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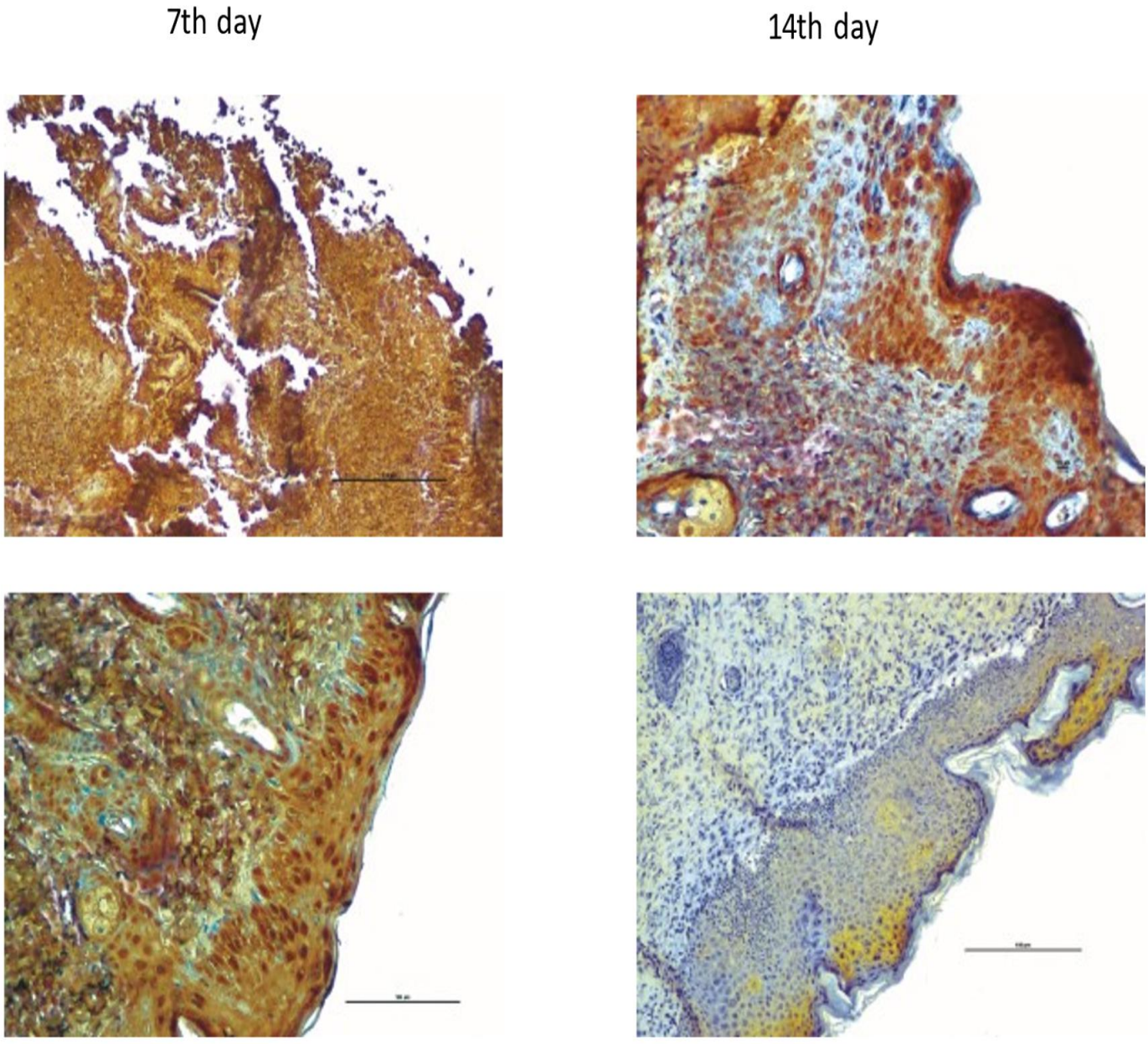

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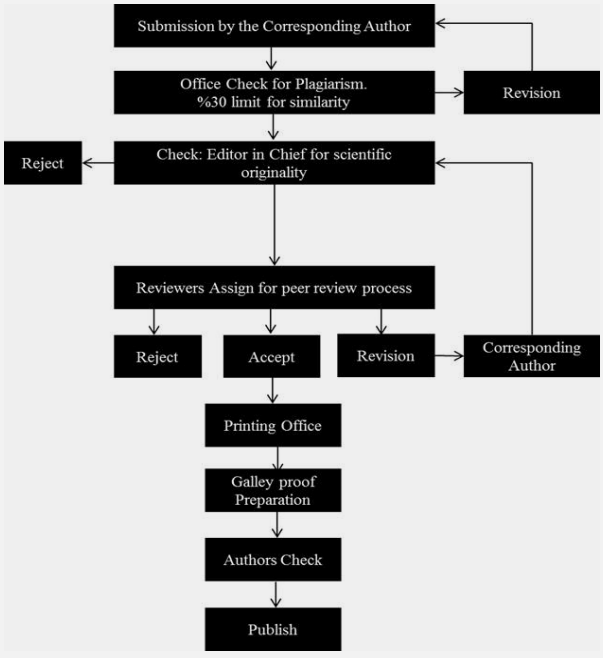

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Contents

Review Articles

Research Articles

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The role of probiotics as gastrointestinal infections treatment and prophylaxis: A review

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ABSTRACT

Objective: Probiotics are products that contain microorganisms capable of supporting symbiotic relations with native microbiota of many environments. They are widely used and studied due to their capacity to improve biological systems' overall health. Many hypothesis exist surrounding the mechanisms by which every microbe labelled as probiotic is the cause by which system health is enhanced by its presence. The aim of this review was to compile article's data concerning the role of different combinations of probiotics used to treat and prevent gastrointestinal conditions, such as antibiotic-associated diarrhea, pseudomembranous colitis, Helicobacter pylori infections, oral, pharyngeal and Salmonella infections. In general, other than presenting excellent safety records, several probiotic combinations registered in clinical trials could prove themselves capable of significantly preventing those infections and some proved to be capable to also treat them once established. The main challenge among the infections studies seems to be oral cavity infections, probably due to microbiota complexity. Nevertheless, probiotics seem to have good prospect for playing a major preventive and protective role in gastrointestinal infections with further investigation to gather sufficient evidence to base treatment protocols.

Keywords: Probiotics; Gastrointestinal Diseases; Clinical Protocols, review

INTRODUCTION

The definition of probiotics was first produced in 1965 by Lilly and Stillwell (1) as bacteria who could increase other bacteria proliferation within biological systems, since then, they have had their definition and usage broadly expanded. Currently updated by Hill et al. in 2014, the meaning of probiotics is now fit to describe microorganisms that can improve the health of a system when ingested insufficient amounts (2).

Therefore, such definition is intended to include their effects on many human systems concerning medical sciences, mainly by impacting the resident microbiota, intestinal epithelium cells, the immune system, and cell-mediated response to infection and stress.

The most studied species include Lactobacillus, Bifidobacterium, and Saccharomyces (3), but even prior to the first steps investigating probiotics' potential, bacteria described as probiotic have been empirically added to dietary supplementation and foods due to the potential beneficial effects their action could produce for human health, and naturally clinical trials have been extensively conducted to measure and improve probiotic consumption and selection (4). Further than that, many studies nowadays have provided evidence to support probiotics' use as treatment and prophylaxis of a large array of infections, such as those produced by Helicobacter pylori, Clostridium difficile on the gastrointestinal tract, Streptococcus mutans on caries and periodontitis, many upper and lower respiratory tract infections, inflammatory bowel disease and diarrhea microbes, as well as other diseases.

Not only typical presentation cases management have shown to be improved by probiotics use, but also multi-drug resistant bacteria infections could potentially be considered for probiotics use, amplifying their relevance since those are universally important public health issues.

Review Article

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The major role probiotics could play, if proven to be effective, is to decrease antibiotic use, which has the catastrophic side-effect of microbial resistance development, other than being a cause of many gastrointestinal conditions themselves, such as very common cases of antibiotic-associated diarrhea (AAB). Mitigating the burdens of diseases and antibiotic use could improve health care morbidity, mortality and cost worldwide (5).

Further on, the interest on probiotics is also inspired by high safety standards extensively recorded on literature (6) (7), even on immunocompromised HIV patients (8) and children (9).

We present this article as an endeavor to produce an updated compilation of current literature concerning probiotic use on several infections and its comparison to traditional antibiotic treatment on terms of prophylactic and treatment effectiveness, safety, and outcomes on gastrointestinal infections.

MATERIAL and METHODS

The present review focused research on well-oriented clinical trials concerning probiotic use on AAD, *Clostridium difficile* infection (CDI), *Helicobacter pylori* infection, *Streptococcus mutans* oral infections, *Streptococcus* and *Bacilli* pharyngeal infections and *Salmonella* infection.

Article research was conducted on PubMed, Scielo, Science Direct and Medline bases. The following key-words were used: "probiotics", "treatment", “prevention", “C*lostridium difficile* infection”, “*Helicobacter pylori*”, “*Salmonella*”, “oral infection”, “microbiota”, “pharynx infection”, “antibiotic" as well as its equivalents in Portuguese. Boxes "AND" and "OR" were selected when they were present.

Enters and records identified in the electronic data banks were exported to the platform Rayaan, used in selection. Studies were initially filtered by title and abstract independently and those selected on a first filtration were evaluated regarding eligibility and inclusion in this review by full-text analysis.

Articles of opinion and isolated case reports were the only automatic exclusion criteria for article analysis, and no case complications were considered as to differ among infection presentations. Articles were also not excluded based on language, date or place of conduction.

RESULTS

1. Probiotics use on Antibiotic-associated diarrhea (AAD)

AAD is a common and undesirable adverse effect of antibiotic treatment and can present itself disregarding previous patient conditions. It occurs in as many as 30% of patients (10) and is characterized by disruption of gut microbiota, decreased intestinal short-chain fatty acid (SCFA) concentrations, accumulation of luminal carbohydrates and colonic bile acids, altered water absorption, and ultimately diarrhea (11).

Several articles evaluated probiotic treatment in children since they are particularly at risk with the incidence of AAD being as high as 35% (12), and all conclusive ones suggested probiotics to be effective in preventing and treating AAD, despite previous theoretical conjectures which suggested that probiotic treatment was a logical flaw on AAD (13).

Compiling thirty-three studies with more than 6300 patients ranging from 0 to 18 years-old, organized in random groups for probiotic treatment, placebo, and no treatment at all it was recorded precise benefit for probiotics. Diarrhea incidence after five days to 12 weeks of follow-up was shown to be 8% in the probiotic group, in comparison to 19% in the control group (risk ratio RR 0.45, 95% confidence interval CI 0.36 to 0.56; I^2 = 57%, 6352 participants; 95% CI 7 to 13). Low dose studies (< 5 billion CFUs per day) showed that the incidence of AAD in the probiotic group was 8% compared to 23% in the control group (4038 participants; RR 0.37; 95% CI 0.30 to 0.46; P = 0.06) and high dose studies (≥ 5 billion CFUs per day) recorded incidence rates of 13% in the probiotic group compared to 23% in control group (4425 participants; RR 0.54; 95% CI 0.42 to 0.70; P <0.00001; I^2 = 68%) (14). The results suggested the effectiveness of treatment to be dose-dependent.

Comparing safety among 24 of those trials (4415 participants) which reported on adverse events, serious adverse events none reported attributable to probiotics.

Although also insensitive due to numerous subgroups consideration, another compilation of 82 randomized trials showed statistically significant AAD reduction results by associating probiotics to antibiotic treatment (relative risk, 0.58; 95% CI, 0.50 to 0.68; P < .001; I(2), 54%) (15).

On the strands used, *Lactobacillus rhamnosus* GG (LGG) had the better record of effectiveness and tolerability on prevention of AAD, and even though *Clostridium difficile* is broadly known to cause around 20% of AAD as an opportunistic pathogen (16), it is better analyzed independently since a 2018 article reported that *Lactobacillus casei* was considered as presenting better efficacy and moderately better tolerance for its infections (17). Another strong recommendation strand is claimed to be *Saccharomyces boulardii*, although evidence is still lacking and current results are still behind LGG (18).

A double-blind randomized study conducted in Australia also confirmed LGG effectiveness combined with *Bifidobacterium lactis* (Bb-12) and *Lactobacillus acidophilus* (La-5). Children were given 200g/day probiotic yogurt versus pasteurized yogurt as placebo, with a narrower population consisting of 72 children of which 70 completed treatment, no severe diarrhea (stool consistency ≥6, ≥3 stools/day for ≥2 consecutive days) episodes were reported in probiotic group while six presented in placebo, and only one minor diarrhea (stool consistency ≥5, ≥2 stools/day for ≥2 days) episode was presented in probiotic group compared to 21 in placebo (19).

2. Probiotics use in *Clostridium difficile* infection (CDI)

The usual *Clostridium difficile* infection causes pseudomembranous colitis, and it is also the leading opportunistic pathogen to cause AAD. Disruption of gastrointestinal microbiota is the most evidence-based infection etiology, since normal conditions usually suffice for preventing *Clostridium* infection (20).

A recent consensus guidelines published by the British Society of Gastroenterology did mention probiotics as

relatively effective treatment to CDI, although it suggested that its use should be restricted to very uncommon scenarios and never as first line treatment or prophylaxis, opposing antibiotics, claiming evidence was lacking (21). Nevertheless, several articles were then conducted, including a Cochrane database article, indicating that guidelines should be revised, since probiotics have the highest quality evidence among cited prophylactic therapies (22).

In order to base this claim, the study compiled thirty-nine studies, concluding that probiotic prophylaxis should reduce the risk of *C. difficile*-associated diarrhea by 60%. The incidence of presentations was 1.5% in the probiotic group and 4.0% in the control (RR 0.40, 95% CI 0.30 to 0.52), which was considered to be statistically significant, even though it noted the heterogeneity of results when pooled.

Even prior to the guideline's publication, many articles suggested reasonable evidence that probiotics could indeed prevent CDI in many cases. New mechanisms were even discovered, suggesting probiotics health promotion worked not only through microbiome protection but also by directly inhibiting pathogen growth, neutralizing toxins, and modulating inflammatory response (23) (24).

The most successful treatment seems to be a multi-strand formula, combining *L. acidophilus* CL1285, *L. casei* LBC80R, *L. rhamnosus* CLR2, Bio-K+, which also showed an excellent safety profile, even in preventing *C. difficile* hospital infections (25).

These recent results should suffice safety evidence that concerned previous researches who found great efficacy outcomes (26) (27) (28) (29).

3. Probiotics use in *Helicobacter pylori* infection (HPI)

Helicobacter pylori infections are relatively common, affect nearly half the world population, and its relevance relies not only on its relation with many gastrointestinal diseases, but also on extragastric manifestations (30). An alternative to antibiotic treatment is usually described as urgent, mainly due to bacterial resistance development (31). Thus, the role of probiotics seems to fit perfectly with *H. pylori* eradication treatment demands.

A recent Chinese study reviewed one hundred and forty results for probiotic eradication therapy in a massive population group (20,215 patients) in order to investigate different probiotics supplementation's effectiveness. All data considered, eradication rates were 84.1% in probiotic group while 70.5% in control, and adverse events rates were 14.4% in probiotic group while 30.1% in control (32).

In general, more than ten strategies of probiotic treatment have experimented and no statistically significant difference was found amidst the strands. Combined therapy did not show better results or tolerance either. Differences among strands seems to rely on treatment length, being *Lactobacillus acidophilus* a slightly better choice in in triple therapy of 7 and 14 days, while *Saccharomyces boulardii* was more applicable for 10-day triple therapy.

Some notable strands properties should also be noted for further investigation, such as *L. pentosus* LPS16, which lactic acid production has been shown to inhibit both drug-sensitive and drug-resistant *H. pylori* strains *in vitro* (33). The same effect could also be obtained from seven *L. bulgaricus* strains (34). The acid-resistant strain *L. johnsonii* No.1088, isolated from gastric juice of healthy volunteers could suppress *H. pylori* both in vitro and in a mouse model, and the heat-killed form of the strain also showed antibacterial effects (35) (36). Many hypotheses suggest mechanisms for the antagonism probiotics exert against *H. pylori*, although molecular studies are still pending for their confirmation (37).

From this review on, few interesting studies were conducted which led to new discoveries and hypothesis concerning probiotics treatment in HPI.

A research conducted on model animals showed a significant association of probiotic treatment with reduction on gastric inflammation secondary to HPI, and also suggested that long-term administration of probiotics might have favorable outcomes in *H. pylori* infection especially by decreasing the risk of development of diseases caused by increased levels of gastric inflammation, such as gastric ulcer (38). Other than that, the study also confirmed probiotics' colonization reduction capability previously observed.

Clinical researchers have yet failed to report on a probiotic treatment that could alone achieve *H. pylori* eradication. This is expected to be the great breakthrough of gastric probiotic therapy, and it is optimistically indicated to happen as further trials are conducted (39).

4. Probiotics use on oral infections

Probiotics are thoroughly studied in oral health problems mainly because periodontal diseases and dental caries are usually treated with systemic use of antimicrobial drugs[39], which can trigger gastrointestinal conditions, as previously exposed, as well as promote bacterial resistance and allergic reactions (40).

Oral microbiota is a delicate subject in the clinics, since the oral cavity is a complex microbiological system that needs homeostasis (41). In order for microorganisms and toxins to attack oral tissues, they are usually organized in a thin film layer deposited on hard oral tissue (enamel and cementum) called biofilm (42), which is described to be the result of bacterial adhesion, aggregation and co-aggregation to colonize the oral cavity (43).

Although action mechanism for probiotic therapy in oral health is still obscure, they are widely correlated with decreased CFU counts of pathogens (44) (45). A review suggests the very interesting hypothesis of the microbiological dynamic of probiotics in the oral cavity competing for adhesion with pathogens, causing the latter's displacement (46).

