to endothelial dysfunction and increased vascular permeability. Pathological endothelial function includes increased secretion of prothrombotic factors into the extracellular matrix, including tissue factor and von Willebrand factor. The hyperglycemia-related endothelial dysfunction affects the function of the organ supplied by dysfunctional vessels. The vascular endothelium is a tissue with versatile effects due to numerous factors modulating the processes occurring in the body. Disturbances in the amount and timing of the secretion of these factors may lead to atherosclerosis and hypertension, which are risk factors for AF.

Diabetic cardiomyopathy is a structural and functional disorder of the left ventricle of the heart. Factors that stimulate cardiac hypertrophy include hyperglycemia and insulin resistance, which is the basis of DM. Diabetic cardiomyopathy is characterized by a pathological structure of the myocardium in the absence of other factors that could lead to it [12]. Glucose transporter type 4 (GLUT4) is responsible for transporting glucose within adipose tissue, liver and muscles. In order to fulfill its role, it must first be incorporated into the cell membrane [13]. In addition to blocking glucose transport within the above-mentioned tissues, disturbances in the pathway responsible for this process, lead to a decrease in the activity of the Ca2+ pump in the sarcoplasmic reticulum [13]. The increase in intracellular Ca2+, additionally intensified by insulin-stimulated coronary endothelial NO synthase (eNOS) (in the case of T2DM, its activity decreases), leads to myocardial stiffness in people with T2DM. The basis of this phenomenon is, among others, the phosphorylation of titin caused by a reduced concentration of NO [12].

Titin is the largest known human protein, weighing approximately 3 MDa, and occurring in 3 cardiac isoforms (N2A, N2B and N2BA). Phosphorylation of titin leads to an increase in the stiff titin isoform N2B/N2BA (compliant) expression ratio. In addition to the ratio of titin isoforms, the stiffness of the myocardium is also influenced by the phosphorylation of its individual elements carried out by protein kinases.

We distinguish between PKA (protein kinase A)-dependent phosphorylation, CaMKII (calmodulin-dependent protein kinase II delta)-dependent phosphorylation, and PKC (protein kinase C)-dependent phosphorylation. PKA (i.e. cyclic adenosine monophosphate (cAMP)-dependent protein kinase) is involved in the phosphorylation of the N2B titin isoform, which leads to a decrease in myocyte stiffness. Reducing the stiffness of the heart walls stimulates the adrenergic system, which in turn increases heart rate and diastolic filling of the atria [14]. Increased participation of the previously mentioned isoform in the structure of the heart muscle translates into problems with cardiac muscle relaxation and increased stiffness. The role of titin, a type of striated muscle myofilament, is to provide stiffness to cardiac myocytes. Its mutations are responsible for 25% of familial dilated cardiomyopathies and 18% of sporadic cases of non-genetic dilated cardiomyopathy (DCM) [15]. Hyperglycemia contributes to abnormal phosphorylation of titin molecules, which affects titin stiffness. This thesis is confirmed by the positive effect of metformin on the development of cardiac diastolic dysfunction [16].

Studies on a mouse heart model showed a reduction in sarcomere passive stiffness as a result of activation of the phosphorylation of the N2B element [16]. Modifications involving the process of titin phosphorylation in patients diagnosed with diastolic heart dysfunction include changes in the activity of protein kinase A (PKCalpha) and phosphorylation of individual components of titin (N2B and PEVK).

In the structure of the previously-mentioned protein, we can distinguish the C-terminal anchored in the M band and the N-terminal associated with the Z disk; a single titin molecule stretches from the Z line to the M line. However, the most important part of titin is related to the I band. Titin within the I band has a stretchable region that generates passive tension when the sarcomere is stretched beyond its Slack length [11]. The contraction f myocytes leads to the generation of a recoil force by the compressed titin molecule, thanks to which the myocytes in the relaxation phase can be rebuilt and regain their original diastolic length [17]. DCM is a pathology within the heart muscle that involves the gradual stretching of muscle fibers, which become thinner and weaker. As a consequence, the heart chambers widen. The TTN gene (codes for titin) is one of the sarcomeric genes whose mutations is one of the most common causes of dilated cardiomyopathy.

