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Article

Treatment of shallow and deep white spot lesions with three different mouthwashes evaluated by laser fluorescence (an in vitro study)

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Abstract
This research aims to find how three different types of mouthwashes affect the depth of artificial white spot lesions. Teeth with various depths of white spot lesions were immersed in either splat mouthwash, Biorepair mouthwash, Sensodyne mouthwash, or artificial saliva (control)twice daily for one minute for 4 weeks and 8 weeks at 37°C. After this immersion procedure, lesion depth was measured using a diagnosed pen score. A one-way analysis of variance, Dunnett T3 and Tukey's post hoc $\alpha = .05$ were used to analyze the testing data. Splat mouthwash enhanced the WSL remineralization and made the lowest ΔF compared with other mouthwashes in shallow and deep enamel after 4 and 8 weeks of treatment. In the repair groups, after 4 weeks of treatment, significant recovery was observed in shallow enamel. Further improvement in shallow WSL after 8 weeks of treatment with biorepair mouthwash was observed compared to Sensodyne and the control group. Splat mouthwash is more effective than other mouthwashes in remineralizing two depths of WSLs at different time points.

Keywords: DIAGNOdent pen, Shallow enamel, Deep enamel, white spot lesion.

Introduction

Rheumatoid arthritis (RA) is a multifaceted inflammatory disease with a significant White-spot lesions (WSLs) are considered one of the most common adverse side effects resulting from orthodontic treatment; 50% of the patients had an effect of this side effect[1]. The occurrence of WSLs happened 1 month after the following fixation of the orthodontic appliance. It remained 5 years after fixed appliance removal, affecting the patients' esthetic appearance and quality of life[2]. Fixation of orthodontic appliance affects oral hygiene, as it provides areas with lowered salivary flow, which permit the adhesion of bacteria and the formation of bacterial biofilms. The acid produced by bacteria will penetrate the enamel with a clinical manifestation of WSLs if the patients cannot maintain good oral hygiene during orthodontic treatment. This WSL appears white opaque, as when the light hits an area demineralization, it scatters differently than the way it hits sound enamel[3]. The treatment of WSL includes a non-invasive approach, such as resin

infiltration or remineralization, and an invasive approach, such as the placement of crowns or veneers or micro-abrasion. The invasive measures involve enamel removal to various degrees, so the dentist should begin with a non-invasive approach[4]. Fluoride application is the most commonly used method for remineralization[5]. After fluoride application, the calcium will bind with fluoride to form calcium fluoride (CaF2), which has a cariostatic effect[6]. During a caries attack, CaF2 will form in plaque or incipient lesions; Caf2 is responsible for releasing fluoride ions when the pH is lowered[7]. Nano-hydroxyapatite is another substance that helped in the deposition of minerals on the external layer of the WSL and only had a limited ability to reduce lesion depth. Nano-HA is a practical repair material and anti-caries agent[8]. Caries detection is still challenging for the dentist since there might be carious lesions in the dentin underneath apparently normal enamel surfaces[9]. The necessity for objective and quantitive methods for caries detection led to the introduction of diode laser fluorescence (LF), which was used to detect the occlusal carious lesions based on laser-induced fluorescence in which the incipient lesions were fluorescent more than the sound tooth when exposed to laser light of specific wavelengths [10]. DIAGNOdent is an easily handheld diode laser device worked by a battery based on quantifying the fluorescence. DIAGNOdent employs a 655-nm modified red diode laser excitation light source. Carious lesions fluoresce more than 680 nm when exposed to this light. This fluorescence is identified and measured by the DIAGNOdent unit, and the reading is displayed as a numerical value on the screen ranging from (0_99) [11].

The null hypotheses were as follows:

There was no significant difference between splat, Biorepair, or Sensodyne mouthwash in remineralizing two depths of WSLs.

The shallow and deep WSL will respond similarly to the remineralizing agents.

Materials and Methods

Two different depths of white spot lesions have been investigated in this study.

- G1 contains teeth with superficial enamel lesions according to the diagnosed pen score (14-20).
- G2 contains teeth with deeper enamel lesions according to the diagnosed pen score (21-29).