A meta-analysis of articles concerning caries development with probiotic therapy recorded a significant decrease in CFU counts of *S. mutans* after bacteriotherapy, which does not happen with the CFU counts of *Lactobacilli*. In addition, after treatment with probiotics, the intervention group had a greater number of patients with low levels of *S. mutans* CFU counts (< 105 CFU/ml) and fewer patients with high levels (> 106 CFU/ml), which does not occur in the control group (47).

On the other hand, a double-blind clinical trial on 96 children divided into three groups receiving probiotics (*B. lactis BB - 12*), xylitol or sorbitol for control, concluded that early

administration of this probiotic strain did not represent its permanent colonization in the oral cavity and that the CFU counts of *S. mutans* were not significantly affected (**48**). In addition, yet another three randomized clinical trials reviewed by Twetman, *et al.* (**49**) using *L. Rhamnosus* and milk as a vehicle for the prevention of dental caries concluded that despite encouraging results and given evidence collected, it is still premature to present probiotics as a preventive clinical recommendation, and indicated the need for long-term follow-up in order to establish needed confirmation for the therapy.

Another meta-analysis of 50 clinical trials suggested that probiotic therapy significantly reduces the *S. mutans* CFU counts (<104 UFC/ml), and that *Bifidobacteria* are the most significant contributor to this effect, but studies that brought up data to this conclusion has a high risk of bias, therefore, forcing researchers to conclude that current clinical evidence is inconsistent in order to make recommendations for the use of probiotics to treat or prevent dental caries (**50**).

Periodontal conditions and caries, being multifactorial diseases (**51),** seem to trouble trials with more variables to consider than it is possible to manage to organize data. That is one review's hypothesis to why despite the evidence, it is still impossible to make a statement towards probiotics recommendations in oral health (**52**).

Many other clinical trials (**53**) were conducted and are currently ingoing investigating other strands in oral health probiotics, such as *S. oralis*, *S. uberis*, *L. salivarius* and *S. rattus*, other pathogens' CFU counts are being considered as *Prevotella intemedia*, *Agregatibacter actinomycetemcomitans* and *Porphyromana gingivalis* and researchers struggle to extend follow-up time. Nevertheless, the main conclusion seems to remain that probiotics cannot replace daily oral hygiene technique (**54**).

5. Probiotics use in pharyngeal infections

Pharyngeal infections, notably the ones caused by *Streptococcus pyogenes*, also produces the previously described structure of biofilm. And as well as in the oral cavity, in the pharynx bacteria in biofilms are less sensitive to host defense mechanisms and antimicrobial agents, due to multiple strategies, that involve modulation of gene expression, controlled metabolic rate, intercellular communication, composition, and 3D architecture of the extracellular matrix (**55**).

In 2012, inspired by the probiotic potential to modulate cavity microbiota to protect it from infections, an *in vitro* research was conducted to experiment and investigate the functional and immunomodulatory properties of the strains *Lactobacillus helveticus* MIMLh5 and *Streptococcus salivarius* ST3 (**56**), which were highlighted previously by other studies (**57**) (**58**). This study concluded that strains MIMLh5 and ST3, alone and in combination, can efficiently adhere to pharyngeal epithelial cells, antagonize *S. pyogenes*, and modulate host innate immunity by inducing potentially protective effects. In addition, it also reported that their combination resulted in a synergistic effect, according to cytokine induction, that might help the host immune system react to potential pathogens while maintaining a balance between pro- and anti-inflammatory cytokines, thus preventing possible exaggerated responses.

Another *in vitro* article observed that S. salivarius 24SMB and S. oralis 89a are able to inhibit the biofilm formation capacity of selected pathogens and even to disperse their pre-formed biofilms. Diffusible molecules secreted by the two streptococci and lowered pH of the medium revealed to be implied in the mechanisms of anti-biofilm activity (**59**).

New strands other than *Streptococcus salivarius*, probiotic candidates *Lactobacillus acidophilus* and *Lactobacillus plantarum* were tested for the same protective properties in a *in vitro* pharynx cosmos, showing promising results together with many other bacteria native to the natural environment (**60**). The article purposes itself to be a preclinical towards future probiotic trials, and no clinical trials were published yet to this review's making date concerning probiotics use in pharyngeal infections that would suffice inclusion criteria, even though given laboratory evidence it poses as a great prospect for a safe upper respiratory infections treatment.

6. Probiotics use in *Salmonella spp.* Infections

Salmonella spp. Infections are one of the leading causes of acute diarrhea worldwide (**61**). As a long known disease, efforts to employ microorganisms therapy, which would today be called probiotic therapy, started even before the first definition of the word itself, in 1959, when Nissle published an article with records of an *E. coli* strand isolated from a soldier which appeared to be resistant to a diarrhea outbreak and seemed to establish persistent intestine colonization, therefore, suggesting its potential to compete with intestinal infections (**62**).

Nissle hypothesis' success mechanisms were explained in 2017, when a trial in rats infected with *S. enterica* serovar Typhimurium showed *E. coli* strain Nissle 1917 outcompeted the pathogen for iron acquisition (**63**) which is established as the most important micronutrient for its virulence (**64**).

Further on, many other probiotics were suggested to alleviate salmonellosis as time progressed. A study identified two non-*Saccharomyces* species - *K. marxianus* and *Metschnikowia gruessii* - as significantly capable of protecting host's intestinal epithelium against disrupting activity from the same *Salmonella* strain (**65**).

Animal trials in newly hatched broiler chicken with a multi-species probiotic consisting of *Lactobacillus crispatus*, *Lactobacillus salivarius*, *Lactobacillus gallinarum*, *Lactobacillus johnsonii*, *Enterococcus faecalis* and *Bacillus amyloliquefaciens* showed better and safer results than traditional antibiotic therapy with oxytetracycline, used as control. Results were significant to the point which probiotic therapy were suggested as preferred choice of treatment (66). Other studies with other strands were also published studying probiotics in poultry and rats, many of which were absolutely successful, through different biochemical mechanisms of pathogen inhibition (**67**) (**68**) (**69**) (**70**).

In fact, microbiota seems to play an extraordinarily important role in *Salmonella* infections, being shown that slight variation in endogenous *Enterobacteriaceae* could importantly determine host's susceptibility to infection, even in genetically similar organisms (**71**). Confirming probiotics effectiveness, several clinical trials in humans replicated *in vitro* and *in vivo* animal trials (**72**). A randomized controlled trial showed *Lactobacillus plantarum* 299 could accelerate

clearance of non-typhoid *Salmonella* and reduce infection-related symptoms, which was influenced by gender (**73**).

Recently, that first *E. coli* strand isolated by Nissle (**62**) was subject to bioengineering and successfully inhibited *Salmonella* colonization via tetrathionate-induced production of microcin H47. The strand seems to greatly outcompete *Salmonella*, using an environmental signal indicative of intestinal inflammation as an inducing molecule, resulting in a considerable increase in fitness advantage (**74**).

CONCLUSION

The quality of evidence for the use of probiotics to treat or prevent gastrointestinal tract infections seems to be uneven. While its usage on AAD, CDI and *Salmonella* infections have shown reasonably positive clinical results and are already incorporated to protocol therapies, it remains unclear which exact mechanisms, microbiological interactions and method approaches could account for many discrepant results in some trials. The main challenge seems to present in oral infections applications, due to the microbiota dynamics complexity.

In assessing safety, probiotics seem to exceed traditional antibiotic treatment, given almost no record of adverse events from all evaluated studies in comparison to the known problematic and often iatrogenic drug therapies. Overall, probiotics seem to have increasingly good prospects in clinical use, even though further research is needed in order to produce evidence for strain selection and effectiveness in specific diseases.

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REFERENCES

1. Lilly DM, Stillwell RH. Probiotics: growth-promoting factors produced by microorganisms. Science. 1965 Feb 12;147(3659):747-8.

2. Hill C., Guarner F., Reid G., Gibson G. R., Merenstein D. J., Pot B., et al. . (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 11, 506–514. 10.1038/nrgastro.2014.66

3. Wilkins T, Sequoia J. Probiotics for Gastrointestinal Conditions: A Summary of the Evidence. Am Fam Physician. 2017 Aug 1;96(3):170-178. PMID: 28762696.

4. Doron S, Gorbach SL. Probiotics: their role in the treatment and prevention of disease. Expert Rev Anti Infect Ther. 2006 Apr;4(2):261-75. doi: 10.1586/14787210.4.2.261. PMID: 16597207.

5. Pattani R, Palda VA, Hwang SW, Shah PS. Probiotics for the prevention of antibiotic-associated diarrhea and Clostridium difficile infection among hospitalized patients: systematic review and meta-analysis. Open Med. 2013 May 28;7(2):e56-67. PMID: 24348885; PMCID: PMC3863752

6. Stadlbauer V. Immunosuppression and probiotics: are they effective and safe? Benef Microbes. 2015;6(6):823-8. doi: 10.3920/BM2015.0065. Epub 2015 Aug 19. PMID: 26287986..

7. Sanders ME, Akkermans LM, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, Huys G, Levy DD, Lutgendorff F, Mack D, Phothirath P, Solano-Aguilar G, Vaughan E. Safety assessment of probiotics for human use. Gut Microbes. 2010 May-Jun;1(3):164-85. doi: 10.4161/gmic.1.3.12127. Epub 2010 Mar 4. PMID: 21327023; PMCID: PMC3023597.

8. Happel AU, Barnabas SL, Froissart R, Passmore JS. Weighing in on the risks and benefits of probiotic use in HIV-infected and immunocompromised populations. Benef Microbes. 2018 Feb 27;9(2):239-246. doi: 10.3920/BM2017.0106. Epub 2018 Jan 18. PMID: 29345159.

9. Jin L, Deng L, Wu W, Wang Z, Shao W, Liu J. Systematic review and meta-analysis of the effect of probiotic supplementation on functional constipation in children. Medicine (Baltimore). 2018 Sep;97(39):e12174. doi: 10.1097/MD.0000000000012174. PMID: 30278490; PMCID: PMC6181519.

10. Barbut F, Meynard JL. Managing antibiotic associated diarrhoea. BMJ. 2002;324(7350):1345-134612052785

11. Mekonnen SA, Merenstein D, Fraser CM, Marco ML. Molecular mechanisms of probiotic prevention of antibiotic-associated diarrhea. Curr Opin Biotechnol. 2020 Feb;61:226-234. doi: 10.1016/j.copbio.2020.01.005. Epub 2020 Feb 19. PMID: 32087535; PMCID: PMC7096272.

12. McFarland LV, Ozen M, Dinleyici EC, Goh S: Comparison of pediatric and adult antibiotic-associated diarrhea and Clostridium difficile infections. World J Gastroenterol 2016, 22: 3078–3104.

13. Gould K, Short G. Probiotics and antibiotic-associated diarrhoea--a logical flaw? J Antimicrob Chemother. 2008 Mar;61(3):761. doi: 10.1093/jac/dkm534. Epub 2008 Jan 28. PMID: 18227093.

14. Guo Q, Goldenberg JZ, Humphrey C, El Dib R, Johnston BC. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database Syst Rev. 2019 Apr 30;4(4):CD004827. doi: 10.1002/14651858.CD004827.pub5. PMID: 31039287; PMCID: PMC6490796.

15. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R, Johnsen B, Shekelle PG. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA. 2012 May 9;307(18):1959-69. doi: 10.1001/jama.2012.3507. PMID: 22570464.

16. Song HJ, Shim KN, Jung SA, Choi HJ, Lee MA, Ryu KH, Kim SE, Yoo K. Antibiotic-associated diarrhea: candidate organisms other than Clostridium difficile. Korean J Intern Med. 2008 Mar;23(1):9-15. doi: 10.3904/kjim.2008.23.1.9. PMID: 18363274; PMCID: PMC2686956

17. Cai J, Zhao C, Du Y, Zhang Y, Zhao M, Zhao Q. Comparative efficacy and tolerability of probiotics for antibiotic-associated diarrhea: Systematic review with network meta-analysis. United European Gastroenterol J. 2018 Mar;6(2):169-180. doi: 10.1177/2050640617736987. Epub 2017 Oct 4. PMID: 29511547; PMCID: PMC5833232

18. Szajewska H, Canani RB, Guarino A, Hojsak I, Indrio F, Kolacek S, Orel R, Shamir R, Vandenplas Y, van Goudoever JB, Weizman Z; ESPGHAN Working Group for ProbioticsPrebiotics. Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children. J Pediatr Gastroenterol Nutr. 2016 Mar;62(3):495-506. doi: 10.1097/MPG.0000000000001081. PMID: 26756877.

19. Fox MJ, Ahuja KD, Robertson IK, Ball MJ, Eri RD. Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blind, randomised, placebo-controlled study. BMJ Open. 2015 Jan 14;5(1):e006474. doi: 10.1136/bmjopen-2014-006474. PMID: 25588782; PMCID: PMC4298112.

20. Mullish BH, Williams HR. Clostridium difficile infection and antibiotic-associated diarrhoea. Clin Med (Lond). 2018 Jun;18(3):237-241. doi: 10.7861/clinmedicine.18-3-237. PMID: 29858434; PMCID: PMC6334067.

21. Lamb CA, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, Hayee B, Lomer MCE, Parkes GC, Selinger C, Barrett KJ, Davies RJ, Bennett C, Gittens S, Dunlop MG, Faiz O, Fraser A, Garrick V, Johnston PD, Parkes M, Sanderson J, Terry H; IBD guidelines eDelphi consensus group, Gaya DR, Iqbal TH, Taylor SA, Smith M, Brookes M, Hansen R, Hawthorne AB. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. Gut. 2019 Dec;68(Suppl 3):s1-s106. doi: 10.1136/gutjnl-2019-318484. Epub 2019 Sep 27. Erratum in: Gut. 2021 Apr;70(4):1. PMID: 31562236; PMCID: PMC6872448.

22. Goldenberg JZ, Yap C, Lytvyn L, Lo CK, Beardsley J, Mertz D, Johnston BC. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev. 2017 Dec 19;12(12):CD006095. doi: 10.1002/14651858.CD006095.pub4. PMID: 29257353; PMCID: PMC6486212.

23. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotic mechanisms of action. Ann Nutr Metab. 2012;61(2):160-74. doi: 10.1159/000342079. Epub 2012 Oct 2. PMID: 23037511.

24. McFarland LV. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. BMJ Open. 2014 Aug 25;4(8):e005047. doi: 10.1136/bmjopen-2014-005047. PMID: 25157183; PMCID: PMC4156804.

25. McFarland LV, Ship N, Auclair J, Millette M. Primary prevention of Clostridium difficile infections with a specific probiotic combining Lactobacillus acidophilus, L. casei, and L. rhamnosus strains: assessing the evidence. J Hosp Infect. 2018 Aug;99(4):443-452. doi: 10.1016/j.jhin.2018.04.017. Epub 2018 Apr 24. PMID: 29702133.

26. Barker AK, Duster M, Valentine S, Hess T, Archbald-Pannone L, Guerrant R, Safdar N. A randomized controlled trial of probiotics for Clostridium difficile infection in adults (PICO). J Antimicrob Chemother. 2017 Nov 1;72(11):3177-3180. doi: 10.1093/jac/dkx254. PMID: 28961980; PMCID: PMC5890711.

27. De Wolfe TJ, Eggers S, Barker AK, Kates AE, Dill-McFarland KA, Suen G, Safdar N. Oral probiotic combination of Lactobacillus and Bifidobacterium alters the gastrointestinal microbiota during antibiotic treatment for Clostridium difficile infection. PLoS One. 2018 Sep 28;13(9):e0204253. doi: 10.1371/journal.pone.0204253. PMID: 30265691; PMCID: PMC6161886.

28. Mills JP, Rao K, Young VB. Probiotics for prevention of Clostridium difficile infection. Curr Opin Gastroenterol. 2018 Jan;34(1):3-10. doi: 10.1097/MOG.0000000000000410. PMID: 29189354; PMCID: PMC6335148.

29. Evans CT, Johnson S. Prevention of Clostridium difficile Infection With Probiotics. Clin Infect Dis. 2015 May 15;60 Suppl 2:S122-8. doi: 10.1093/cid/civ138. PMID: 25922397.

30. Gravina AG, Zagari RM, De Musis C, Romano L, Loguercio C, Romano M. Helicobacter pylori and extragastric diseases: A review. World J Gastroenterol. 2018 Aug 7;24(29):3204-3221. doi: 10.3748/wjg.v24.i29.3204. PMID: 30090002; PMCID: PMC6079286.

31. Suzuki S, Esaki M, Kusano C, Ikehara H, Gotoda T. Development of Helicobacter pylori treatment: How do we manage antimicrobial resistance? World J Gastroenterol. 2019 Apr 28;25(16):1907-1912. doi: 10.3748/wjg.v25.i16.1907. PMID: 31086459; PMCID: PMC6487377.

32. Wang F, Feng J, Chen P, Liu X, Ma M, Zhou R, Chang Y, Liu J, Li J, Zhao Q. Probiotics in Helicobacter pylori eradication therapy: Systematic review and network meta-analysis. Clin Res Hepatol Gastroenterol. 2017 Sep;41(4):466-475. doi: 10.1016/j.clinre.2017.04.004. Epub 2017 May 25. PMID: 28552432.

33. Zheng P.X., Fang H.Y., Yang H.B., Tien N.Y., Wang M.C., Wu J.J. Lactobacillus pentosus strain LPS16 produces lactic acid, inhibiting multidrug-resistant Helicobacter pylori. J. Microbiol. Immunol. Infect. 2016;49:168–174. doi: 10.1016/j.jmii.2014.04.014.

34. Boyanova L., Gergova G., Markovska R., Yordanov D., Mitov I. Bacteriocin-like inhibitory activities of seven Lactobacillus delbrueckii subsp. bulgaricus strains against antibiotic susceptible and resistant Helicobacter pylori strains. Lett. Appl. Microbiol. 2017;65:469–474. doi: 10.1111/lam.12807.

35. Aiba Y., Nakano Y., Koga Y., Takahashi K., Komatsu Y. A highly acid-resistant novel strain of Lactobacillus johnsonii No. 1088 has antibacterial activity, including that against Helicobacter pylori, and inhibits gastrin-mediated acid production in mice. Microbiologyopen. 2015;4:465–474. doi: 10.1002/mbo3.252.