Analysis of data from a global health research network (mainly from the United States) and concerning 634885 patients diagnosed with cardiomyopathy in the years 2002--2020, revealed the relationship between mortality among patients and cardiomyopathy with co-existing AF [18]. The examined patient group included people diagnosed with dilated, hypertrophic and restrictive cardiomyopathy. The overall mortality rate in patients with DCM and AF was 7.1%, while in patients with DCM without AF it was 4.9% (odds ratio 1.36, 95% CI 1.27-1.46, $p < 0.0001$). These results were statistically significant and indicate a meaningful difference in mortality between the 2 patient groups. A higher risk of death within 1 year from the diagnosis was observed in patients with AF and DCM compared to those diagnosed with cardiomyopathy without AF. The results of this analysis indicate the frequent occurrence of AF in patients with cardiomyopathy and support a worse prognosis among these patients [18].

Conclusions

The relationship between DM and AF does not appear to result from a single direct mechanism, but rather from the cumulative effect of multiple pathological processes, including structural, electrical and autonomic remodeling. However, people with DM are at a higher risk of developing AF. This is supported by the impact of large fluctuations of glycemia on blood vessels. The resulting inflammation, oxidative stress and endothelial dysfunction lead to both minor and major changes in vascular structure and function. A malfunctioning circulatory system places an additional burden on the heart, which may result in scarring and remodeling. Importantly, AF in patients with T2DM should be regarded less as the consequence of one specific pathway and more as the outcome of numerous overlapping processes. In this context, AF may serve both as a marker of advanced cardiovascular degeneration and as an independent disease entity associated with a worse prognosis. Therefore, reducing the risk of arrhythmic complications depends not only on early diagnosis and treatment of AF itself, but also on comprehensive management of DM. Optimal glycemic control and pharmacotherapy, particularly metformin, may indirectly reduce the incidence of AF by slowing down the progression of adverse cardiovascular remodeling.

Conflicts of interest

None.