Three brands of mouthwash solutions were used: biorebair mouthwash (zinc hydroxyapatite), Sensodyne mouthwash (sodium fluoride, sodium phosphate, contain 0,048%sodium fluoride {217ppm}) and splat mouthwash (calcium/magnesium/zinc hydroxyapatite and calcium lactate). Artificial saliva is used as control media. 80 sound human permanent premolars that had been extracted for orthodontic purposes were selected in this study. These selected teeth were intact with no enamel cracks, decays, fillings, surface irregularities, or any enamel abnormality like hypoplastic enamel and without any previous treatment with chemical agents such as bleaching material. The remnants of blood and calculus were removed from the teeth, and polishing the buccal surfaces of the teeth was done with a fluoride-free pumice and rubber cup. Chloramine T

trihydrate bacteriostatic _bactericidal solution was used for storing the teeth for one week, then stored in distilled water at room temperature until use. Each tooth was poured into a cold cure acrylic mold. All the teeth were covered with acid resistance nail varnish except a small region in the buccal surface of each tooth, which was covered by adhesive tape with 6 mm × 6 mm dimension to form a window at which a white spot lesion will be created. The adhesive tape was removed after the nail varnish coating was completed. DIAGNOdent pen (KaVo, Biberach, Germany) was used to check the samples.

The device was calibrated against its ceramic standards before each measurement, as per the recommendation of the manufacturer. The probe was used to scan the buccal window of the teeth, keeping the pen in close touch with the tooth surface and moving it around the window to get the greatest fluorescence area. The baseline (T0) fluorescence values of the samples were then recorded.

All teeth were then immersed in a demineralizing solution (2.2 mM calcium chloride, 2.2 mM monopotassium phosphate, and 1 M potassium hydroxide were mixed to make the demineralizing solution with the addition of 0.05 mM acetic acid to create an acidic medium with a pH of 5) for all the day. Every day, the solution was changed until the frosty white appearance showed. The buccal window was evaluated with a DIAGNOdent™ pen (KaVo, Bibberach, Germany) day after day. If the lesion depth was insufficient, the teeth were immersed again in the demineralizing solution. The teeth were divided into two significant groups, 40 teeth each, according to the readings of the DIAGNOdent™: shallow enamel group and deep enamel group. Then, each leading group was subdivided into four subgroups, 10 teeth each according to the remineralizing agent used. The samples' demineralization readings (T1) fluorescence values were then recorded. After that, the specimens were washed with DDW and then stored in artificial saliva, which was changed every day [12]**.** All samples were removed daily from the artificial saliva and immersed in a small plastic mold containing mouthwash (twice daily for one minute). Then, the samples were rinsed with de-ionized water and returned to artificial saliva, which was renewed daily.

The vibrator will be used (to vibrate the mold) to simulate the action of the mouthwash inside the oral cavity.

DIAGNOdent Pen scores will be measured at baseline (T0), after induction of WSL (T1), after 4 weeks of treatment (T2), and after 8 weeks of treatment (T3)

Results

Using Shapiro Wilk test, the fluorescence difference in each phase (ΔFBD, ΔFBR1, ΔFBR2, ΔFR1R2) among mouthwashes and enamel lesion depth was shown to be generally distributed at $p>0.05$ (Table 1).

Variable	Mouthwash	Enamel lesion depth					
		Shallow			Deep		
		Statistic	df	P value	Statistic	df	P value
ΔFBD	Splat MW	0.984	10	0.983	0.859	10	0.075
	Biorepair MW	0.969	10	0.878	0.954	10	0.713
	Sensodyne MW	0.953	10	0.703	0.935	10	0.498
	Control	0.899	10	0.215	0.909	10	0.277
ΔFBR1	Splat MW	0.872	10	0.107	0.911	10	0.290
	Biorepair MW	0.929	10	0.442	0.964	10	0.830
	Sensodyne MW	0.850	10	0.058	0.932	10	0.463
	Control	0.848	10	0.055	0.946	10	0.626
ΔFBR2	Splat MW	0.943	10	0.588	0.891	10	**0.**173
	Biorepair MW	0.934	10	0.484	0.848	10	0.055
	Sensodyne MW	0.943	10	0.588	0.966	10	0.850
	Control	0.934	10	0.484	0.928	10	0.425

Table 1: Normality test (Shapiro-Wilk test) of fluorescence difference among mouthwashes and enamel lesion depth.

The baseline measurements showed no significant difference in inflorescence between mouthwashes and enamel lesion depth (Table 2).