36. Aiba Y., Ishikawa H., Tokunaga M., Komatsu Y. Anti-Helicobacter pylori activity of non-living, heat-killed form of lactobacilli including Lactobacillus johnsonii No.1088. FEMS Microbiol. Lett. 2017;364 doi: 10.1093/femsle/fnx102.

37. Ji J, Yang H. Using Probiotics as Supplementation for Helicobacter pylori Antibiotic Therapy. Int J Mol Sci. 2020 Feb 8;21(3):1136. doi: 10.3390/ijms21031136. PMID: 32046317; PMCID: PMC7037652.

38. Chakravarty K, Gaur S. Role of Probiotics in Prophylaxis of Helicobacter pylori Infection. Curr Pharm Biotechnol. 2019;20(2):137-145. doi: 10.2174/1389201020666190227203107. PMID: 30827235.

39. Becker DE. Drug allergies and implications for dental practice. AnesthProg. 2013;60:188–97.

40. Becker DE. Antimicrobial drugs. AnesthProg. 2013;60:111–22

41. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Diseases. 2012;18:109–20.

42. Zijnge V, van Leeuwen MB, Degener JE, Abbas F, Thurnheer T, Gmür R. Oral biofilm architecture on natural teeth. PLoS One. 2010;5:e9321

43. Piwat S, Sophatha B, Teanpaisan R. An assessment of adhesion, aggregation and surface charges of Lactobacillus strains derived from the human oral cavity. LettApplMicrobiol. 2015;61:98–105.

44. Laleman I, Yilmaz E, Ozcelik O, Haytac C, Pauwels M, Herrero ER. The effect of a streptococci containing probiotic in periodontal therapy: a randomized controlled trial. J Clin Periodontol. 2015;42:1032–41.

45. Morales A, Carvajal P, Silva N, Hernandez M, Godoy C, Rodriguez G. Clinical Effects of Lactobacillus rhamnosus in Non-Surgical Treatment of Chronic Periodontitis: A Randomized Placebo-Controlled Trial With 1-Year Follow-Up. J Periodontol. 2016 Aug;87(8):944–52.

46. Faran Ali SM, Tanwir F. Oral microbial habitat a dynamic entity. J Oral Biol Craniofac Res. 2012;2:181–7.

47. Laleman I, Detailleur V, Slot DE, Slomka V, Quirynen M, Teughels W. Probiotics reduce mutans streptococci counts in humans: a systematic review and meta-analysis. Clin Oral Investig. 2014;18:1539–52.

48. Taipale T, Pienihäkkinen K, Salminen S, Jokela J, Söderling E. Bifidobacteriumanimalis subsp. lactis BB-12 administration in early childhood: a randomized clinical trial of effects on oral colonization by mutans streptococci and the probiotic. Caries Res. 2012;46:69–77.

49. Twetman S, Keller MK. Probiotics for caries prevention and control. Adv Dent Res. 2012;24:98–102.

50. Gruner D, Paris S, Schwendicke F. Probiotics for managing caries and periodontitis: Systematic Review and Meta-Analysis. J Dent. 2016;48:16–25.

51. Iheozor-Ejiofor Z, Worthington HV, Walsh T, O'Malley L, Clarkson JE, Macey R. Water fluoridation for the prevention of dental caries. Cochrane Database Syst Rev. 2015;6:CD010856.

52. Seminario-Amez M, López-López J, Estrugo-Devesa A, Ayuso-Montero R, Jané-Salas E. Probiotics and oral health: A systematic review. Med Oral Patol Oral Cir Bucal. 2017 May 1;22(3):e282-e288. doi: 10.4317/medoral.21494. PMID: 28390121; PMCID: PMC5432076.

53. Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y, Yamaki K. Improvement of periodontal condition by probiotics with Lactobacillus salivariusWB21: a randomized, double-blind, placebo-controlled study. J Clin Periodontol. 2008;35:897–905

54. Morales A, Carvajal P, Silva N, Hernandez M, Godoy C, Rodriguez G. Clinical Effects of Lactobacillus rhamnosus in Non-Surgical Treatment of Chronic Periodontitis: A Randomized Placebo-Controlled Trial With 1-Year Follow-Up. J Periodontol. 2016 Aug;87(8):944–52.

55. Alves-Barroco C, Paquete-Ferreira J, Santos-Silva T, Fernandes AR. Singularities of Pyogenic Streptococcal Biofilms - From Formation to Health Implication. Front Microbiol. 2020 Dec 23;11:584947. doi: 10.3389/fmicb.2020.584947. PMID: 33424785; PMCID: PMC7785724.

56. Taverniti V, Minuzzo M, Arioli S, Junttila I, Hämäläinen S, Turpeinen H, Mora D, Karp M, Pesu M, Guglielmetti S. In vitro functional and immunomodulatory properties of the Lactobacillus helveticus MIMLh5-Streptococcus salivarius ST3 association that are relevant to the development of a pharyngeal probiotic product. Appl Environ Microbiol. 2012 Jun;78(12):4209-16. doi: 10.1128/AEM.00325-12. Epub 2012 Apr 13. PMID: 22504812; PMCID: PMC3370528.

57. Wescombe PA, et al. 2011. Salivaricin 9, a new lantibiotic produced by Streptococcus salivarius. Microbiology 157:1290–1299

58. Guglielmetti S, et al. 2010. Oral bacteria as potential probiotics for the pharyngeal mucosa. Appl. Environ. Microbiol. 76:3948–3958

59. Bidossi A, De Grandi R, Toscano M, Bottagisio M, De Vecchi E, Gelardi M, Drago L. Probiotics Streptococcus salivarius 24SMB and Streptococcus oralis 89a interfere with biofilm formation of pathogens of the upper respiratory tract. BMC Infect Dis. 2018 Dec 13;18(1):653. doi: 10.1186/s12879-018-3576-9. PMID: 30545317; PMCID: PMC6292094.

60. Humphreys GJ, McBain AJ. Antagonistic effects of Streptococcus and Lactobacillus probiotics in pharyngeal biofilms. Lett Appl Microbiol. 2019 Apr;68(4):303-312. doi: 10.1111/lam.13133. Epub 2019 Mar 13. PMID: 30776138.

61. Popa GL, Papa MI. Salmonella spp. infection - a continuous threat worldwide. Germs. 2021 Mar 15;11(1):88-96. doi: 10.18683/germs.2021.1244. PMID: 33898345; PMCID: PMC8057844.

62. Nissle A. Explanations of the significance of colonic dysbacteria & the mechanism of action of E. coli therapy (mutaflor) Medizinische. 1959;4:1017–1022.

63. Deriu E, Liu JZ, Pezeshki M, Edwards RA, Ochoa RJ, Contreras H, Libby SJ, Fang FC, Raffatellu M. Probiotic bacteria reduce salmonella typhimurium intestinal colonization by competing for iron. Cell Host Microbe. 2013 Jul 17;14(1):26-37. doi: 10.1016/j.chom.2013.06.007. PMID: 23870311; PMCID: PMC3752295.

64. Crouch ML, Castor M, Karlinsey JE, Kalhorn T, Fang FC. Biosynthesis and IroC-dependent export of the siderophore salmochelin are essential for virulence of Salmonella enterica serovar Typhimurium. Molecular microbiology. 2008;67:971–983.

65. Smith IM, Baker A, Arneborg N, Jespersen L. Non-Saccharomyces yeasts protect against epithelial cell barrier disruption induced by Salmonella enterica subsp. enterica serovar Typhimurium. Lett Appl Microbiol. 2015 Nov;61(5):491-7. doi: 10.1111/lam.12481. Epub 2015 Sep 22. PMID: 26280244.

66. Neveling DP, van Emmenes L, Ahire JJ, Pieterse E, Smith C, Dicks LMT. Effect of a Multi-Species Probiotic on the Colonisation of Salmonella in Broilers. Probiotics Antimicrob Proteins. 2020 Sep;12(3):896-905. doi: 10.1007/s12602-019-09593-y. PMID: 31784950.

67. Carter A, Adams M, La Ragione RM, Woodward MJ. Colonisation of poultry by Salmonella Enteritidis S1400 is reduced by combined administration of Lactobacillus salivarius 59 and Enterococcus faecium PXN-33. Vet Microbiol. 2017 Feb;199:100-107. doi: 10.1016/j.vetmic.2016.12.029. Epub 2016 Dec 26. PMID: 28110775.

68. Levkut M, Pistl J, Lauková' A, Revajová V, Herich R, Sevcíková Z, Strompfová V, Szabóová R, Kokincáková T. Antimicrobial activity of Enterococcus faecium ef 55 against Salmonella enteritidis in chicks. Acta Vet Hung. 2009 Mar;57(1):13-24. doi: 10.1556/AVet.57.2009.1.2. PMID: 19457770.

69. de Oliveira JE, van der Hoeven-Hangoor E, van de Linde IB, Montijn RC, van der Vossen JM. In ovo inoculation of chicken embryos with probiotic bacteria and its effect on posthatch Salmonella susceptibility. Poult Sci. 2014 Apr;93(4):818-29. doi: 10.3382/ps.2013-03409. PMID: 24706958.

70. Mazkour S, Shekarforoush SS, Basiri S, Nazifi S, Yektaseresht A, Honarmand M. Effects of two probiotic spores of Bacillus species on hematological, biochemical, and inflammatory parameters in Salmonella Typhimurium infected rats. Sci Rep. 2020 May 15;10(1):8035. doi: 10.1038/s41598-020-64559-3. PMID: 32415253; PMCID: PMC7229222.

71. Velazquez EM, Nguyen H, Heasley KT, Saechao CH, Gil LM, Rogers AWL, Miller BM, Rolston MR, Lopez CA, Litvak Y, Liou MJ, Faber F, Bronner DN, Tiffany CR, Byndloss MX, Byndloss AJ, Bäumler AJ. Endogenous Enterobacteriaceae underlie variation in susceptibility to Salmonella infection. Nat Microbiol. 2019 Jun;4(6):1057-1064. doi: 10.1038/s41564-019-0407-8. Epub 2019 Mar 25. PMID: 30911125; PMCID: PMC6533147.

72. Gut AM, Vasiljevic T, Yeager T, Donkor ON. Salmonella infection - prevention and treatment by antibiotics and probiotic yeasts: a review. Microbiology (Reading). 2018 Nov;164(11):1327-1344. doi: 10.1099/mic.0.000709. Epub 2018 Aug 23. PMID: 30136920.

73. Lönnermark E, Lappas G, Friman V, Wold AE, Backhaus E, Adlerberth I. Effects of probiotic intake and gender on nontyphoid Salmonella infection. J Clin Gastroenterol. 2015 Feb;49(2):116-23. doi: 10.1097/MCG.0000000000000120. PMID: 24667592.

74. Palmer JD, Piattelli E, McCormick BA, Silby MW, Brigham CJ, Bucci V. Engineered Probiotic for the Inhibition of Salmonella via Tetrathionate-Induced Production of Microcin H47. ACS Infect Dis. 2018 Jan 12;4(1):39-45. doi: 10.1021/acsinfecdis.7b00114. Epub 2017 Sep 20. PMID: 28918634; PMCID: PMC5766358.

MSD

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Awake Prone Positioning in SARS-CoV-2 (Covid-19) Non-Intubated Patients with Acute Respiratory Failure in Adult Population: A Literature Review

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ABSTRACT

Objective: This article aims to review the current evidence in Covid-19 patients with acute respiratory failure who required awake prone positioning as a therapeutic intervention.

I searched the literature on MEDLINE, PubMed, Cochrane Library, Google Scholar, and other databases. I found several studies that analyzed and shared the data regarding awake prone positioning in the Covid-19 patients, with duration ranging from 12-16 hours.

I found that the major criteria used included PaO2/FiO2 ratio, SpO2/FiO2, respiratory rate, heart rate. I also searched the suggested indications, contra-indications, complications, and outcomes of those patients.

Awake proning showed improvement in lung mechanics and oxygenation, but no benefit in outcome in the majority of studies.

Currently, the data is unclear to determine the overall benefit. Further controlled trials are needed.

Keywords: Covid-19, prone positioning, awake, major criteria, outcome, SARS-CoV-2

INTRODUCTION

Traditionally, the prone position has been proposed as a treatment of severe ARDS patients with hypoxemia. Its use started in the 1970s after a study done by Bryan in 1974 in the pediatric population showed benefits (1).

Several meta-analyses and systemic reviews have been published in Non-Covid patients, but all were done in intubated patients.

In 2011, a meta-analysis of 48 studies was done, which found that a statistically significant mortality benefit was found in patients with severe ARDS only (2).

In 2014, a systematic review of 11 trials determined that ventilated patients with lower tidal volumes, in combination with prone positioning, reduced mortality of about one additional patient in eleven (3).

The Large Observational Study to Understand the Global Impact of Severe Acute Respiratory Failure (LUNG-SAFE) looked at the use of prone position during the study period of 2014. At that time, proning was used for 7% of all ARDS patients and 14% of the most severe cases (4).

Guidelines have been published by The ESICM and Surviving Sepsis Campaign on the Management of Critically Ill Adults with Coronavirus Disease 2019 (COVID-19) in 2020 (5). These recommended the use of proning (6).

Covid-19 pandemic has further increased the interest of the medical community to further study the effect of proning on the outcome of those patients as they develop severe pneumonia with ARDS. Hospitals have been overwhelmed with patients requiring invasive ventilation who failed non-invasive methods. This lead to a growing interest in awake proning protocols to avoid intubation and improve outcomes.

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Effects of body position on lung mechanics

The beneficial effects of proning in ARDS have several mechanisms (**7, 8**).

The most severely affected lung regions in ARDS are at the lower and the basal zones. They have the worse V/Q (Ventilation/Perfusion) mismatch or even shunt physiology in the most severe cases. In supine position, most of the oxygenated blood goes to these areas due to the gravity effect. However, ventilation is less affected by the gravitational force. This leads to severe oxygenation failure due to a reduction in the gas exchange, requiring a higher FiO2 and Peep (**9, 10**). Proning these patients has positive physiological effects on respiratory system mechanics (**11**).

In the supine position, the intra-abdominal compartment pressure exceeds five times the intra-thoracic cavity pressure, leading to decrease compliance of respiratory system (CRS) (**12**). It can further decrease with associated intra-abdominal sepsis. In the prone position, the weight of the body on the lungs and diaphragm is reduced, which improves the respiratory system compliance. There is a reversal of posterior alveolar atelectasis, over inflation of anterior alveoli, and V/Q mismatch when the patient is changed from supine to prone position

The risk of barotrauma is also reduced.

When patients are in the prone position, the drainage of secretions may be improved with decreased chances of ventilator-associated pneumonia.

Covid-19 and Proning

As the pandemic is worsening, severe ARDS in Covid-19 patients is similar to the "Classical" ARDS described in other conditions. It is the single most crucial pathology which determines the outcome.

Historically, proning has been done in the intubated patients. This practice is labour-intensive and requires trained nurses and other staff. Recent studies on Covid-19 proning have focused more on awake proning. The first study about proning benefits in awake non-intubated Covid-19 patients was done in China by Sun Q, Qiu H, Huang M, et al. (**13**). Several protocols have been developed since for awake proning Covid-19 patients. Awake proning is a relatively safe intervention, which has shown improvement in oxygenation in conscious patients who failed to improve with non-invasive ventilation (HFNC, BiPAP/CPAP). It can be easily used in patients admitted to the wards and even at home by trained family members.

The suggested indications of awake proning include hemodynamically stable patients, oxygen saturation less than 92, when the hypoxemia becomes refractory to simple oxygen therapy, SpO2/FiO2 ratio less than 315, PaO2/FiO2 ratio less than 300, a respiratory rate more than 30, heart rate more than 120, conscious and able to move in bed with minimal assistance, pregnancy 1st and 2nd trimesters while fetal status is being monitored (**14**, **15**).

There are several suggested contra-indications which include hemodynamic instability with MAP less than 65, Difficult airway, severe respiratory distress especially if hypercapnia with the possibility of immediate intubation unless mechanical ventilation is not available, low GCS, 3rd trimester of pregnancy, spinal diseases, raised intracranial pressure, acute abdomen, difficult airway. Proning should be avoided in patients with multi-organ failure (**15**).

Most studies did not report any major complications of awake proning. However, some reported complications include dislodgement of lines, devices, and drains. Facial edema, increase in intra-abdominal and intracranial pressures, related to nutritional difficulties, nausea, vomiting, anxiety, and intolerance. A concern has been raised that awake proning may delay intubation and mechanical ventilation in ARDS patients. This could lead to poorer outcomes (**15**).

If a patient develops cardiac arrest during awake proning, "Reverse CPR" should be performed till the patient can be safely changed back to supine position (**16**).

Several studies have been done to assess the impact of awake or self proning.

Around seven recent studies have been done which studied awake prone positioning in Covid-19 non- intubated patients. A study was done by Caputo. et al, showed a reduction in intubation by 64%. However, 36% of patients were intubated within 72 hours, and of these, 38% (n = 7) were intubated within the first hour. However, other studies have not shown any significant reduction in intubation rates. Most of those studies were limited by the fact that the duration of awake proning sessions was much shorter than compared to intubated patients with ARDS and no major trial has been done. Further studies and trials are required to determine a beneficial impact.

More than the duration of a single prone positioning course, multiple short prone positioning sessions might improve tolerance and lead to better outcomes. Use pillows to improve comfort, and trained staff should help to reposition if the patient has physical difficulty moving. Published data does not allow us to determine for which patients prone positioning may be beneficial, or the best duration and frequency of the prone positioning sessions (**17**).

Before starting the awake prone positioning, it is important to explain the procedure to the patient, and he should be reassured. This would improve tolerance. Secure all lines, devices, tubes, and drains. In patients with mild hypoxemia, it may be better to do "Assisted Proning" rather than "Self Proning", so the patient can conserve his energy and use it for breathing efforts.

The patient should be kept nil by mouth in case urgent intubation is needed and also to minimize the risk of aspiration in the prone position.