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References

1. Iwasaki Y, Nishida K, Kato T, Nattel S. Atrial Fibrillation Pathophysiology. Circulation [Internet]. 2011 Nov 15;124(20):2264–74. Available from: https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.111.019893
2. Pellman J, Sheikh F. Atrial Fibrillation: Mechanisms, Therapeutics, and Future Directions. Compr Physiol [Internet]. 2015 Apr;5(2):649–65. Available from: https://onlinelibrary.wiley.com/doi/10.1002/j.2040-4603.2015.tb00626.x
3. Karam BS, Chavez-Moreno A, Koh W, Akar JG, Akar FG. Oxidative stress and inflammation as central mediators of atrial fibrillation in obesity and diabetes. Cardiovasc Diabetol [Internet]. 2017 Dec 29;16(1):120. Available from: https://cardiab.biomedcentral.com/articles/10.1186/s12933-017-0604-9
4. Tseng C-H. Metformin Use Is Associated With a Lower Incidence of Hospitalization for Atrial Fibrillation in Patients With Type 2 Diabetes Mellitus. Front Med [Internet]. 2021 Feb 22;7. Available from: https://www.frontiersin.org/articles/10.3389/fmed.2020.592901/full
5. Lorenzo-Almorós A, Casado Cerrada J, Álvarez-Sala Walther L-A, Méndez Bailón M, Lorenzo González Ó. Atrial Fibrillation and Diabetes Mellitus: Dangerous Liaisons or Innocent Bystanders? J Clin Med [Internet]. 2023 Apr 14;12(8):2868. Available from: https://www.mdpi.com/2077-0383/12/8/2868
6. Senoner T, Dichtl W. Oxidative Stress in Cardiovascular Diseases: Still a Therapeutic Target? Nutrients [Internet]. 2019 Sep 4;11(9):2090. Available from: https://www.mdpi.com/2072-6643/11/9/2090
7. Chang S-H, Wu L-S, Chiou M-J, Liu J-R, Yu K-H, Kuo C-F, et al. Association of metformin with lower atrial fibrillation risk among patients with type 2 diabetes mellitus: a population-based dynamic cohort and in vitro studies. Cardiovasc Diabetol [Internet]. 2014 Dec 10;13(1):123. Available from: https://cardiab.biomedcentral.com/articles/10.1186/s12933-014-0123-x
8. Mascarenhas L, Downey M, Schwartz G, Adabag S. Antiarrhythmic effects of metformin. Hear Rhythm O2 [Internet]. 2024 May 1;5(5):310–20. Available from: https://doi.org/10.1016/j.hroo.2024.04.003
9. Obońska K, Grąbczewska Z, Fisz J, Kubica J. Cukrzyca i dysfunkcja śródbłonka – krótkie spojrzenie na złożony problem [in Polish]. Folia Cardiol [Internet]. 2011;6(2):109–16. Available from: https://journals.viamedica.pl/folia_cardiologica/article/view/23604
10. Duffy AM, Bouchier-Hayes DJ, Harmey JH. Vascular endothelial growth factor (VEGF) and its role in non-endothelial cells: autocrine signalling by VEGF. In: Madame Curie Bioscience Database [Internet] [Internet]. Landes Bioscience; 2013. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6482/
11. Granzier H, Labeit S. Cardiac titin: an adjustable multi-functional spring. J Physiol [Internet]. 2002 Jun;541(2):335–42. Available from: https://physoc.onlinelibrary.wiley.com/doi/10.1113/jphysiol.2001.014381
12. Zhang Q, Fang W, Ma L, Wang Z-D, Yang Y-M, Lu Y-Q. VEGF levels in plasma in relation to metabolic control, inflammation, and microvascular complications in type-2 diabetes. Medicine (Baltimore) [Internet]. 2018 Apr;97(15):e0415. Available from: https://journals.lww.com/00005792-201804130-00073
13. Jia G, Hill MA, Sowers JR. Diabetic Cardiomyopathy. Circ Res [Internet]. 2018 Feb 16;122(4):624–38. Available from: https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.117.311586
14. Lewartowski B, Mackiewicz U. Cardiac titin, the giant sarcomeric protein in health and disease. Kardiol Pol [Internet]. 2014 Mar 20;72(3):215–22. Available from: https://journals.viamedica.pl/kardiologia_polska/article/view/77718
15. Schultheiss H-P, Fairweather D, Caforio ALP, Escher F, Hershberger RE, Lipshultz SE, et al. Dilated cardiomyopathy. Nat Rev Dis Prim [Internet]. 2019 May 9;5(1):32. Available from: https://www.nature.com/articles/s41572-019-0084-1
16. Tharp C, Mestroni L, Taylor M. Modifications of Titin Contribute to the Progression of Cardiomyopathy and Represent a Therapeutic Target for Treatment of Heart Failure. J Clin Med [Internet]. 2020 Aug 26;9(9):2770. Available from: https://www.mdpi.com/2077-0383/9/9/2770
17. Vikhorev PG, Vikhoreva NN, Yeung W, Li A, Lal S, dos Remedios CG, et al. Titin-truncating mutations associated with dilated cardiomyopathy alter length-dependent activation and its modulation via phosphorylation. Cardiovasc Res [Internet]. 2022 Jan 7;118(1):241–53. Available from: https://academic.oup.com/cardiovascres/article/118/1/241/5949014
18. Buckley BJR, Harrison SL, Gupta D, Fazio-Eynullayeva E, Underhill P, Lip GYH. Atrial Fibrillation in Patients With Cardiomyopathy: Prevalence and Clinical Outcomes From Real-World Data. J Am Heart Assoc [Internet]. 2021 Dec 7;10(23). Available from: https://www.ahajournals.org/doi/10.1161/JAHA.121.021970