Mouthwashes	Enamel lesion depth								T-test	
	Shallow				Deep				T value	P value
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD		
Splat MW	4.000	8.000	6.200	1.135	5.000	8.000	6.300	0.949	0.214	0.833
Biorepair MW	5.000	8.000	6.600	0.843	5.000	8.000	6.200	1.033	0.949	0.355
Sensodyne MW	5.000	8.000	6.000	1.054	5.000	8.000	6.500	1.080	1.048	0.309
Control	5.000	8.000	6.200	0.919	5.000	8.000	6.100	0.876	0.249	0.806
ANOVA	F=0.640				F=0.299					
	P=0.594 NS				P=0.826 NS					

Table 2: Descriptive and statistical test of fluorescence baseline among mouthwashes and enamel lesion depth.

Mouthwash	Enamel lesion depth								T-test	
	Shallow				Deep				T value	P value
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD		
Splat MW	6.000	14.000	10.000	2.211	15.000	21.000	17.900	2.025	8.332	**0.000**
Biorepair MW	7.000	13.000	9.500	2.838	17.000	23.000	19.700	1.829	9.553	**0.000**
Sensodyne MW	8.000	12.000	10.000	1.155	15.000	21.000	18.000	2.000	10.954	**0.000**
Control	8.000	13.000	11.000	1.491	15.000	23.000	18.300	2.751	7.378	**0.000**
ANOVA	F=0.960				F=1.464					
	P=0.422				P=0.241					

Table 3: Descriptive and statistical test of fluorescence change among mouthwashes and enamel lesion depth from baseline to demineralization readings (ΔFBD).

Mouthwash	Enamel lesion depth								T-test	
	Shallow				Deep				T value	P value
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD		
Splat MW	6.000	14.000	10.000	2.211	15.000	21.000	17.900	2.025	8.332	0.000
Biorepair MW	7.000	13.000	9.500	2.838	17.000	23.000	19.700	1.829	9.553	0.000
Sensodyne MW	8.000	12.000	10.000	1.155	15.000	21.000	18.000	2.000	10.954	0.000
Control	8.000	13.000	11.000	1.491	15.000	23.000	18.300	2.751	7.378	0.000
ANOVA	F=0.960				F=1.464					
	P=0.422				P=0.241					

Table 4: Descriptive and statistical test of fluorescence change among mouthwashes and enamel lesion depth ΔFBR1.

Mouthwash	Enamel lesion depth								T-test	
	Shallow				Deep				T value	P value
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD		
Splat MW	3.000	7.000	5.600	1.265	8.000	16.000	11.200	2.616	6.094	0.000
Biorepair MW	4.000	13.000	7.800	3.048	12.000	22.000	16.500	3.100	6.328	0.000
Sensodyne MW	6.000	12.000	9.500	1.958	13.000	19.000	16.600	1.776	8.493	0.000
Control	8.000	12.000	10.300	1.703	12.000	22.000	16.900	3.414	5.471	0.000
ANOVA	F=9.821				F=9.595					
	P=.000				P=.000					

Table 5: Multiple pairwise comparisons of fluorescence change among mouthwashes in shallow and deep enamel ΔFBR1 Dunnett T3.

Mouthwash	Enamel lesion depth								T-test	
	Shallow				Deep				T value	P value
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD		
Splat MW	1.000	4.000	2.500	1.434	6.000	12.000	8.200	1.989	7.352	0.000
Biorepair MW	4.000	10.000	5.800	2.348	10.000	20.000	13.600	3.718	5.610	0.000
Sensodyne MW	5.000	10.000	8.200	1.751	11.000	17.000	13.700	1.703	7.120	0.000
Control	7.000	11.000	9.100	1.449	10.000	19.000	15.200	3.011	5.773	0.000
ANOVA	F=27.330				F=12.689					
	P=.000				P=.000					

Table 6: Descriptive and statistical test of fluorescence change among mouthwashes and enamel lesion depth ΔFBR2.