Monitoring of these patients is the most important aspect. Various recommendations have been suggested. One of them is the ROX index (Respiratory rate – OXygenation). It is the ratio of SpO2/FiO2 to RR. An improvement in the index is suggestive of fewer chances of intubation. (**18**) More experience is needed to assess this index in Covid-19 patients.

In contrast to awake proning, intubated patients have shown improved oxygenation and a reduction in mortality with moderate to severe ARDS.

Early vs. Late Prone Positioning

Early proning is done during the period when oxygen is supplied by simple methods like nasal cannula or face mask, while late proning is the period when a patient is using HFNC, CPAP or BIPAP.

The studies have shown variable results, but overall fail to demonstrate any benefit regarding “Early vs Late Proning”. (**19**)

CONCLUSION

Awake-prone positioning has shown improvement in oxygenation, but any benefit after re-supination and impact on mortality and length of stay remains unclear.

Due to its relative ease of use, and low sides-effects, prone positioning for non-intubated patients has been widely applied and studied in Covid-19 patients, whether in medical wards or emergency rooms. Association with NIV or HFNC is suggested to improve the benefits of respiratory status.

It is encouraging to see the evidence showing improved oxygenation but more evidence is needed on clinical outcomes, such as mortality or intubation rates.

Studies failed to determine the best duration and frequency of prone positioning, and tolerance of prolonged sessions remains a concern.

Abbreviations:

PaO_2/FiO_2: Arterial oxygen partial pressure/fractional inspired oxygen

SpO_2: Oxygen Saturation by pulse oximeter

ARDS: Adult Respiratory Distress Syndrome

ESICM: European Society of Intensive Care Medicine

GCS: Glasgow Coma Scale

HFNC: High Flow Nasal Cannula

BIPAP: Bi-level Positive Airway pressure

CPAP: Continuous Positive Airway Pressure

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REFERENCES

1. Bryan AC. Conference on the scientific basis of respiratory therapy. Pulmonary physiotherapy in the pediatric age group. Comments of a devil's advocate. Am Rev Respir Dis. 1974 Dec;110(6 Pt 2):143-4. doi: 10.1164/arrd.1974.110.6P2.143. PMID: 4440945.

2. Abroug F, Ouanes-Besbes L, Dachraoui F, Ouanes I, Brochard L. An updated study-level meta-analysis of randomised controlled trials on proning in ARDS and acute lung injury. Critical Care. 2011 Feb;15(1):1-9.

3. Sud S, Friedrich JO, Adhikari NK, Taccone P, Mancebo J, Polli F, Latini R, Pesenti A, Curley MA, Fernandez R, Chan MC. Effect of prone positioning during mechanical ventilation on mortality among patients with acute respiratory distress syndrome: a systematic review and meta-analysis. Cmaj. 2014 Jul 8;186(10):E381-90.

4. Jump up to:a b c d e Guérin, Claude (2017), "Prone position", in Davide Chiumello (ed.), Acute Respiratory Distress Syndrome, Springer, pp. 73–84, ISBN 9783319418520

5. Davenport L. Top 10 must-dos in ICU in COVID-19 include prone ventilation. Medscape Medical News. 2020(March 21,).

6. Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, Oczkowski S, Levy MM, Derde L, Dzierba A, Du B. Surviving Sepsis Campaign: guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). Intensive care medicine. 2020 May;46(5):854-87.

7. Coppo A, Bellani G, Winterton D, Di Pierro M, Soria A, Faverio P, Cairo M, Mori S, Messinesi G, Contro E, Bonfanti P. Feasibility and physiological effects of prone positioning in non-intubated patients with acute respiratory failure due to COVID-19 (PRON-COVID): a prospective cohort study. The Lancet Respiratory Medicine. 2020 Aug 1;8(8):765-74.

8. Koulouras V, Papathanakos G, Papathanasiou A, Nakos G. Efficacy of prone position in acute respiratory distress syndrome patients: a pathophysiology-based review. World journal of critical care medicine. 2016 May 4;5(2):121.

9. Nyrén S, Radell P, Lindahl SG, Mure M, Petersson J, Larsson SA, Jacobsson H, Sánchez-Crespo A. Lung ventilation and perfusion in prone and supine postures with reference to anesthetized and mechanically ventilated healthy volunteers. The Journal of the American Society of Anesthesiologists. 2010 Mar 1;112(3):682-7.

10. Glenny RW, Robertson HT. Determinants of pulmonary blood flow distribution. Compr Physiol. 2011 Jan;1(1):39-59.

11. Kallet RH, Katz JA. Respiratory system mechanics in acute respiratory distress syndrome. Respir Care Clin N Am. 2003 Sep;9(3):297-319.

12. Froese AB. Gravity, the belly, and the diaphragm: you can't ignore physics. Anesthesiology. 2006 Jan;104(1):193-6.

13. Sun Q, Qiu H, Huang M, Yang Y. Lower mortality of COVID-19 by early recognition and intervention: experience from Jiangsu Province. Annals of intensive care. 2020 Dec;10(1):1-4.

14. Bower G, He H. Protocol for awake prone positioning in COVID-19 patients: to do it earlier, easier, and longer. Critical Care. 2020 Dec;24(1):1-3.

15. Stilma W, Åkerman E, Artigas A, Bentley A, Bos LD, Bosman TJ, De Bruin H, Brummaier T, Buiteman-Kruizinga LA, Carcò F, Chesney G. Awake proning as an adjunctive therapy for refractory hypoxemia in non-intubated patients with COVID-19 acute respiratory failure: guidance from an international group of healthcare workers. The American Journal of Tropical Medicine and Hygiene. 2021 May;104(5):1676.

16. Costa LM, Nunes RA, Scudeler TL. Cardiopulmonary Resuscitation in Prone Position. International Journal of Cardiovascular Sciences. 2021 Feb 22;34:315-8.

17. Telias I, Katira BH, Brochard L. Is the prone position helpful during spontaneous breathing in patients with COVID-19?. Jama. 2020 Jun 9;323(22):2265-7.

19. Dubosh NM, Wong ML, Grossestreuer AV, et al. Early, awake proning in emergency department patients with COVID-19. Am J Emerg Med. 2021;46:640-645. doi:10.1016/j.ajem.2020.11.074

18. Suliman LA, Abdelgawad TT, Farrag NS, Abdelwahab HW. Validity of ROX index in prediction of risk of intubation in patients with COVID-19 pneumonia. Advances in Respiratory Medicine. 2021;89(1):1-7.

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The Coronavirus Disease 2019 pandemic as a threat to reproductive health and fetal life

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ABSTRACT

Objective: Covid-19 disease has spread rapidly across the world since its first appearance in 2019. At the beginning of the pandemic, Covid-19 disease was thought to affect only the respiratory system, although it has since been realized that it causes numerous transient or permanent problems in various body systems. One of these effects involves the reproductive system.

Several studies have investigated the effects of Covid-19 disease on the female and male reproductive systems. Embryological life depends on the fertilization of a healthy mature oocyte, a healthy mature sperm, and the continuation of pregnancy. The purpose of this article is to examine the effects of Covid-19 disease on the male and female reproductive systems and embryological life through a review of the current literature.

Keywords: Covid-19, Embryo, Reproductive Health, Pandemic

INTRODUCTION

The covid-19 disease first appeared in China in December 2019 (1). Coronavirus causes various health problems, such as pneumonia, acute respiratory tract problems, kidney damage, myocardial dysfunction, and gastrointestinal diseases (2). Studies have also reported that Covid-19 can cause changes in the hypothalamic-pituitary-gonadal axis (3).

This axis is sensitive to insufficient sleep and to psychological and physical stress. Pulsatile release of hormones is necessary for a regular menstrual cycle, and irregularities in hormone secretion may result in menstrual disorders (4). Studies have investigated the effects of Covid-19 on male reproductive health, sperm parameters, and testicular tissue (5, 6). The regular functioning of the female and male reproductive systems is very important for a healthy embryological life (7).

Covid-19 Disease and Its Effects on the Female Reproductive System

The female menstrual cycle is regulated by the hypothalamic-pituitary-ovarian axis, through the positive and negative feedback of hormones on these structures (8). The changes caused by Covid-19 in the hypothalamic-pituitary-gonadal axis will directly affect the menstrual cycle (3).

Covid-19 affects the target cell by binding to angiotensin-converting enzyme (ACE) 2 via the surface spike protein (9). ACE2 mRNA transcripts have been detected in the ovaries of reproductive age and postmenopausal women (10). ACE2 is a key enzyme in the axis that plays a synergistic role in the balance between Ang II and Ang-(1-7) levels. Ang II induces steroid secretion, facilitates follicle development and oocyte maturation, plays a role in ovulation, and maintains corpus luteum progression (11-15).

In addition, ACE2 mRNA has also been detected in the human and rat uterus (16, 17). Ang II initiates menstruation through spiral artery vasoconstriction (18).

The balance between Ang II and Ang-(1-7) plays a role in the regulation of myometrial activity and endometrial regeneration (16, 19). The normal function of Ang II in the endometrium is also essential for regular menstrual cycles (20). For all these reasons, Covid-19 affects the uterus, ovary, and oocyte and disrupts the functioning of the female reproductive system.

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Covid-19 Disease and Its Effects on the Male Reproductive System

The male reproductive system is a complicated system consisting of the testicles, penis, duct systems, and accessory glands. The main task of the testicles is the production of sperm and the release of the hormone androgen. Sperm is stored and matured in the ducts. The production of non-sperm ejaculate substances occurs in the accessory glands. The principle factor in the formation of good-quality spermatozoa is proper spermatogenesis. Spermatogenesis is defined as the process of maturation of diploid male germ cells by meiosis and their differentiation into haploid male gamete cells. Spermatogenesis starts at puberty, once sperm maturation begins, and continues to the end of life. Four mature sperm are formed from each primary spermatocyte. A disruption in this system causes oligospermia, azoospermia, asthenospermia, and cryptorchidism, events that play a major role in male infertility (**21-23**).

ACE2 is expressed in the testes and epididymis, especially in Leydig cells, Sertoli cells, and spermatogonia (**24**). Covid-19 causes high fever and a possible cytokine storm, symptoms that affect male fertility. An increase in testicular temperature causes oxidative stress and sperm DNA fragmentation by increasing levels of reactive oxygen species (**25**). Postmortem macroscopic and microscopic analyses of the testes of 12 patients who died from Covid-19 in one study revealed damage in 11 cases, and that the mean Leydig cell count was significantly lower compared to that of a control group (**26**). In a recent study of 119 male patients of reproductive age, semen analysis of male patients diagnosed with Covid-19 revealed decreased sperm motility, sperm morphological disorders, and loss of libido. In addition, serum luteinizing hormone (LH) levels were significantly higher in the Covid-19 group compared to the control group, although no significant change was observed in serum testosterone levels. The authors concluded that gonadal functions were affected by the higher serum LH level and lower serum testosterone/LH ratio compared to the control group (**27**). Studies on this subject are still ongoing, and it is clear that the male reproductive system is under threat by Covid-19.

Effects of Covid-19 Disease in the Embryonic and Fetal Period

Fertilization is a complex molecular chain of events in which oocyte and sperm nuclei and cytoplasmic components come together to form a zygote (**28, 29**). Fertilization begins with sperm capacitation, attachment of sperm to the zona pellucida, induction of the acrosome reaction, crossing of the perivitelline space, and fusion with the oolemma. The subsequent process involves the completion of the second meiotic division in the oocyte, the expulsion of the second polar body, the activation of the oocyte, the decondensation of the sperm nucleus and maternal chromosomes, and finally the cytoplasmic migration of the pronuclei. Processes including receptor-ligand interaction, ion-channel modulations, membrane fusion, and proteolysis occur during fertilization (**28-30**).

After fertilization, the oocyte becomes a blastocyst and adheres to the surface of the endometrium. The blastocyst implants into the endometrium on the seventh day. Implantation is followed by the formation of the placenta, which will support the embryo until the end of pregnancy (**31, 32**). The embryonic period, which is particularly sensitive to external factors (between the third and eighth weeks) involves the formation of numerous tissues and organ systems. The embryonic period is followed by the fetal period, which will continue until birth.

ACE2 is expressed in the placenta, placental villi, syncytiotrophoblast, cytotrophoblast, endothelium, and vascular smooth muscle of primary and secondary villi. It is also expressed in the maternal stroma, intravascular trophoblast, and decidual cells. ACE2 is also found in the arterial and venous endothelium and the smooth muscle of the umbilical cord (**33**). ACE2 reaches its highest levels during early pregnancy, and is expressed in the primary and secondary decidual region and luminal and glandular epithelial cells. ACE2 has been observed in the placenta and amniotic and yolk sac epithelium during late pregnancy (**34-36**). During pregnancy, Ang2, ACE2, and Ang-(1-7) are principally involved in regulating blood pressure and fetal development. They also interact to maintain normal uterine physiology (**37**).

Ang-(1-7) and ACE2 are also thought to act as a local autocrine/paracrine regulator in the early (angiogenesis, apoptosis and growth) and late (uteroplacental blood flow) events of pregnancy (35). ACE2 controls the blood pressure balance in the pregnant woman (34). One previous study observed suppressed plasma Ang-(1-7) levels in pre-eclamptic women compared with normal pregnancies (38). Finally, low levels of ACE2 and Ang-(1-7) in the placenta have been associated with intrauterine growth retardation (36, 39).

RESULTS

Covid-19 disease exhibits its effect by binding to ACE receptors. It affects numerous body systems, such as the lungs, heart, kidneys, nervous system, and skin. The effect mechanism of the virus and its acute and chronic phase effects on different organs are still the subject of investigation.

Studies of male infertility after Covid-19 have shown that the disease exerts a deleterious effect on the male reproductive system. Covid-19 can also impair female reproductive functions, causing infertility, menstrual irregularity, and fetal distress. It has also been shown to infect the ovary, uterus, vagina, and placenta.

CONCLUSION

We therefore recommend that couples planning pregnancies be protected against Covid-19 infection. Close monitoring of the fetus and pregnancy is also highly important.

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REFERENCES

1. Li D, Jin M, Bao P, Zhao W, Zhang S. Clinical characteristics and results of semen tests among men with coronavirus disease 2019. JAMA network open. 2020 May 1;3(5):e208292.

2. Gupta A, Madhavan MV, Sehgal K, et al. Extrapulmonary manifestations of COVID-19. Nat Med. 2020;26(7):1017- 1032. doi:10.1038/s41591-020-0968-3.

3. Mauvais-Jarvis F, Klein SL, Levin ER. Estradiol, progesterone, immunomodulation, and COVID-19 outcomes. Endocrinology. 2020;161(9):bqaa127. doi:10.1210/endocr/bqaa127.

4. Prado RCR, Silveira R, Asano RY. SARS-CoV-2 (COVID-19) pandemic and a possible impact in the future of menstrual cycle research. Health Sci Rep. 2021;4(2):e276. doi:10.1002/ hsr2.276.

5. Yang M, Chen S, Huang B, Zhong JM, Su H, Chen Y. J et al. Pathological findings in the testes of COVID19 patients: clinical implications. Eur Urol Focus. 2020;6:1124-9.

6. Li H, Xiao X, Zhang J, Zafar MI, Wu C, Long Y et al. Impaired spermatogenesis in COVID-19 patients. E ClinicalMedicine. 2020;28:100604.

7. Sadler TW. Langman's Medical Embryology. 12th Edition. USA: Lippincott Williams & Wilkins. 2012; 1-128.

8. Treloar AE. Variation of the human menstrual cycle through reproductive life. Int J Fertil. 1967;12:77-126.

9. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The lancet. 2020 Feb 22;395(10224):565-74.

10. Reis FM, Bouissou DR, Pereira VM, Camargos AF, dos Reis AM, Santos RA. Angiotensin-(1-7), its receptor Mas, and the angiotensin-converting enzyme type 2 are expressed in the human ovary. Fertility and sterility. 2011 Jan 1;95(1):176-81.

11. Hayashi KG, Acosta TJ, Tetsuka M, Berisha B, Matsui M, Schams D, Ohtani M, Miyamoto A. Involvement of angiopoietin-tie system in bovine follicular development and atresia: messenger RNA expression in theca interna and effect on steroid secretion. Biology of reproduction. 2003 Dec 1;69(6):2078-84.

12. Ferreira R, Gasperin B, Rovani M, Santos J, Barreta M, Bohrer R, Price C, Bayard Dias Gonçalves P. Angiotensin II signaling promotes follicle growth and dominance in cattle. Endocrinology. 2011 Dec 1;152(12):4957-65.

13. Stefanello JR, Barreta MH, Porciuncula PM, Arruda JN, Oliveira JF, Oliveira MA, Gonçalves PB. Effect of angiotensin II with follicle cells and insulin-like growth factor-I or insulin on bovine oocyte maturation and embryo development. Theriogenology. 2006 Dec 1;66(9):2068-76.

14. Guo B, Zhang XM, Li SJ, Tian XC, Wang ST, Li DD, Liu DF, Yue ZP. Expression and regulation of Ang-2 in murine ovaries during sexual maturation and development of corpus luteum. Molecular Biology. 2012 Nov;46(6):802-8.

15. Sugino N, Suzuki T, Sakata A, Miwa I, Asada H, Taketani T, Yamagata Y, Tamura H. Angiogenesis in the human corpus luteum: changes in expression of angiopoietins in the corpus luteum throughout the menstrual cycle and in early pregnancy. The Journal of Clinical Endocrinology & Metabolism. 2005 Nov 1;90(11):6141-8.

16. Vaz-Silva J, Carneiro MM, Ferreira MC, Pinheiro SV, Silva DA, Silva AL, Witz CA, Reis AM, Santos RA, Reis FM. The vasoactive peptide angiotensin-(1–7), its receptor Mas and the angiotensin-converting enzyme type 2 are expressed in the human endometrium. Reproductive sciences. 2009 Mar;16(3):247-56.

17. Brosnihan KB, Bharadwaj MS, Yamaleyeva LM, Neves LA. Decidualized pseudopregnant rat uterus shows marked reduction in Ang II and Ang-(1-7) levels. Placenta. 2012 Jan 1;33(1):17-23.