Mouth wash		Shallow enamel lesion		Deep enamel lesion	
		MD	P value	MD	P value
Splat MW	Biorepair MW	-3.300	**0.001**	-5.400	**0.007**
	Sensodyne MW	-5.700	**0.000**	-5.500	**0.000**
	Control	-6.600	**0.000**	-7.000	**0.000**
Biorepair MW	Sensodyne MW	-2.400	**0.024**	-.100	0.999
	Control	-3.300	**0.001**	-1.600	0.864
Sensodyne MW	Control	-.900	0.675	-1.500	0.679

Table 7: Multiple pairwise comparisons of fluorescence change among mouthwashes in shallow and deep enamel ΔFBR2 Tukey HSD.

Discussion

Because WSLs are considered one of the most adverse side effects of fixed orthodontic treatment, it is vital to know the best treatment modalities and the effectiveness of the agents that produce remineralization. However, no previous research had evaluated the ability of the mouthwashes to remineralize two depths of WSLs. Therefore, this study aimed to examine the effectiveness of three different mouthwashes in remineralizing two depths of WSLs using an in vitro study.

The change in lesion fluorescence was used to determine the effect of the mouthwashes. The null hypotheses were rejected based on the findings since the differences in the remineralization capabilities were seen among the mouthwashes, and the different lesion depths responded differently. Splat mouthwash was better in reducing the fluorescence loss in both shallow and deep lesions as it had the lowest mean values compared to repair mouthwash, Sensodyne mouthwash, and control (Artificial saliva).

Fluoride treatment strengthens the mineral surface layer by the strong affinity of fluoride to hydroxyapatite, preventing further mineral loss from the lesion. Previous studies have shown that fluoride-containing mouth rinse only remains in situ for up to 24 hours.[13] However, the possible time point remained undetermined. In the splat groups, the result showed that it had the best improvement among the remaining mouthwashes. Significant fluorescence improvement was shown after 4 weeks of treatment, with further improvements at 8 weeks for both depths. Its superior ability in remineralizing WSLs could be attributed to its composition (calcium/magnesium/zinc hydroxyapatite and calcium lactate), which provides the enamel surface with calcium and phosphate ions, promoting more homogeneous remineralization throughout the body of the lesion. Regarding bio-repair mouthwash, results showed that it significantly improves shallow enamel after 4 weeks and 8 weeks of treatment, which may be related to the fact that it also contains hydroxyapatite.

Although there is a reduction in the values of Δf in Sensodyne mouthwash and the control group after 4 weeks and 8 weeks of treatment compared to baseline values, these values are not clinically and statistically significant.

The findings of this study, which demonstrate the superiority of splat mouthwash and repair mouthwash, were due to its composition (nano-hydroxyapatite). In some research, they found that hydroxyapatite was superior to fluoride in remineralizing WSL.[14]

The remineralization of WSLs by the artificial saliva with NaF treatment groups was a slow process, as it depended on the deposition of calcium ions.[15]

The differences among the samples of the control groups from baseline to the 8th week imply that natural remineralization of WSLs happened. Previous research [16,17] indicated that the solution of artificial saliva, frequently utilized as a negative control group in vitro study, demonstrated the capacity for remineralizing WSLs. Therefore, the reduction in fluorescence change is likely linked to the daily changing of the artificial saliva solution at a pH level between 6.5 and 6.

There was no significant difference in fluorescence between mouthwashes and enamel lesion depth in the baseline measurements. After demineralization, the greater the WSL, the more ΔF recorded, and the result was significant between shallow and deep enamel. This may be related to the more significant dissolution of mineral crystals in demineralized teeth. After the application of the remineralizing mouthwashes, a significant difference still existed. Based on this, the second null hypothesis was also rejected. Although this study could not imitate real-life situations exactly, the results of this study have the potential to supply helpful information on the remineralization efficiency of different brands of mouthwashes for two WSLs depths. Further, in vivo studies would be advantageous to validate treatment efficacy regarding different brands of mouthwashes on different WSL depths during and after orthodontic therapy.

Conclusion

Splat mouthwash containing hydroxyapatite was superior to the other tested mouthwashes in promoting white spot lesion remineralization. It could regain the original fluorescence of the shallow lesions and considerably reduce the fluorescence change for the deep lesions.

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Conflict of interest

None

References

1. Ahsan, H., Hasan, M. Y., & Ahmad, R. Role of free radicals in autoimmune diseases. In Translational Autoimmunity. 2022; (pp. 317-324). Academic Press.
2. Albarzinji, N., Ismael, S. A., & Albustany, D. Association of rheumatoid arthritis and its severity with human leukocytic antigen-DRB1 alleles in Kurdish region in North of Iraq. BMC rheumatology, 2022; 6(1), 1-5.

3. Albarzinji, N., Ismael, S. A., & Albustany, D. Association of rheumatoid arthritis and its severity with human leukocytic antigen-DRB1 alleles in Kurdish region in North of Iraq. BMC rheumatology,2022; 6(1), 1-5.
4. Aldabbagh, K. A. O., & Al-Bustany, D. A. Relationship of serum copper and HLADR4 tissue typing to disease activity and severity in patients with rheumatoid arthritis: A cross-sectional study. Annals of Medicine and Surgery, 2022; 73, 103193.
5. Alghamdi, M. F., & Redwan, E. M. Advances in the diagnosis of autoimmune diseases based on citrullinated peptides/proteins. Expert review of molecular diagnostics,2021; 21(7), 685-702.
6. Aljoboury, B. A., Alta'ee, A. H., & Alrubaie, S. J. Association of HLA-DRB1 Gene Polymorphism in Rheumatoid Arthritis Patients in Babylon Province, Iraq. Indian Journal of Forensic Medicine & Toxicology, 2020;14(3).
7. AL-TIMIMI, D. J., SHAFEEQ, S. S., AHMED, I. H., & AL-QADI, R. A. FREQUENCY OF HLA-DRB1/DQB1 ALLELES AMONG TYPE 1 DIABETES PATIENTS IN DUHOK, KURDISTAN REGION (IRAQ). Duhok Medical Journal, 2019; 13(1), 66-73.
8. Ash, D., & Roy, T. Immunogenetics in the diagnosis of clinical disorders. In Clinical Applications of Immunogenetics, 2022; (pp. 35-56). Academic Press.
9. Ash, D., & Roy, T. Immunogenetics in the diagnosis of clinical disorders. In Clinical Applications of Immunogenetics, 2022; (pp. 35-56). Academic Press.
10. Begovich, A. B., Carlton, V. E., Honigberg, L. A., Schrodi, S. J., Chokkalingam, A. P., Alexander, H. C., ... & Gregersen, P. K. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. The American Journal of Human Genetics, 2004; 75(2), 330-337.
11. Bhagavatham, S. K. S., Potikuri, D., & Sivaramakrishnan, V. Adenosine deaminase and cytokines associated with infectious diseases as risk factors for inflammatory arthritis and methotrexate as a potential prophylactic agent. Medical Hypotheses, 2022; 159, 110751.
12. Bian, B., Couvy-Duchesne, B., Wray, N. R., & McRae, A. F. The role of critical immune genes in brain disorders: insights from neuroimaging immunogenetics. Brain Communications, 2022.
13. Birga, A. M., Ning, L., & Huang, J. The Genetic Variants of HLA-DRB1 Alleles and the Chance of Developing Rheumatoid Arthritis: Systematic Review and meta-analysis , 2021.
14. Bodmer, W., & Bonilla, C. Common and rare variants in multifactorial susceptibility to common diseases. Nature genetics, 2008; 40(6), 695-701.
15. Brewerton, D. A., Hart, F. D., Nicholls, A., Caffrey, M., James, D. C. O., & Sturrock, R. D. Ankylosing spondylitis and HL-A 27. The Lancet, 1973; 301(7809), 904-907.
16. Chamani, S., Moossavi, M., Naghizadeh, A., Abbasifard, M., Majeed, M., Johnston, T. P., & Sahebkar, A. Immunomodulatory effects of curcumin in systemic autoimmune diseases. Phytotherapy Research, 2022.
17. Chasman, D. I., Hyde, C. L., Giulianini, F., Danning, R. D., Wang, E. Q., Hickling, T., ... & Loomis, A. K. Genome-wide pharmacogenetics of anti-drug antibody response to bococizumab highlights key residues in HLA DRB1 and DQB1. Scientific reports, 2022; 12(1), 1-11.
18. Chia, R., Saez-Atienzar, S., Murphy, N., Chiò, A., Blauwendraat, C., International Myasthenia Gravis Genomics Consortium, ... & Traynor, B. J. Identification of genetic risk loci and prioritization of genes and pathways for myasthenia gravis: a genome-wide association study. Proceedings of the National Academy of Sciences, 2022; 119(5), e2108672119.
19. Clanchy, F. I., Borghese, F., Bystrom, J., Balog, A., Penn, H., Taylor, P. C., ... & Williams, R. O. Disease status in human and experimental arthritis, and response to TNF blockade, is associated with MHC class II invariant chain (CD74) isoform expression. Journal of Autoimmunity, 2022; 128, 102810.
20. Conforti, A., Di Cola, I., Pavlych, V., Ruscitti, P., Berardicurti, O., Ursini, F., ... & Cipriani, P. Beyond the joints, the extra-articular manifestations in rheumatoid arthritis. Autoimmunity Reviews, 2021; 20(2), 102735.
21. Dracou, C., Constantinidou, N., & Constantopoulos, A. Juvenile chronic arthritis profile in Greek children. Pediatrics International, 1998; 40(6), 558-563.
22. Firdous, P., Nissar, K., & Ali, S. Rheumatoid arthritis: immunogenetic factors and immune therapies. In Clinical Applications of Immunogenetics, 2022; (pp. 279-307). Academic Press.