18. Li XF, Ahmed A. Dual role of angiotensin II in the human endometrium. Human reproduction. 1996 Oct 1;11(suppl_2):95-108.

19. Vaz-Silva J, Tavares RL, Ferreira MC, Honorato-Sampaio K, Cavallo IK, Santos RA, dos Reis AM, Reis FM. Tissue specific localization of angiotensin-(1–7) and its receptor Mas in the uterus of ovariectomized rats. Journal of molecular histology. 2012 Oct 1;43(5):597-602.

20. Li XF, Ahmed A. Expression of angiotensin II and its receptor subtypes in endometrial hyperplasia: a possible role in dysfunctional menstruation. Laboratory investigation; a journal of technical methods and pathology. 1996 Aug 1;75(2):137-45.

21. Moore K.L, Persaud T.V.N. The Devoloping Human Clinically Oriented Embryology. 8th ed, Saunders, 2007: 262-270.

22. Eskandari M, Ghalyanchi Langeroudi A, Zeighami H, Rostami A, Kazemi M, Eyni H, Shokri S. Co-administration of ginseng and ciprofloxacin ameliorates epididymo-orchitis induced alterations in sperm quality and spermatogenic cells apoptosis following infection in rats. Andrologia. 2017 Apr;49(3):e12621.

23. Lin CY, Chen CY, Yu CH, Yu IS, Lin SR, Wu JT, Lin YH, Kuo PL, Wu JC, Lin SW. Human X-linked intellectual disability factor CUL4B is required for post-meiotic sperm development and male fertility. Scientific reports. 2016 Feb 2;6(1):1-7.

24. Wang Z, Xu X. scRNA-seq profiling of human testes reveals the presence of the ACE2 receptor, a target for SARS-CoV-2 infection in spermatogonia, Leydig and Sertoli cells. Cells. 2020 Apr;9(4):920. doi: 10.3390/cells9040920.

25. Albani E, Castellano S, Gurrieri B, Arruzzolo L, Negri L, Borroni EM, Levi-Setti PE. Male age: negative impact on sperm DNA fragmentation. Aging (Albany NY). 2019 May 15;11(9):2749. doi: 10.18632/aging.101946.

26. Yang M, Chen S, Huang BO, Zhong JM, Su H, Chen YJ, Cao Q, Ma L, He J, Li XF, Li X. Pathological findings in the testes of COVID-19 patients: clinical implications. European urology focus. 2020 Sep 15;6(5):1124-9.

27. Ma L, Xie W, Li D, Shi L, Ye G, Mao Y, Xiong Y, Sun H, Zheng F, Chen Z, Qin J. Evaluation of sex-related hormones and semen characteristics in reproductive-aged male COVID-19 patients. Journal of medical virology. 2021 Jan;93(1):456-62.

28. Dietzel E. Sperm-oocyte interaction. J Reprod Biol Endocrinol. 2018;2 (2): - 59 - 60.

29. Georgadaki K, Khoury N, Spandidos DA, Zoumpourlis V. The molecular basis of fertilization. International journal of molecular medicine. 2016 Oct 1;38(4):979-86.

30. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. Gametogenesis, fertilization, and first week. Larsens's human embryology. 4th ed. Philadelphia: Churchill Livingstone. 2009:15-50.

31. Moore Keith L. The Developing Human Clinically Oriented Embryology. 4. Edition, W.B. Saunders Company, Philadelphia, sayfa: 31-34.

32. Ross MH, Pawlina W. Histology. Lippincott Williams & Wilkins; 2006.

33. Valdes G, Neves LA, Anton L, Corthorn J, Chacon C, Germain AM, Merrill DC, Ferrario CM, Sarao R, Penninger J, Brosnihan KB. Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta. 2006 Feb 1;27(2-3):200-7.

34. ER P. The expression and localization of the human placental prorenin/renin-angiotensin system throughout pregnancy: roles in trophoblast invasion and angiogenesis. Placenta. 2011;32:956-62.

35. Neves LA, Stovall K, Joyner J, Valdés G, Gallagher PE, Ferrario CM, Merrill DC, Brosnihan KB. ACE2 and ANG-(1-7) in the rat uterus during early and late gestation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2008 Jan;294(1):R151-61.

36. Ghadhanfar E, Alsalem A, Al-Kandari S, Naser J, Babiker F, Al-Bader M. The role of ACE2, angiotensin-(1–7) and Mas1 receptor axis in glucocorticoid-induced intrauterine growth restriction. Reproductive Biology and Endocrinology. 2017 Dec;15(1):1-9.

37. Hering L, Herse F, Geusens N, Verlohren S, Wenzel K, Staff AC, Brosnihan KB, Huppertz B, Luft FC, Muller DN, Pijnenborg R. Effects of circulating and local uteroplacental angiotensin II in rat pregnancy. Hypertension. 2010 Aug 1;56(2):311-8.

38. Brosnihan KB, Neves LA, Anton L, Joyner J, Valdes G, Merrill DC. Enhanced expression of Ang-(1-7) during pregnancy. Brazilian Journal of Medical and Biological Research. 2004;37:1255-62.

39. Jing Y, Run-Qian L, Hao-Ran W, Hao-Ran C, Ya-Bin L, Yang G, Fei C. Potential influence of COVID-19/ACE2 on the female reproductive system. Molecular human reproduction. 2020 Jun;26(6):367-73.

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Precision medicine to identify optimal diagnostic and therapeutic interventions for Parkinson's Disease

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ABSTRACT

Objective: Parkinson's disease, the second most common neurodegenerative disorder afflicting 10 million people worldwide and the fourteenth leading cause of death in the United States, is caused by the death of dopaminergic neurons that regulate movement in the substantia nigra pars compacta. Mechanisms contributing to the development of Parkinson's disease in vulnerable individuals include protein misfolding, protein aggregation, and mitochondrial dysfunction. In order to develop guidelines for clinicians to utilize precision medicine to develop treatment plans to address the specific needs of individuals with Parkinson's disease and related conditions, we have developed algorithms for diagnosis and treatment based on their view of available knowledge. We reviewed the key literature on the pathogenesis of Parkinson's disease on PubMed and google scholar in order to propose guidelines for the development of diagnostic and therapeutic interventions for people with Parkinson's disease and related conditions. In about 25 percent of patients, clinicians incorrectly diagnose Parkinson's disease. Causes of misdiagnosis include a lack of algorithms and inadequate use of diagnostic modalities. Four main mechanisms that may contribute to the development of Parkinson's disease (misfolding of alpha-synuclein, mitochondrial dysfunction, dysfunctional ubiquitin proteasomal pathways, and abnormal autophagy) and different diagnostic modalities (structured interview and examination, laboratory assessments, neuropathology, genetic testing, neuroimaging) will form the basis for our algorithm for the diagnosis and treatment of Parkinson's disease and related conditions. Clinicians, administrators, policy planners, advocates, and other concerned individuals will benefit from the adoption of our guidelines for the diagnosis and treatment of Parkinson's disease and related conditions.

Keywords: Precision Medicine, Parkinson's Disease, Algorithms, Basal ganglia, Neurodegenerative disorders, DAT-SPECT, L-DOPA, Dopamine agonists, Amyloid Protein, Proteosomes, Neuroimaging, Accelerometer, CT, MRI, PET, Transcranial Sonograghy, Neurosurgery, Pallidotomy, Thalamotomy, Sargramostism, Coenzyme Q10, Genetic Testing, Polymerase Chain reaction, Ubiquitination Assay, PRKN gene

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INTRODUCTION

Pathogenesis of the Parkinson's Disease

a) Alpha-synuclein protein misfolding

Alpha-synuclein is a cerebral protein normally found in an insoluble fibril. It is an abundant presynaptic protein that binds to negatively charged phospholipids, which also function as SNARE –complex chaperone which contributes to the pathogenesis of Parkinson disease due to its misfolding (1).Experiments were carried out on rodents and found the abnormal neurotoxic alpha-synuclein protein is found in an oligomeric form rather than the mature insoluble fibrils (2). Protein folding and refolding of the misfolded proteins occurs via a group of molecules known as chaperones and co-chaperones such as Hsp70 and Hsp90 and their Co Chaperone Hsp 40 (3). Syncluein misfolding can be detected via a molecular test called Protein Misfolding Cyclic Amplification that will be discussed in detail (4).

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Developing drugs that enhance chaperone and co-chaperone molecular systems may help reduce protein misfolding in some patients with Parkinson's disease (3).

b) Dysfunctional ubiquitin-proteasome system

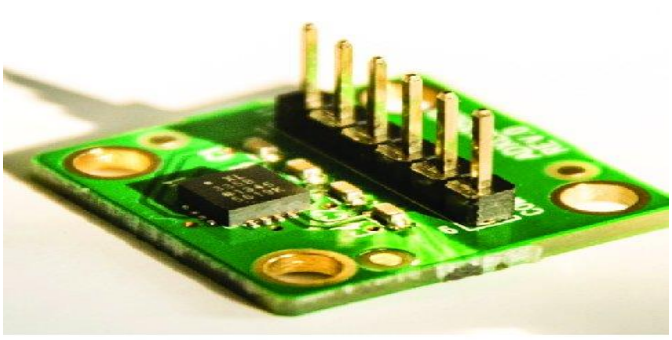
Proteosomes are organelles responsible for protein turnover. Diminished activity of these organelles may result in abnormal protein accumulation in the basal ganglia and cause damage which may contribute to the development of Parkinson's disease (5). Decreased expression of proteasomal components has been identified in the SNpc of the brain in patients with Parkinson's disease (6). Ubiquitination Assay for Parkin gene can be tested in autosomal recessive cases of PD (7).

c) Mitochondrial dysfunction

Mitochondrial dysfunction has been found in many cases of idiopathic or hereditary Parkinson's disease (8).The neuro-toxic alpha-synuclein in its oligomeric form can interact with a mitochondrial receptor resulting in increased oxidative damage due to reduced mitochondrial respiration (9).Genetic testing can help diagnose hereditary cases with mitochondrial dysfunction as many genes involved have been detected as SNCA gene mutation in Autosomal dominant cases while Parkin gene mutation in Autosomal recessive cases. Developing drugs that target Mitochondrial Complexes to resolve the dysfunction may enhance symptoms in PD (10).

Diagnostic Modalities

a) The most recent simple diagnostic modality: Tri-axial accelerometer (11).



McKay, James Brasic, and their colleagues at Johns Hopkins medical school developed a low cost simple objective method to diagnose and grade Parkinson's disease (11). This technique involves objective quantitative assessment of tremors in extremities of patients with Parkinson's disease.

Low-cost accelerometers are attached to the upper and lower limbs to generate a continuous three diminutions representation of the movements. Patients and controls were assessed through a test-retest method. The degree of tremors is rated clinically by trained examiners. The output of the accelerometers can be interpreted using Fast Fourier Transforms and Continuous Wavelet transforms by experts for diagnosis and therapy (12). The use of this device for clinical practice is not FDA approved yet (11).

b) Transcranial sonography

Sonography is a simple, low-cost and widely available imaging modality and it can be used for diagnosing Parkinson's disease with high accuracy. Pooled sensitivity and specificity of transcranial -sonography for diagnosing Parkinson's disease is 84% and 85%. This is calculated from the meta-analysis of 39 studies involving 3123 patients (13). Usually, it is not used for diagnosis unless there is an associated condition such as epilepsy or stroke.

c) Genetic and molecular testing

1) RT-Polymerase chain reaction (RT-PCR)

This method can detect genetic mutations in hereditary cases of PD. It can be used for screening and diagnosis. This method uses heat to denature DNA then annealing occurs via Reverse Transcription DNA polymerase enzyme to amplify the gene of interest (14).

2) Protein Misfolding Cyclic Amplification

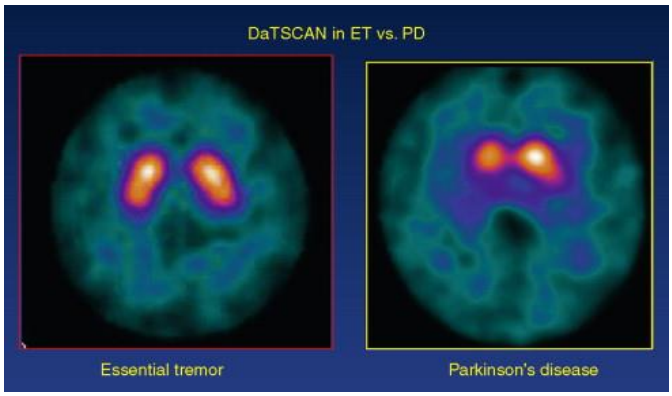
This test uses ultrasound waves to degrade proteins such as alpha-synucleininto small pieces so they can be amplified and studied. This method can be used to detect misfolded alpha-synuclein protein with high sensitivity and specificity in patients with multiple system atrophy and Parkinson's disease (15).

3) Ubiquitination assay for ligase enzyme

This method can be used in Autosomal recessive cases with Parkin gene mutations. The concept relies on detecting the activity of the Parkinubiquitin ligase enzyme which is important for protein turnover (16).

d) Molecular imaging methods

1) In single-photon emission computed tomography (SPECT) (33).



SPECT is a method of molecular imaging in which a gamma ray-emitting radioactive isotope is tagged to a molecule of interest. In patients with Parkinson's disease, labeled cocaine derivatives such as Ioflupane for DAT-SPECT is most widely used, others include 123I-β-CIT and 123I-FP-CIT (N-ω-fluoropropyl-2β-carboxymethoxy-3β-(4-iodophenyl tropane). They label the presynaptic dopamine reuptake sites. Patients with Parkinson's disease show reduced uptake of these substances in the basal ganglia (17). A subtype of SPECT called 123I-β-CIT SPECT was 100% sensitive and specific for the diagnosis in younger patients (age <55 years). In older patients (age >55 years), specificity was substantially lower (68.5%) (18).

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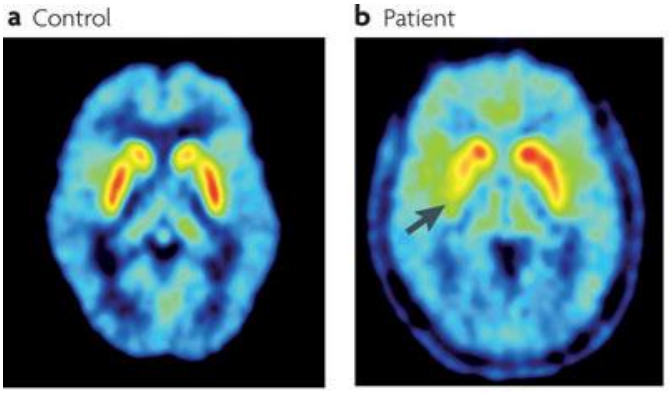
A major disadvantage of SPECT, when compared to other methods of nuclear imaging such as PET, include the limited resolution of images for the visualization of basal ganglia in PD (19).

2) Positron emission tomography (34)

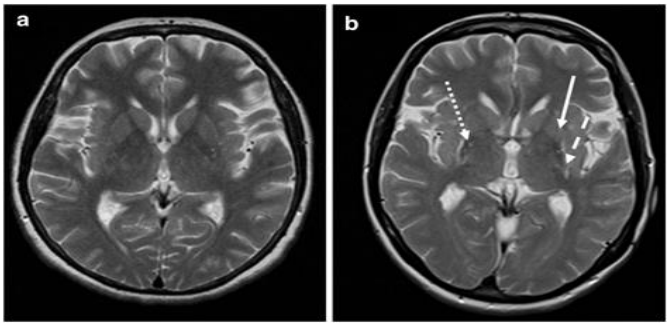


PET is another method of molecular imaging in which a positron-emitting radioactive isotope is tagged to a molecule of interest. Fluorine is injected intravenously into patients with Parkinson's disease. Fluorine can be attached to either DOPA or deoxyglucose. Flurodopa F18 is taken by the presynaptic neurons in the basal ganglia particularly the caudate and putamen. However, fluorodeoxyglucose is taken by active cells to be trapped in the tissues during the imaging study. Reduced uptake of Flurodopa is seen in Parkinson's disease (20).

One study showed that the use of F-DOPA PET to discover the pathogenesis of Parkinson's disease showed that F-DOPA uptake correlated with increased bradykinesia and rigidity, not with tremors which show that the pathogenesis of tremors is different from the pathogenesis of bradykinesia and rigidity (21).

It can be used if the diagnosis is inconclusive by DAT-SPECT.A meta-analysis calculated the pooled sensitivity of PET in diagnosing Parkinson's disease as 88% and specificity as 92 % (22). A major disadvantage of PET imaging is the short half-lives of the radioactive isotopes which do not allow much time for imaging. Another major disadvantage is the high cost of a PET scan.

3) Magnetic resonance imaging (35).



MRI is a method of imaging in which high magnetic field strengths are used to excite the hydrogen atoms in water molecules. MRI can demonstrate structural changes(atrophy) in the basal ganglia in patients with Parkinson's disease as illustrated in Image B.MRI is superior to CT, because of better resolution and sensitivity to identify structural brain pathology (23). A meta-analysis study published in 2021 calculated pooled sensitivity and specificity of using MRI in PD. The pooled sensitivity was found as 92% and pooled specificity as 90% (24). Usually, it is used for diagnosis only if there is an associated condition like stroke or epilepsy.

4) Magnetic resonance spectroscopy

MRS can be used to quantify the intermediary metabolites in small volumes of brain tissue. Patients with Parkinson's disease show decreased concentration of Dopamine in the basal ganglia. This method is usually used for research purposes (25).