23. García, M. V., Barrandeguy, M. E., & Prinz, K. Contemporary climate influence on variability patterns of Anadenanthera colubrina var. cebil, a key species in seasonally dry tropical forests. Journal of Forestry Research, 2022; 33(1), 89-101.
24. Gonzalez-Gay, M. A., Garcia-Porrua, C., & Hajeer, A. H. Influence of human leukocyte antigen-DRB1 on the susceptibility and severity of rheumatoid arthritis. In Seminars in arthritis and rheumatism, 2002; Vol. 31, No. 6, pp. 355-360). WB Saunders.
25. Gregersen, P.; Sliver, J. & Winchester , R. The shared epitope hypothesis - an approach to understanding the molecular genetics of rheumatoid arthritis Susceptibility. Arthritis Rheum, 1987; 30: 1205-1213.
26. Heide, G. Human platelet antigen (HPA)-1a alloimmunization-Why only blame it on the platelets, 2021.
27. Horinouchi, T., Nozu, K., & Iijima, K. An updated view of the pathogenesis of steroid-sensitive nephrotic syndrome. Pediatric Nephrology, 2022; 1-9.
28. Dmitry Olegovich Bokov, Abduladheem Turki Jalil, Forat H. Alsultany, Mustafa Z. Mahmoud, Wanich Suksatan, Supat Chupradit, Maytham T. Qasim & Parvaneh Delir Kheirollahi Nezhad. Ir-decorated gallium nitride nanotubes as a chemical sensor for recognition of mesalamine drug: a DFT study, Molecular Simulation, 2022. DOI: 10.1080/08927022.2021.2025234
29. Ansari, M.J., Jasim, S.A., Taban, T.Z. et al. Anticancer Drug-Loading Capacity of Green Synthesized Porous Magnetic Iron Nanocarrier and Cytotoxic Effects Against Human Cancer Cell Line. J Clust Sci (2022). https://doi.org/10.1007/s10876-022-02235-4
30. Huldani Huldani, Saade Abdalkareem Jasim, Dmitry Olegovich Bokov, Walid Kamal Abdelbasset, Mohammed Nader Shalaby, Lakshmi Thangavelu, Ria Margiana, Maytham T. Qasim. Application of extracellular vesicles derived from mesenchymal stem cells as potential therapeutic tools in autoimmune and rheumatic diseases, International Immunopharmacology,Volume 106, 2022, 108634, ISSN 1567-5769, https://doi.org/10.1016/j.intimp.2022.108634.
31. Zadeh, Firoozeh Abolhasani, et al. "Cytotoxicity evaluation of environmentally friendly synthesis Copper/Zinc bimetallic nanoparticles on MCF-7 cancer cells." Rendiconti Lincei. Scienze Fisiche e Naturali (2022): 1-7.
32. Hafsan Hafsan,Dmitry Bokov,Walid Kamal Abdelbasset,Mustafa M. Kadhim,Wanich Suksatan,Hasan Sh. Majdi,et al . Dietary Dracocephalum kotschyi essential oil improved growth, haematology, immunity and resistance to Aeromonas hydrophila in rainbow trout (Oncorhynchus mykiss), 2022. https://doi.org/10.1111/are.15829
33. Marinović, I., Čečuk-Jeličić, E., Perković, D., Marasović Krstulović, D., Aljinović, J., Šošo, D., ... & Martinović Kaliterna, D. Association of HLA-DRB1 alleles with rheumatoid arthritis in Split-Dalmatia County in southern Croatia. Wiener klinische Wochenschrift,2022; 1-8.
34. Marinović, I., Čečuk-Jeličić, E., Perković, D., Marasović Krstulović, D., Aljinović, J., Šošo, D., ... & Martinović Kaliterna, D. (2022). Association of HLA-DRB1 alleles with rheumatoid arthritis in Split-Dalmatia County in southern Croatia. Wiener klinische Wochenschrift, 2022; 1-8.
35. Mikhaylenko, D. S., Kuznetsova, E. B., Musatova, V. V., Bure, I. V., Deryagina, T. A., Alekseeva, E. A., ... & Nemtsova, M. V. Genetic and Clinical Factors Associated with Olokizumab Treatment in Russian Patients with Rheumatoid Arthritis. Journal of Personalized Medicine,2022; 12(4), 641.
36. Mikhaylenko, D. S., Kuznetsova, E. B., Musatova, V. V., Bure, I. V., Deryagina, T. A., Alekseeva, E. A., ... & Nemtsova, M. V. Genetic and Clinical Factors Associated with Olokizumab Treatment in Russian Patients with Rheumatoid Arthritis. Journal of Personalized Medicine, 2022; 12(4), 641.
37. Muazzam, A. G., Mansoor, A., Ali, L., Siddiqi, S., Hameed, A., Ajmal, M., & Mazhar, K. Association of HLA-DRB1 and-DQB1alleles and haplotypes with rheumatoid arthritis in a Pakistani population. Arthritis Research & Therapy, 2013; 15(4), 1-9.
38. Mukhtar, M., Nadeem Sheikh, D., Andleeb Batool, D., Khawar, M. B., Naz Fatima, D., & Mehmood, R. Novel functional polymorphism on PADI-4 gene and its association with arthritis onset. Saudi Journal of Biological Sciences, 2022; 29(2), 1227.
39. Nabi, N., Khan, M. S., Shah, A., Wani, J. A., & Majid, S. Human leukocyte antigen-typing: a significant immunogenetic application in clinical medicine. In Clinical Applications of Immunogenetics , 2022; pp. 245-265. Academic Press.
40. Nagafuchi, Y., Shoda, H., Sumitomo, S., Nakachi, S., Kato, R., Tsuchida, Y., ... & Yamamoto, K. Immunophenotyping of rheumatoid arthritis reveals a linkage between HLA-DRB1 genotype, CXCR4 expression on memory CD4+ T cells and disease activity. Scientific reports, 2016; 6(1), 1-11.