Our Algorithm for Diagnosis: Algorithm for Diagnosis of PD (ADPD)

Diagnosis of PD (ATPD)	
Step 1	**1. History of Present Illness:** History of Bradykinesia with/without static tremors and or rigidity. It is crucial to differentiate PD from other similar conditions such as • Supranuclear palsy which is characterized by gaze palsy seen early in the disease. • Shy-Dragersyndromewhichis characterized by autonomic dysfunction and • Cortical base degeneration which is characterized by early cortical signs as speech deficits and cognitive decline. **2. History of Traumatic brain injury if present.** **3. History of any chronic illness.** **4. Family history of PD and or other movement disorders.**
Step 2	**Clinical examination:** • Inspection: Static regular tremors like Bell rolling. • Tone:Rigidity of upper and or lower extremities. • Gait:Bradykinesia and characteristic shuffling gait.
Step 3	**Use of mechanical accelerometer.** • Simple and cheap method. • It allows accurate grading of tremors. If diagnosed: Step 4
Step 4	**With a family history.** • Genetic and molecular testing (RT-PCR,Ubiquitination Assay for ligase enzyme,etc) **Without Family history** a) If no other condition is associated • DAT SPECT. • Reliable and widely available Imaging Modality. b) If another condition associated (epilepsy or stroke) • Transcranial Sonography • CT • MRI

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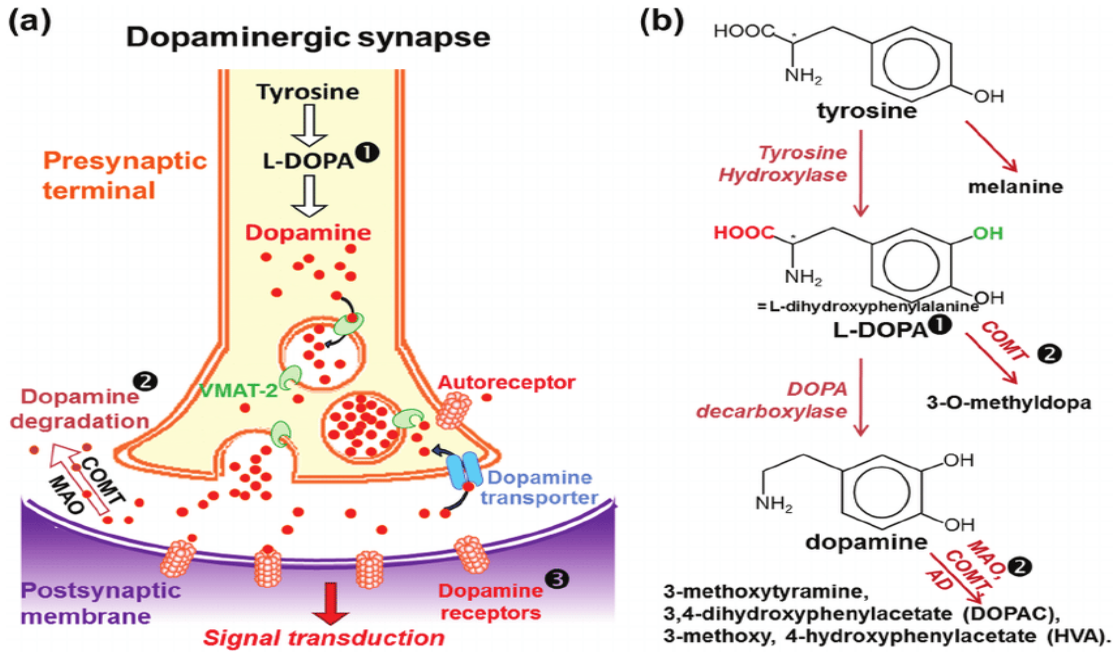

Figure: formation of Dopamine in the basal ganglia (36)

Medications used for Parkinson's disease:

Most medications used for Parkinson's disease target the neuro-transmitter; Dopamine which is formed in the basal ganglia in normal individuals.

To understand the mechanisms of these medications, we must discuss how Dopamine is formed normally in the basal ganglia.

Tyrosine is the amino acid used for the formation of Dopamine and other neurotransmitters as adrenaline and noradrenaline.

The picture below illustrates the steps for the formation of Dopamine in the basal ganglia

a) L-DOPA

L-DOPA is the most potent and is the first line of treatment for Parkinson's disease, especially in the early stages. It provides DOPA that is decarboxylated in the body to Dopamine. It is highly effective but it has many adverse effects. Combing it with Carbidopa helps prevents decarboxylation outside the brain which reduces adverse effects (**26, 27**).

b) Dopamine agonists

Drugs such as Pramipexole can be used to treat Parkinson's disease as they stimulate the dopaminergic 2 receptors which are located in the basal ganglia (**27, 28**).

c) Catechol methyl-transferase inhibitor

Tolcapone and Entacapone are drugs that work via inhibiting the enzyme Catechol Methyl-transferase thus increasing dopamine availability in the basal ganglia.

d) Monoamine oxidase inhibitors

Drugs that inhibit MAO –B such as Selegiline help increase the availability of Dopamine in the basal ganglia in a step different from COMT inhibitors. Both classes have similar efficacy (**27, 28**).

A meta-analysis summarized 45 clinical trials on 9000 patients to compare the efficacy of adjuvant drugs such as dopamine agonists, catechol-methyl-transferase inhibitors to L-DOPA. Dopamine agonists were found to be more effective than catechol-methyl-transferase inhibitors and MAO-B inhibitors which have similar efficacy. Drugs of the same class were not different in efficacy except Tolcapone which was found to be more effective than entacapone (**29**).

Experimental drugs

a) Carbenoxolone

Laboratory Experiments showed that the drug carbenoxolonein a low dose enhances the activity of Hsp 90 in rat models which results in the reduction of misfolded alpha-synucleinprotein in neurons and improvement in motor functions (**29**)

b) Coenzyme Q10(Ubiquinol-10)

Coenzyme Q10 serves as a co-factor for complexes 1,2 and 3 for the electron transport chain in the mitochondria. It can be of significant benefit for some patients with PD.A clinical study showed that it might be helpful in patients taking L-DOPA with wear-off symptoms (**30**).

c) Sargramostism(GM-CSF):

GM-CSF is a cytokine produced by T- regulatory cells and it plays a role in PD by protecting against basal ganglia degeneration (**10, 30**). Drugs targeting the immune system like Sargramostism has shown promise in animal model of Parkinson's disease and human clinical trials (**31**).

Surgical methods

a) Deep Brain Stimulation

The concept relies on stimulating the subthalamic nucleus which inhibits signals from the basal ganglia. It is an invasive method but showed marked improvement in many patients.

b) Thalamotomy

The concept relies on destruction of the thalamus. Benefit for tremors only.

c) Pallidotomy

The Concept relies on destruction of globus pallidum. Benefit for tremors, bradykinesia,and levodopa-induced dyskinesias. Bilateral procedures are not recommended.

d) Subthalamotomy

The concept relies on destruction of the subthalamicnucleas. Under development; may reduce tremor, rigidity, bradykinesia, and levodopa-induced dyskinesias. Not recommended for bilateral use.

Other surgical methods include focused ultrasound thalamotomies, and cell transplantation therapies (**32, 33**).

Our Algorithm for Treatment of PD: Algorithm for Treatment of PD (ATPD)

Treatment of PD (ATPD)	
Step 1	Exercise and rehabilitation medicine. L-DOPA or carbidopa. If no improvement(monitor degree of static tremors by accelerometer /monitor other signs of PD.
Step 2	Increase dose of L-DOPA/carbidopa. Maintain exercise andrehabilitationmedicine. If no improvement or wear-off symptoms (Monitor degree of static tremors by accelerometer /monitor other signs of PD.
Step 3	Maximize dose of L-DOPA/carbidopa with or without another class of medication such as MAO-B inhibitor or COMT inhibitor. Use of co-enzyme(Ubiquinol-10). Maintain exercise and rehabilitationmedicine. If no improvement, Monitor degree of static tremors by accelerometer /monitor other signs of PD.
Step 4	MAO-B inhibitors with or without COMT Inhibitors. Maintain exercise and rehabilitation medicine. If no improvement (monitor degree of static tremors by accelerometer /monitor other signs of PD.
Step 5	Deep brain stimulation (invasive method).
Step 6	Surgical methods including radiofrequency or radiosurgery procedures(pallidotomy,thalamotomy or subthalamotomy).
Step 7	Potential new treatments including immunotherapy, gene therapy or cell transplantation.

CONCLUSION

Clinicians, administrators, policy planners, advocates, and other concerned individuals will benefit from the adoption of our algorithms for the diagnosis and treatment of Parkinson's disease.

Author Contributions: TE, ABS, MAMA, JRB: Search of the literature, performed the analysis, wrote the manuscript, and designed the article for submission.

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REFERENCES

1. Burré J, Vivona S, Diao J, Sharma M, Brunger AT, Südhof TC. Properties of native brain α-synuclein. Nature. 2013;498(7453):E4-6; discussion E6-7.
2. Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A. 2011;108(10):4194–9.
3. Jung BC, Lim Y-J, Bae E-J, Lee JS, Choi MS, Lee MK, et al. Amplification of distinct α-synuclein fibril conformers through protein misfolding cyclic amplification. Exp Mol Med. 2017;49(4):e314–e314.
4. Kalia SK, Kalia LV, McLean PJ. Molecular chaperones as rational drug targets for Parkinson's disease therapeutics. CNS Neurol Disord Drug Targets. 2010;9(6):741–53.
5. McKinnon C, Tabrizi SJ. The ubiquitin-proteasome system in neurodegeneration. Antioxid Redox Signal. 2014;21(17):2302–21.
6. McNaught KSP, Belizaire R, Jenner P, Olanow CW, Isacson O. Selective loss of 20S proteasome α-subunits in the substantia nigra pars compacta in Parkinson's disease. Neurosci Lett. 2002;326(3):155–8.
7. Schlossmacher MG, Shimura H. Parkinson's disease: assays for the ubiquitin ligase activity of neural Parkin. Methods Mol Biol. 2005;301:351–69.
8. Moon HE, Paek SH. Mitochondrial dysfunction in Parkinson's disease. Exp Neurobiol. 2015;24(2):103–16.
9. Di Maio R, Barrett PJ, Hoffman EK, Barrett CW, Zharikov A, Borah A, et al. α-Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease. Sci Transl Med. 2016;8(342):342ra78-342ra78.
10. Thomas B, Beal MF. Mitochondrial therapies for Parkinson's disease: Mitochondria and Parkinson's Disease. Mov Disord. 2010;25(S1):S155–60.
11. McKay GN, Harrigan TP, Brašić JR. A low-cost quantitative continuous measurement of movements in the extremities of people with Parkinson's disease. MethodsX. 2019;6:169–89.
12. Brasic J. Signal processing of quantitative continuous measurement of movements in the extremities. Mendeley; 2020.
13. Tao A, Chen G, Deng Y, Xu R. Accuracy of transcranial sonography of the substantia nigra for detection of Parkinson's disease: A systematic review and meta-analysis. Ultrasound Med Biol. 2019;45(3):628–41.
14. Wang J, Liu Y, Chen T. Identification of key genes and pathways in Parkinson's disease through integrated analysis. Mol Med Rep. 2017;16(4):3769–76.

15. Shahnawaz M, Mukherjee A, Pritzkow S, Mendez N, Rabadia P, Liu X, et al. Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy. Nature. 2020;578(7794):273–7.

16. Benamer HTS, Oertel WH, Patterson J, Hadley DM, Pogarell O, Höffken H, et al. Prospective study of presynaptic dopaminergic imaging in patients with mild parkinsonism and tremor disorders: part 1. Baseline and 3-month observations: Presynaptic Dopaminergic Imaging. Mov Disord. 2003;18(9):977–84.

17. Eerola J, Tienari PJ, Kaakkola S, Nikkinen P, Launes J. How useful is [I123]β-CIT SPECT in clinical practice? Journal of Neurology, Neurosurgery and Psychiatry. 2005;76(9):1211–1216,.

18. Wang L, Zhang Q, Li H, Zhang H. SPECT molecular imaging in Parkinson's disease. J Biomed Biotechnol. 2012;2012:412486.

19. Vingerhoets FJ, Schulzer M, Calne DB, Snow BJ. Which clinical sign of Parkinson's disease best reflects the nigrostriatal lesion? Ann Neurol. 1997;41(1):58–64.

20. Otsuka M, Ichiya Y, Kuwabara Y, Hosokawa S, Sasaki M, Yoshida T, et al. Differences in the reduced 18F-Dopa uptakes of the caudate and the putamen in Parkinson's disease: correlations with the three main symptoms. J Neurol Sci. 1996;136(1–2):169–73.

21. Gu S-C, Ye Q, Yuan C-X. Metabolic pattern analysis of 18F-FDG PET as a marker for Parkinson's disease: a systematic review and meta-analysis. Rev Neurosci. 2019;30(7):743–56.

22. Brooks DJ. Morphological and functional imaging studies on the diagnosis and progression of Parkinson's disease. J Neurol. 2000;247 Suppl 2:II11-8.

23. Cho SJ, Bae YJ, Kim J-M, Kim HJ, Baik SH, Sunwoo L, et al. Iron-sensitive magnetic resonance imaging in Parkinson's disease: a systematic review and meta-analysis. J Neurol [Internet]. 2021; Available from: http://dx.doi.org/10.1007/s00415-021-10582-x

24. Clarke CE, Lowry M. Systematic review of proton magnetic resonance spectroscopy of the striatum in parkinsonian syndromes. Eur J Neurol. 2001;8(6):573–7.

25. LeWitt PA. Levodopa therapy for Parkinson's disease: Pharmacokinetics and pharmacodynamics: Levodopa Pharmacokinetics and Pharmacodynamics. Mov Disord. 2015;30(1):64–72.

26. Radhakrishnan DM, Goyal V. Parkinson's disease: A review. Neurol India. 2018;66(Supplement):S26–35.

27. Armstrong MJ, Okun MS. Diagnosis and treatment of Parkinson disease: A review: A review. JAMA. 2020;323(6):548–60.

28. Thakur P, Nehru B. Long-term heat shock proteins (HSPs) induction by carbenoxolone improves hallmark features of Parkinson's disease in a rotenone-based model. Neuropharmacology. 2014;79:190–200.

29. Yoritaka A, Kawajiri S, Yamamoto Y, Nakahara T, Ando M, Hashimoto K, et al. Randomized, double-blind, placebo-controlled pilot trial of reduced coenzyme Q10 for Parkinson's disease. Parkinsonism Relat Disord. 2015;21(8):911–6.

30. Gendelman HE, Zhang Y, Santamaria P, Olson KE, Schutt CR, Bhatti D, et al. Evaluation of the safety and immunomodulatory effects of sargramostim in a randomized, double-blind phase 1 clinical Parkinson's disease trial. NPJ Parkinsons Dis [Internet]. 2017;3(1). Available from: http://dx.doi.org/10.1038/s41531-017-0013-5

31. Lee DJ, Dallapiazza RF, De Vloo P, Lozano AM. Current surgical treatments for Parkinson's disease and potential therapeutic targets. Neural Regen Res. 2018;13(8):1342–5.

32. Olanow CW, Brin MF, Obeso JA. The role of deep brain stimulation as a surgical treatment for Parkinson's disease. Neurology. 2000;55(12 Suppl 6):S60-6.

33. Wang L, Zhang Q, Li H, Zhang H. SPECT molecular imaging in Parkinson's disease. J Biomed Biotechnol. 2012;2012:412486.

34. Loane C, Politis M. Positron emission tomography neuroimaging in Parkinson's disease. Am J Transl Res. 2011;3(4):323–41.

35. Heim B, Krismer F, De Marzi R, Seppi K. Magnetic resonance imaging for the diagnosis of Parkinson's disease. J Neural Transm (Vienna). 2017;124(8):915–64. .

36. Jones DR, Moussaud S, McLean P. Targeting heat shock proteins to modulate α-synuclein toxicity. Ther Adv Neurol Disord. 2014;7(1):33–51

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The effect of different anesthesia applications on serum Pentraxin-3 levels: a randomized prospective study

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ABSTRACT

Objective: Pentraxin-3 (PTX3) is a biomarker shown to correlate with the severity of infections. It is a good indicator of mortality and is useful in monitoring treatment success. However, there is inadequate information about the factors affecting PTX3 levels. This study aimed to investigate the effects of different anaesthesia types on serum PTX3 levels.

Materials/Patients and Methods: Serum PTX3 levels were obtained from patients who were under general anaesthesia (GA) and spinal anaesthesia (SA) for a caesarean section (C-section). Blood Samples were collected preoperatively at 6 h and 24 h postoperatively. Biomarkers such as C-reactive protein, white blood cells, neutrophils and lymphocytes were also assessed as biomarkers.

Results: No difference was found in the preoperative serum PTX3 levels among the participants ($p > 0.05$). A significant increase was observed when the preoperative PTX3 levels (0.16 ng/mL) were compared with the postoperative levels at 6 h (0.25 ng/mL) and 24 h (0.54 ng/mL) in the GA group. No significant change was found in the PTX3 levels at 0–6–24 h measurements in the SA group. Nevertheless, the GA group was found to be significantly higher than the SA group at 6 h and 24 h postoperatively ($p < 0.05$). Additionally, No correlation was observed between PTX3 levels and other biomarkers.

Conclusions: This study showed that when coupled with C-section, GA increased the PTX3 levels postoperatively compared with the PTX3 levels during the preoperative period. No significant change was observed with SA. The PTX-3 levels should be considered to increase in association with GA in suspected infectious and inflammatory cases. Therefore, regional anaesthesia should be preferred.

Keywords: Biomarker, Pentraxin-3, General Anesthesia, Spinal Anesthesia

INTRODUCTION

The pentraxin (PTX) family, an acute-phase reactant, has a discoid structure consisting of cyclic pentamers that are highly stable proteins. It has subgroups of long and short PTXs. The standard acute-phase protein, C-reactive protein (CRP), was first defined in the 1930s, with the serum amyloid component (SAP) constituting the short PTXs and pentraxin-3 (PTX3) being the prototype of long PTXs (1). The main structural feature of PTX3 is the presence of an amino-terminal domain, which is 174 amino acids in length, unlike CRP and SAP.

There are differing features not only in the structure of the CRP but also in the source of its secretion. PTX3 is secreted by macrophages, dendritic cells, neutrophils, fibroblasts and vascular endothelial cells within an inflammatory zone, and CRP is secreted by hepatocytes (2). The source of production or secretion of PTX3 depends on the type of stimulant.

PTX3 levels increase in both acute and chronic diseases (3). Recent studies have reported that PTX3 is correlated with the severity of the disease (4, 5), is a good indicator of mortality (6, 7) and is useful in monitoring treatment success (4).