41. Nyman, S.; mottonnen, T.; Hermann, R.; Tuokko, J.; Luukkanen, R.;Hakala, M.;Hannonen, P.;Korpela, M.;Toivanen, A. & Ilonen, J. HLA-DR-DQ haplotypes & genotypes in Finnish patients with rheumatoid arthritis. Ann. Rheum. Dis. 2004 ; 63: 1406-1412.
42. Padyukov, L. Genetics of rheumatoid arthritis. In Seminars in Immunopathology, 2022; (pp. 1-16). Springer Berlin Heidelberg.
43. Panhuber, A., Lamorte, G., Bruno, V., Cetin, H., Bauer, W., Erber, A., ... & Koneczny, I. Genetic risk factors for pathogenic IgG4 autoantibodies: a systematic review and meta-analysis of HLA class II associations in patients with IgG4 autoimmune diseases, 2021.
44. Pascual, M.; Nieto, A.; Lopez-Nevot, M.; Ramal, L. & Caballero, A. Rheumatoid arthritis in southern Spain: toward elucidation of a unifying role of the HLA class II region in disease predisposition. Arthritis Rheum. 2021; 44: 307-14.
45. Petrovská, N., Prajzlerová, K., Vencovský, J., Šenolt, L., & Filková, M. The pre-clinical phase of rheumatoid arthritis: From risk factors to prevention of arthritis. Autoimmunity Reviews, 2021; 20(5), 102797.
46. Regueiro, C., Casares-Marfil, D., Lundberg, K., Knevel, R., Acosta-Herrera, M., Rodriguez-Rodriguez, L., ... & Gonzalez, A. HLA–B* 08 Identified as the Most Prominently Associated Major Histocompatibility Complex Locus for Anti–Carbamylated Protein Antibody–Positive/Anti–Cyclic Citrullinated Peptide–Negative Rheumatoid Arthritis. Arthritis & Rheumatology, 2021; 73(6), 963-969.
47. Ria, F., Pirolli, D., Di Sante, G., Righino, B., Gremese, E., Gervasoni, J., ... & De Rosa, M. C. Selective Inhibitors of T Cell Receptor Recognition of Antigen–MHC Complexes for Rheumatoid Arthritis. ACS medicinal chemistry letters, 2019; 10(4), 644-649.
48. Rojas-Villarraga, A., Diaz, F. J., Calvo-Páramo, E., Salazar, J. C., Iglesias-Gamarra, A., Mantilla, R. D., & Anaya, J. M. Familial disease, the HLA-DRB1 shared epitope and anti-CCP antibodies influence time at appearance of substantial joint damage in rheumatoid arthritis. Journal of autoimmunity, 2009; 32(1), 64-69.
49. Rosetti, F., Madera-Salcedo, I. K., Rodríguez-Rodríguez, N., & Crispín, J. C. Regulation of activated T cell survival in rheumatic autoimmune diseases. Nature Reviews Rheumatology, 2022; 1-13.
50. Røyrvik, E. C., & Husebye, E. S. The genetics of autoimmune Addison disease: past, present and future. Nature Reviews Endocrinology, 2022; 1-14.
51. Scavuzzi, B. M., van Drongelen, V., & Holoshitz, J. HLA-G and the MHC Cusp Theory. Frontiers in Immunology,2021; 706.
52. Tan, L. K., Too, C. L., Diaz-Gallo, L. M., Wahinuddin, S., Lau, I. S., Heselynn, H., ... & Padyukov, L. The spectrum of association in HLA region with rheumatoid arthritis in a diverse Asian population: evidence from the MyEIRA case-control study. Arthritis research & therapy,2021; 23(1), 1-13.
53. van der Horst, IE.; Visser, H.;Hazes ,J.; Breedveld, F. ; Verduyn, W. & Schreuder, G. HLA-DQ associated predisposition to and dominant HLA-DR associated protection against rheumatoid arthritis. Human Immunol. 1999 ;60:152-8.
54. van der Woude, D., & van der Helm-van, A. H. Update on the epidemiology, risk factors, and disease outcomes of rheumatoid arthritis. Best Practice & Research Clinical Rheumatology, 2018; 32(2), 174-187.
55. van der Woude, D., & van der Helm-van, A. H. Update on the epidemiology, risk factors, and disease outcomes of rheumatoid arthritis. Best Practice & Research Clinical Rheumatology, 2018; 32(2), 174-187.
56. Vecellio, M., Wu, H., Lu, Q., & Selmi, C. The multifaceted functional role of DNA methylation in immune-mediated rheumatic diseases. Clinical rheumatology, 2021; 40(2), 459-476.
57. Vetchinkina, E. A., Mikhaylenko, D. S., Kuznetsova, E. B., Deryagina, T. A., Alekseeva, E. A., Bure, I. V., ... & Nemtsova, M. V. Genetic Factors of Predisposition and Clinical Characteristics of Rheumatoid Arthritis in Russian Patients. Journal of personalized medicine,2021; 11(6), 469.
58. Weyand, C. M., Hicok, K. C., Conn, D. L., & Goronzy, J. J. The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. Annals of Internal Medicine, 1992; 117(10), 801-806.
59. Yang, Z., Liu, W., Yan, T., & Liu, R. HLA-DPB1 rs9277535 polymorphism is associated with rheumatoid arthritis risk in a Chinese Han population. Aging (Albany NY), 2021; 13(8), 11696.
60. Yelamos, J., Raül Garcia-Lozano, J., Moreno, I., Aguilera, I., Francisca Gonzalez, M., Garcia, A., ... & Sanchez, B. Association of HLA–DR4-Dw15 (DRB1* 0405) and DR10 with rheumatoid arthritis in a

Spanish population. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology, 1993; 36(6), 811-814.
61. Zhang, T. Perinatal risk factors for mental disorders in the offspring and in their mothers, 2022.

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