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Moreover, it is a better biomarker that provides earlier warning than CRP and is an indicator of organ dysfunction (8). These advantages observed in infectious cases suggest that PTX3 may become a more widely used marker in the future. However, to date, there is inadequate information about the factors affecting PTX3 levels.

The direct pharmacologic effects of agents used during anaesthesia in terms of the type, duration and depth of anaesthesia and their effects on stress response and the immune system have been studied previously (9–11). Therefore, it is possible that anaesthesia affects PTX3 levels. In this study, we aimed to investigate the effects of two types of anaesthesia applications, general anaesthesia (GA) and spinal anaesthesia (SA), on PTX3 levels.

MATERIALS and METHODS

Study design. The study was approved by the Institutional Ethics Committee of the Clinical Research of Canakkale Onsekiz Mart University (Protocol No: 2015-04, Date: February 18, 2015). The study was conducted in 2015-2016 at Canakkale Onsekiz Mart University Faculty of Medicine Hospital and Canakkale Public Hospital. All participants were pregnant at term and older than 18 years. The patients were excluded if they had an emergency operation, active infectious disease, malignancy, or preeclampsia. The exclusion criteria were age younger than 18 years, emergency surgery, active infection, steroid therapy and malignancy. Sixty patients were divided into two groups.

GA group (n = 30): Caesarean section (C-section) under the GA group. Following pre-oxygenation, 0.5 mg/kg of lidocaine, 2–3 mg/kg of propofol and 0.6 mg/kg of rocuronium bromide were intravenously administered to the patients during the induction of anaesthesia. After the umbilical cord was clamped, 1–2 mg/kg−1 of fentanyl was administered to the patient. Anaesthesia was maintained with 50% N2O and 0.5–0.7 minimal alveolar concentration (MAC) for sevoflurane. Inhalation anaesthesia was terminated at the end of the operation, and decurarization was performed.

SA group (n = 30): C-section under the SA group. Fluid infusion with a balanced crystalloid solution of 15 mL kg−1 was administered to the patients before anaesthesia to prevent maternal hypotension. A total of 10–12 mg of 0.5% hyperbaric bupivacaine + 25 μg fentanyl (total volume of 2.5 ± 0.3 mL) mixture was administered using a 25‐27 G spinal needle for SA. Surgery was performed when the sensory block reached T6‐T8. Intraoperatively, if the systolic arterial pressure decreased to ⩽ 90 mmHg, an intravenous (i.v.) bolus of 5 mg of ephedrine was administered until the systolic blood pressure increased to 100 mmHg. If the heart rate was ⩽ 55 beats min−1, 0.5 mg of i.v. atropine was administered. Age, weight, gravida, parity, gestational age, foetal gender, birth weight and 1–5 min Apgar values of all patients were recorded. Neonatal Apgar evaluation was performed by paediatricians.

Blood samples: For basal values, serum samples were obtained from patients who were about to deliver through C-section during preoperative examination. A second set of serum samples was obtained at 6 h postoperatively, and a third set of serum samples were obtained from all patients at 24 h following the delivery. White blood cell (WBC), neutrophil, lymphocyte, CRP and PTX3 values were measured from the blood samples.

Measurement of plasma PTX3: The samples for the analysis of PTX3 levels were stored at −80°C in small specimen containers. The samples were studied following the completion of serum collection in the biochemistry laboratory of the Canakkale Onsekiz Mart University Faculty of Medicine Hospital. Serum PTX3 levels were determined quantitatively using an enzyme-linked immunosorbent assay (ELISA) using an ELISA microplate strip washer (ELX50; BioTek Instruments, Vinooski, VT, USA) and an ELISA microplate reader (ELX808; BioTek Instruments, USA). PTX3 was analysed using a commercially available kit from MyBioSource Diagnostics (MyBioSource, Inc., San Diego, CA, USA). The minimum detectable dose of PTX3 was up to 0.06 ng/mL. The intra- and inter-assay coefficients of variation for PTX3 were 8% and 12%, respectively. Correlation between laboratory tests results. The correlation of PTX3 with other inflammation indicators, such as WBC, neutrophil, lymphocyte and CRP values, was evaluated for each group.

Statistical analysis: The obtained data were compared using IBM SPSS Statistics 19 software owned by Canakkale Onsekiz Mart University. The variables were investigated using visual (histogram and probability plots) and analytical methods (Kolmogorov–Smirnov test) to determine whether they were normally distributed. Descriptive analyses were presented using the means and standard deviations for normal variables. As the WBC, neutrophil, lymphocyte, CRP and PTX3 values and the patient data were normally distributed; Student's t-test was used to compare these parameters between the SA and GA groups. A t-test was used for the intergroup statistical comparison of the PTX3 levels. The correlation of PTX3 levels with the WBC, neutrophil, lymphocyte and CRP values was evaluated using the Spearman correlation test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Patients' characteristics: A significant intergroup difference was not observed in the data related to pregnancy, such as the demographic features of patients, mother's weight, gravida, parity, gestational age, and information related to the baby, such as gender, birth weight and Apgar score. The data are summarised in **Table 1**. The duration of surgery was approximately 50 min for the GA group and 45 min for the SA group. Hypotension was observed in two patients in the SA group and was treated with 5–10 mg of i.v. ephedrine.

Routine laboratory analyses: The mean CRP levels were higher (normal range, 5 mg/L) than those of non-pregnant healthy adults in all groups. Moreover, the CRP levels were higher in consecutive measurements. In the intragroup comparison of CRP levels between the SA and GA groups, all differences were statistically significant ($p < 0.05$). In the intergroup comparison, significant differences were found in the CRP levels between the SA and GA groups at 6 h and 24 h postoperatively. Significant intergroup differences were not found in the WBC, neutrophil, or lymphocyte values. The results are summarised in **Table 2**.

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Table 1. Demographic characteristics of C-section patients under GA or SA, data related to pregnancy and information related to the baby after birth.

	Spinal Anesthesia (n=30)	General Anesthesia (n=30)	p value
Age (years)	27.3 ± 6.2	28.2 ± 7.1	0.84
Maternal weight (kg)	80.6 ± 9.1	73 ± 8,7	0.35
Gravida	2.3 ± 2.1	2.2 ± 1.9	0.70
Parity	1.2 ± 2.0	1.0 ± 0.9	0.14
Delivery (weeks)	38.3 ± 2.6	36.6 ± 3.3	0.17
Male (baby), n (%)	17 (57)	21 (70)	
Birth weight (g)	3475 ± 960	3664 ± 745	0.08
APGAR score 1. min	6.9 ± 1.5	6.2 ± 1.2	0.272
APGAR score 5. min	9.0 ± 1.0	8.5 ± 1.2	0.368

Table 2. Intragroup and intergroup statistical comparison of the WBC, neutrophil, lymphocyte, CRP and PTX3 results.

Parameters		Spinal Anesthesia	General Anesthesia	*p* value
WBC count (x10^3/mL)	*T1 T2 T3*	7.93 ± 1.73 7.43 ± 2.67 8.34 ± 2.64	7.61 ± 1.68 8.16 ± 2.83 8.20 ± 2.60	0.07 0.35 0.26
Neutrophil (x10^3/mL)	*T1 T2 T3*	4.65 ± 1.47 5.17 ± 1.96 5.23 ± 1.91	4.51 ± 1.42 5.3 ± 1.83 4.61 ± 1.37	0.18 0.62 0.51
Lymphocyte (x10^3/mL)	*T1 T2 T3*	2.09 ± 0.89 1.82 ± 1.53 2.23 ± 0.92	2.07 ± 0.83 2.41 ± 1.08 2.28 ± 0.84	0.81 0.16 0.35
CRP (mg/dL)	*T1 T2 T3*	6.5 ± 1.5 9.5 ± 1.7 # 31.6 ± 9.8 # ¥	5.9 ± 1.6 13.1 ± 2.7 # 19.2 ± 5.5 # ¥	0.10 *0.00** *0.00**
Pentraxin-3 (ng/mL)	*T1 T2 T3*	0.13 ± 0.06 0.12 ± 0.04 0.15 ± 0.04 ¥	0.16± 0.06 0.25 ± 0.08 # 0.54 ± 0.09 # ¥	0.83

WBC, white blood cell; CRP, C-reactive protein; T1, before anesthesia; T2, 6 hour after anesthesia; T3, 24 hour after anesthesia.
*: p<0.05 in the intergroup comparison
#: p<0.05 compared to preoperative values in the intragroup comparison
¥: p<0.05 compared to 6th hour after operation values in the intragroup comparison

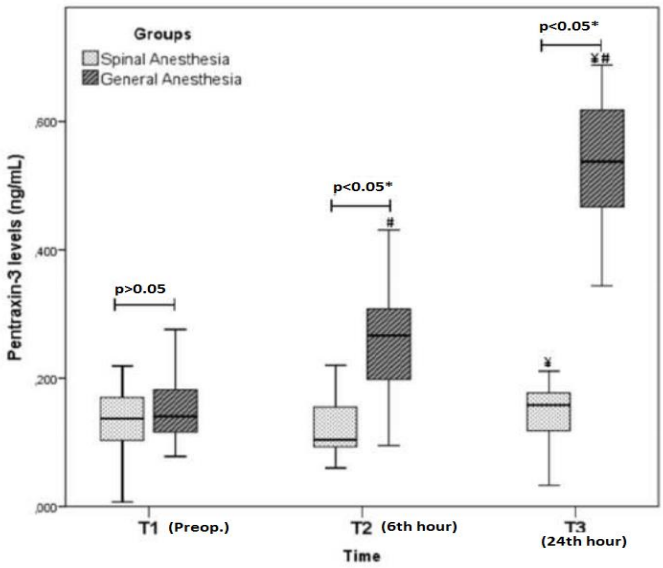

Figure 1. The results of intragroup and intergroup statistical comparison of pentraxin- 3 levels of both groups obtained at the preoperative and postoperative 6 and 24 hour.
*: p<0.05 in the intergroup comparison
#: p<0.05 compared to preoperative values in the intragroup comparison
¥: p<0.05 compared to 6 hour after operation values in the intragroup comparison

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Table 3. Correlation between the PTX3 levels and the WBC, neutrophil, lymphocyte and CRP values.

Parameters		Spinal Anesthesia	General Anesthesia
Pentraxin-3 - WBC	*T1*	0.68	0.87
	T2	0.95	0.85
	T3	0.27	0.61
Pentraxin-3 - Neutrophil	*T1*	0.16	0.93
	T2	0.77	0.87
	T3	0.41	0.30
Pentraxin-3 - Lymphocyte	*T1*	0.17	0.52
	T2	0.53	0.45
	T3	0.93	0.05
Pentraxin-3 - CRP	*T1*	*0.01**	*0.03**
	T2	*0.02**	*0.04**
	T3	0.61	0.09

WBC, white blood cell; CRP, C-reactive protein; T1, before anesthesia; T2, 6 hour after anesthesia; T3, 24 hour after anesthesia; *:p<0.05.

Serum PTX3 levels: The PTX3 values preoperatively were comparable between the two groups ($p > 0.05$). In the intragroup comparison, the decrease in the SA group was not statistically significant ($p > 0.05$), but the increase in the GA group was statistically significant ($p < 0.05$) at 6 h postoperatively compared with the preoperative values.

The PTX3 levels were significantly higher at 24 h postoperatively than at 6 h postoperatively in all groups ($p < 0.05$). The PTX3 levels increased significantly in the GA group, but the increase in the SA group was not statistically significant 24 h postoperatively compared with the preoperative values (**Table 2**). In the intergroup comparison, GA was significantly higher than SA at both 6 h and 24 h postoperatively ($p < 0.05$, **Figure 1**).

Correlation between laboratory test results: The correlation of the calculated differences between the effects of pre- and post-anaesthesia on the PTX3 levels with other inflammatory markers is shown in **Table 3**.

The intragroup changes in the PTX3 levels in both the SA and GA groups did not correlate with the change in the WBC, neutrophil, lymphocyte and CRP measurements ($p > 0.05$, **Table 3**).

DISCUSSION

In this study, PTX3, which is considered a new acute-phase reactant, was observed to be significantly elevated in patients undergoing a C-section through GA during the postoperative period compared with the preoperative period.

PTX3 is a good indicator of the inflammatory response. The setting of an infection has been shown to correlate with the severity of disease (**12**). In addition, it correlates with the success of treatment after antibiotic therapy (**13, 14**). In a 2012 study, Bastrup et al. (**12**) showed that PTX3 levels in patients diagnosed with systemic inflammatory response syndrome (SIRS) correlated with the severity of SIRS, sepsis, severe sepsis, septic shock and mortality. In 2013, Kleber et al. (**15**) found that PTX3 acted as a secondary acute-phase reactant in patients exposed to polytrauma, with the levels significantly increased in a period of 24 h.

These studies emphasise the clinical importance of PTX3 levels in both infectious diseases and trauma. In our study, the PTX3 levels increased more in the GA group than in the SA group. It is advisable to use regional anaesthesia for suspected infectious cases that will undergo surgical procedures. However, data about other factors affecting PTX3 levels are inadequate.

Anaesthesia implementations affect the immune system (**9**). However, to the best of our knowledge, to date, no clinical studies have been conducted on the effects of anaesthesia on PTX3 levels.

In this study, we examined two patient groups giving birth through SA and GA. The PTX3 levels increased significantly in the postoperative period compared with the preoperative period in the GA group. Conversely, the PTX3 levels did not significantly increase in the postoperative period compared with the preoperative period in the SA group.

This effect of GA on the levels of an inflammatory marker (PTX3) may be related to several factors. Research on the effects of volatile anaesthetics on the immune system has generally consisted of animal studies (**16–18**). Volatile anaesthetics in vitro exhibited a dose-dependent inhibitory effect on neutrophil function, reduced the release of cytokines from mononuclear cells, reduced the cell proliferation of lymphocytes and induced apoptosis in lymphocytes (**19–21**). However, measuring the effects of anaesthesia and surgery alone on the immune system in humans is difficult. Many factors, such as medical history and demographic characteristics of the patient, type of surgery, tissue properties and duration of operation, may affect the immune response. Different anaesthetic agents created different effects on the immune system in a study attempting to minimise the effect of surgery in humans (**22**). The effects of volatile anaesthetics on lymphocyte function have been reported to be highly variable. They also inhibit neutrophil chemotaxis and microbicidal oxidative functions in humans (**16, 23**). The effects of propofol have been observed to be generally protective. Mitsuhata et al. demonstrated that sevoflurane application led to the release of cytokines, such as interleukin (IL)-1β and tumour necrosis factor-α by Natural Killer (NK) and NK-like cells (**24**). Nitric oxide administration was

associated with depression in neutrophil function and decreased production of mononuclear cells (28). Our study suggests that the PTX3 increase in group GA may be related to the use of sevoflurane and nitrous oxide as inhalation anaesthesia. Kitamura et al. (25) found that sevoflurane caused lymphopenia in human studies comparing the use of sevoflurane and propofol, whereas propofol was protective against lymphopenia. Schneemilch et al. (16) showed a significant increase in IL-6 levels during and after inhalation anaesthesia.

Their study compared the effects of total i.v. anaesthesia (propofol and sufentanil) with those of inhalational anaesthesia (sevoflurane, fentanyl and N_2O) on the immune system. We used the induction of anaesthesia with propofol and the maintenance of anaesthesia with sevoflurane in the GA group. The increase observed in PTX3 levels, which is consistent with the literature, is likely to be related to sevoflurane. Moreover, we used fentanyl as an analgesic in the GA group. Fentanyl generally increases the number of NK cells but does not contribute to cell activity, which may have affected the PTX3 increase (22).

In our study, to reduce external factors, we included C-section cases with similar durations of surgery, those that avoided the use of drugs because of pregnancy, younger age groups and those that had no additional diseases. Pregnant women who had preeclampsia with high PTX3 levels due to endothelial dysfunction were also excluded from the study (26).

Compared with GA, regional anaesthesia was shown to have a minimal effect on patients' immune system in two recent studies (27, 28). However, there is insufficient information about the effects of regional anaesthesia on PTX3 levels. To the best of our knowledge, our study is the first to investigate PTX3 levels and regional anaesthesia. Again, there is inadequate information on the effect of surgical procedures on PTX3 levels. Nevertheless, no significant difference in PTX3 levels was observed between treated and untreated patients diagnosed with SIRS and sepsis in Bastrup et al.'s study (12). In our study, the insignificant increase in PTX3 levels in the SA group suggests that both SA and surgery slightly affect PTX3 levels. The factors that reduce stress in patients, such as being awake during SA, hearing the sound of a baby and watching the baby, should also not be disregarded.

CONCLUSIONS

During a C-section, patients who were investigated for the effects of GA and regional anaesthesia on PTX3 levels showed the following results:

1. PTX3 levels in the postoperative period compared with the preoperative period significantly increased in patients undergoing a C-section under GA.

2. Changes in PTX3 levels were not observed in the postoperative period compared with the preoperative period in patients undergoing a C-section under SA.

3. The levels of PTX3, which is accepted as an acute-phase reactant, are likely to be elevated in association with GA in the setting of suspected infectious and inflammatory cases. Therefore, regional anaesthesia should be preferred.

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A–research concept and design;
B–collection and/or assembly of data;
C–data analysis and interpretation;
D–writing the article;
E–critical revision of the article;
F–final approval of article;.

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REFERENCES

1. Garlanda C, Bottazzi B, Salvatori G, De Santis R, Cotena A, Deban L. Pentraxins in innate immunity and inflammation. Novartis Found Symp 2006; 79: 80-86.

2. Doni A, Mantovani G, Porta C, Tuckermann J, Reichardt HM, Kleiman A. Cell-specific regulation of PTX3 by glucocorticoid hormones in hematopoietic and nonhematopoietic cells. J Biol Chem 2008; 283:29983-92.

3. Bottazzi B, Inforzato A, Messa M, Barbagallo M, Magrini E, Garlanda C. The pentraxins PTX3 and SAP in innate immunity, regulation of inflammation and tissue remodelling. J Hepatol 2016; 64(6): 1416-27.

4. Aksungur N, Ozogul B, Ozturk N, Arslan S, Karadeniz E, Korkut E. Prognostic importance of pentraxin 3 levels in acute cholesistitis. Ulus Travma Acil Cerrahi Derg 2015; 21: 380-384.

5. Locatelli M, Ferrero S, Martinelli Boneschi F, Boiocchi L, Zavanone M, Maria Gaini S. The long pentraxin PTX3 as a correlate of cancer-related inflammation and prognosis of malignancy in gliomas. J Neuroimmunol 2013; 260: 99-106.

6. Mauri T, Bellani G, Patroniti N, Coppadoro A, Peri G, Cuccovillo I. Persisting high levels of plasma pentraxin 3 over the first days after severe sepsis and septic shock onset are associated with mortality. Intensive Care Med 2010; 36: 621-29.

7. Usitalo-Seppala R, Huttunen R, Aittoniemi J, Koskinen P, Leino A, Vahlberg T. Pentraxin 3 (PTX3) is associated with severe sepsis and fatal disease in emergency room patients with suspected infection: a prospective cohort study. PLoS One 2013; 8(1): e53661.

8. Liu S, Qu X, Liu F, Wang C. Pentraxin 3 as a prognostic biomarker in patients with systemic inflammation or infection. Mediators Inflamm 2014; 421-29.

9. MStevenson GW, Hall SC, Rudnick S, Seleny FL, Stevenson HC. The effect of anesthetic agents on the human immune response. Anesthesiology 1990; 72: 542-52.

10. López-Andrade Jurado A, Almazán Duro A, Martín Ruiz JL, Samaniego Muñoz F, López-Andrade Jurado MA, del Campo Iglesias A. Immune response in the surgical patient: effect of anesthesia and blood transfusion. Rev Esp Anestesiol Reanim 2000; 47: 67-80.

11. Kilic R, Yasar MA, Avci L, Demirel I, Yasar D. The Effects of Using Epidural Anesthesia with General Anesthesia on Plasma Levels of Cytokins and Cortizol in Patients with Lower Abdominal Surgery. Firat Med J 2005; 10: 59-63

12. Bastrup-Birk S, Skjoedt MO, Munthe-Fog L, Strom JJ, Ma YJ, Garred P. Pentraxin-3 serum levels are associated with disease severity and mortality in patients with systemic inflammatory response syndrome. PLoS One 2013; 9:8(9):e73119.

13. Huttunen R, Hurme M, Aittoniemi J, Huhtala H, Vuento R, Laine J. High plasma level of long pentraxin 3 (PTX3) is associated with fatal disease in bacteremic patients: a prospective cohort study. PLoS One 2011; 10;6(3):e17653.

14. de Kruif MD, Limper M, Sierhuis K, Wagenaar JF, Spek CA, Garlanda C. PTX3 predicts severe disease in febrile patients at the emergency department. J Infect 2010; 60: 122-27.

15. Kleber C, Becker CA, Schmidt-Bleek K, Schaser KD, Haas NP. Are pentraxin 3 and transsignaling early markers for immunologic injury severity in polytrauma? A pilot study. Clin Orthop Relat Res 2013; 471: 2822-30.

16. Hamra JG, Yaksh TL. Halothane inhibits T cell proliferation and interleukin-2 receptor expression in rats. Immunopharmacol Immunotoxicol 1996; 18: 323-36.

17. Lewis RE Jr, Cruse JM, Hazelwood J. Halothane-induced suppression of cell-mediated immunity in normal and tumor-bearing C3Hf/He mice. Anesth Analg 1980; 59: 666-671.

18. Markovic SN, Knight PR, Murasko DM. Inhibition of interferon stimulation of natural killer cell activity in mice anesthetized with halothane or isoflurane. Anesthesiology 1993; 78: 700–06.

19. Homburger JA, Meiler SE. Anesthesia drugs, immunity, and long-term outcome. Curr Opin Anaesthesiol 2006; 19(4):423-28.

20. Matsuoka H, Kurosawa S, Horinouchi T, Kato M, Hashimoto Y. Inhalation anesthetics induce apoptosis in normal peripheral lymphocytes in vitro. Anesthesiology 2001; 95:1467-72.

21. Brand JM, Kirchner H, Poppe C, Schmucker P. The effects of general anesthesia on human peripheral immune cell distribution and cytokine production. Clin Immunol Immunopathol 1997; 83: 190-94.

22. Schneemilch CE, Ittenson A, Ansorge S, Hachenberg T, Bank U. Effect of 2 anesthetic techniques on the postoperative proinflammatory and anti-inflammatory cytokine response and cellular immune function to minor surgery. J Clin Anesth 2005; 17: 517-27.

23. Lippa S, De Sole P, Meucci E, Littarru GP, De Francisci G, Magalini SI. Effect of general anesthetics on human granulocyte chemiluminescence. Experientia 1983; 39: 1386-88.

24. Mitsuhata H, Shimizu R, Yokoyama MM. Suppressive effects of volatile anesthetics on cytokine release in human peripheral blood mononuclear cells. Int J Immunopharmacol 1995; 17(6): 529-34.

25. Kitamura T, Ohno N, Bougaki M, Ogawa M, Yamada Y. Comparison of the effects of sevoflurane and propofol on changes in leukocyte-count induced by surgical stress. Masui 2008; 57(8):968-72.

26. Cetin I, Cozzi V, Pasqualini F, Nebuloni M, Garlanda C, Vago L. Elevated maternal levels of the long pentraxin 3 (PTX3) in preeclampsia and intrauterine growth restriction. Am J Obstet Gynecol 2006; 194(5), 1347-53.

27. Kun L, Tang L, Wang J, Yang H, Ren J. Effect of Combined General/Epidural Anesthesia on Postoperative NK Cell Activity and Cytokine Response in Gastric Cancer Patients Undergoing Radical Resection. Hepatogastroenterology 2014; 61(132):1142-47.

28. Kaye AD, Patel N, Bueno FR, Hymel B, Vadivelu N, Kodumudi G. Effect of opiates, anesthetic techniques, and other perioperative factors on surgical cancer patients. Ochsner J 2014; 14(2): 216-28.

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Comparative results of hernia repair from surgeons with different approaches at a tertiary medical center

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ABSTRACT

Objective: At present, there is still no method that can be called the gold standard in hernia repair. The main objective of this study was to review the rates, including pain and recurrence, of the most important complications for patient dissatisfaction across different methods applied by different surgeons.

Material and Methods: Four hundred twenty one patients who were operated on by three surgeons were retrospectively reviewed with respect to the main complications of pain and recurrence. Self-adhesive mesh and Lichtenstein repairs were performed by the same surgeon, whereas Bassini and transabdominal preperitoneal (TAPP) repairs were performed by separate surgeons.

Results: In all repair types, there were a significant difference between visual analogue scale (VAS) pain scores on the first postoperative day compared to VAS scores at the one-month mark ($p<0.001$). The difference between VAS scores in the first month according to repair types was statistically significant ($p<0.001$). There was a significant difference between repair types and development of chronic pain ($p<0.001$). Recurrence rates also showed a statistically significant difference amongst repair types ($p=0.001$).

Conclusion: Although the Lichtenstein and laparoscopic methods are superior in terms of recurrence compared to the Bassini method, chronic pain complications from the Bassini method appear to be acceptable

Keywords: Postherniorаphy pain, Self-adhesive mesh, Bassini repair, Standard mesh, Postherniorаphy recurrence

INTRODUCTION

Choosing the optimal approach for herniorrhaphy is still a debatable subject. The preferred methods for herniorrhaphy are tension-free techniques because they have low recurrence and complication rates (1). Four different methods were conducted by three surgeons from different generations were compared for complication rates, including pain and recurrence. Opposed to recurrences, the prevalence of chronic postoperative groin pain (CPGI), also described as ongoing pain three months after surgery, is still a very important matter. CPGI rates range from 15% to 53%. Surgical approaches that prevent chronic herniorrhaphy pain are still discussed in detail, and avoiding CPGI has become a crucial point of interest in surgical studies that deal with inguinal hernia repair (2,3).

The main parameter for assessment in this study was pain. Pain is a personal experience, so it is, therefore, difficult to characterise it clearly. Pain has been described by the International Association for the Study of Pain as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain has also been described as discomfort that is unrelated to postoperative infection or previously present causes apart from surgery (4). Different studies have reported the prevalence of continuing pain following hernia repair to be between 10% and 30% based on established criteria (5).

This study also reviewed complications other than pain, including recurrence; wound infection, and seroma or haematoma formation, according to the different techniques used by the three surgeons. The main objective was to present the results and differences between the three generations.

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MATERIALS and METHODS

Four hundred twenty one patients who were operated on by three surgeons from different eras at the Yenimahalle research and training hospital located in the city of Ankara Turkey between 2016 and 2018 were retrospectively reviewed. All the hernia repair techniques mentioned in the study were performed using standard methods under either general or spinal anaesthesia, according to their type. Self-adhesive mesh and Lichtenstein repairs were performed by the same surgeon, whereas Bassini and transabdominal preperitoneal (TAPP) repairs were performed by separate surgeons. The following were obtained from each patient's record: age, gender, hernia side, hernia type, repair type, postoperative day one and one-month VAS scores, presence of chronic pain at the third month follow-up, wound infection, seroma, haematoma, and recurrence rates. Pain resistance to treatment was another parameter considered in this study. Pain levels on the first day of surgery were measured in the hospital, whereas pain levels at the first and third months were either assessed over the phone or during follow-up visits. Patients were asked to "scale" their "present" pain from 0 to 10 using the VAS. During follow-up visits, pain presence, severity using the VAS (if present), characteristics (including burning, stinging, and shooting) , and localisation were reviewed. Pain seen in the first 24 hours was deemed acute postoperative pain, whereas pain with a VAS score $\geqslant$3 three months after surgery was deemed chronic pain. All patients were called for a follow-up examination by their surgeon on the seventh day after surgery to check for seromas, haematomas, and wound infections.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows 21.0 IBM SPSS Statistics. Parameters without a normal distribution and comparison of VAS scores with ordinal distribution according to repair type were assessed using the Kruskal-Wallis test. Statistically significant results were compared using the Mann-Whitney U test and evaluated using Bonferroni correction. For comparison of qualitative results related with repair types, the chi-square test or Fisher's exact test were used to assess the differences between the results. The Wilcoxon test was used to assess the statistical significance level of the differences within groups in each repair type for the first postoperative day and first month VAS scores. The confidence interval was set at 95% with a level of statistical significance of $p<0.05$.

RESULTS

There were a total of 421 patients in this study. The surgeon who graduated before 1990 (considered the old generation) and still preferred the Bassini technique operated on 114 patients. The second surgeon who graduated in the 2000s (considered the middle generation) operated on 115 patients using regular mesh and 144 patients using self-adhesive mesh techniques.

The third surgeon who graduated after 2010 (considered the new generation) operated on 48 patients with the TAPP technique. The median age was 46 years old, with the youngest patient being 19 years old and the oldest being 74 years old. Three hundred eighty-six patients were male, while the remaining 35 were female. Two hundred thirty-five patients had right-sided hernias, 158 had left-sided hernias, and 28 had bilateral hernias. Eighty-three patients had scrotal hernias. The median hospitalisation period was one day, except for one TAPP patient who was followed up for seven days as hematoma developed due to bleeding during surgery. **Table 1** shows the descriptive statistics of the patients according to their repair types.

VAS scores in total and according to repair type on the first day and first month of surgery are presented in **Table 2** and **Table 3**.

Twenty-six out of 421 developed treatment-resistant pain. There was a significant difference in developing treatment-resistant pain according to repair type ($p=0.008$). Paired group comparisons revealed a significant difference between standard mesh and self-adhesive mesh repair ($p=0.006$).

In all repair types, there was a significant difference between postoperative day one and one-month VAS scores ($p<0.001$). One-month VAS scores were significantly lower for all repair types.

There was also a significant difference in postoperative day one VAS scores according to repair type (**Table 2**). Paired comparisons for repair types showed significant differences (except for the Bassini-standard mesh repair comparison, $p=0.014$).

The difference between one-month VAS scores according to repair types was found to be statistically significant ($p<0.001$) (**Table 3**). Paired comparisons for repair types revealed significant differences between the Bassini and self-adhesive mesh techniques ($p=0.001$), Bassini and TAPP techniques ($p=0.001$), and regular repair and TAPP repair ($p<0.001$).

Thirty-four out of 421 patients developed chronic pain. There was a significant difference between repair types and the development of chronic pain ($p<0.001$). Paired comparisons showed a significant difference between the Bassini and standard repair techniques ($p=0.008$), standard repair and TAPP repair ($p=0.008$), and the standard repair and self-adhesive mesh repair ($p<0.001$).

Recurrence rates also showed a significant difference amongst repair types ($p=0.001$). Paired comparisons revealed a significant difference between Bassini and self-adhesive mesh repair ($p=0.002$) and Bassini and standard repair ($p=0.001$). No statistically significant difference was found between repair types in terms of postoperative complications ($p=0.45$).

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Table 1: General Features

	Bassini Repair n= 114	Self-Gripping Mesh (PROGRIP™) Repair n=144	Lichtenstein Repair n= 115	Laparascopic TAPP Repair n=48
Age (Range)- year	46 (19-74)	47 (22-74)	47(23-72)	49(25-68)
Gender (M/F)				
Male (386/100%)	106(27.5%)	135(35%)	100(25.9%)	45(11.7%)
Female (35/100%)	8(22.9%)	9(25.7%)	15(42.9%)	3(8.6%)
Total (421/100%)	114(27.1%)	144(34.2%)	115(27.3%)	48(11.4%)
Hospitalization time (day)	1 (1-3)	1 (1-2)	1 (1-2)	1 (1-7)
Hernia Type				
Direct hernia	54(47.4%)	54(37.5%)	59(51.3%)	3(6.3%)
Indirect hernia	54(47.4%)	86(59.7%)	50(43.5%)	18(37.5%)
Bilateral hernia	5(4.4%)	2(1.4%9)	2(1.7%)	12(25%)
Femoral hernia	1(0.9%)	2(1.4%)	4(3.5%)	1(2.1%)
Recurrent hernia	0	0	0	14(29.2%)
Hernia Side				
Right hernia	70(61.4%)	76(52.8%)	77(67.0%)	12(25.0%)
Left hernia	44(38.6%)	68(47.2%)	38(33.0%)	8(16.7%)
Bilateral hernia	0	0	0	28(58.3%)
Recurrents				
Yes	15(13.2%)	4(2.8%)	2(1.7%)	4(8.3%)
No	99(86.8%)	140(97.2%)	113(98.3%)	44(91.7%)
Treatment Resistant Pain				
Yes	9 (7.9%)	4(2.8%)	13 (11.3%)	0
No	105 (92.1%)	140(97.2%)	102(88.7%)	48(100%)
Complications				
Hematoma	11(9.6%)	4(2.8%)	9(7.8%)	1(2.1%)
Seroma	11(9.6%)	24(16.7%)	19(16.5%)	11(22.9%)
Wound İnfection	8(7.0%)	0	6(22.9%)	0

Table 2: Postoperative 1. day VAS

Hernia Repair type	VAS (Visual Analog Score)						
	VAS2	VAS3	VAS4	VAS5	VAS6	VAS7	VAS8
Bassini Repair	0	2(2.1%)	6(6.3%)	41(43.6%)	53(58.9%)	11(45.8%)	1(33.3%)
Self-Gripping Mesh (PROGRIP™) Repair	14(77.8%)	77(80.2%)	50(52.1%)	2(2.1%)	1(1.1%)	0	0
Lichtenstein Repair	0	3(3.1%)	22(22.9%)	42(44.7%)	34(37.8%)	13(54.2%)	1(33.3%)
Laparascop ic TAPP Repair	4(22.2%)	14(14.6%)	18(18.8%)	9(9.6%)	2(2.2%)	0	1(33.3%)
Total	18(100%)	96(100%)	96(100%)	94(100%)	90(100%)	24(100%)	3(100%)

Table 3: Postoperative 1. month VAS

Hernia Repair Type	VAS (Visual Analog Score)					
	VAS0	VAS1	VAS2	VAS3	VAS4	VAS5
Bassini Repair	7(63.6%)	13(18.6%)	36(19.5%)	28(26.2%)	21(53.8%)	9(100%)
Self-Gripping Mesh (PROGRIP™) Repair	1(9.1%)	19(27.1%)	85(45.9%)	35(32.7%)	4(10.3%)	0
Lichtenstein Repair	3(27.3%)	16(22.9%)	47(25.4%)	36(33.6%)	13(33.3%)	0
Laparascopic TAPP Repair	0	22(31.4%)	17(9.2%)	8(7.5%)	1(2.6%)	0

DISCUSSION

This study is one of the rare studies comparing the methods of 3 different generations of surgeons. This study compares the older bassini hernia repair with self-adhesive mesh repair, standard mesh repair, and, currently, the more commonly used laparoscopic hernia repair. Although there are studies that compare the two previous methods, we have a surgeon in our clinic from the older generation who still uses the Bassini technique, even though newer generations of surgeons don't use it, so we were able to compare four different methods and their outcomes.

The main objective parameters were pain and recurrence. We evaluated long-term comparative results for pain as recommended by the European Hernia Society (**6**), and found a treatment- resistant pain rate of 6 %. Most patients localised their pain within the pubic tubercle. Postoperative inguinal pain is one of the most important complications seen in hernia repair surgery today (**7**). The main reason for groin pain is either injury or irritation to the genitofemoral, ilioinguinal, or iliohypogastric nerves which innervate the structures within the inguinal channel (**8**).

Post-herniorrhaphy pain can be acute or chronic. Acute pain is frequent in almost all hernia surgeries and changes from light to moderate severity during rest and movement (9). Chronic postoperative treatment-resistant pain rates have been reported as 3% and 10–12% (10). Current evidence suggests the aetiology of CPGI may be perioperative nerve damage, postoperative fibrosis, or mesh-related fibrosis.

Self-adhesive materials were manufactured to prevent the damage caused by invasive equipment, such as sutures and staples (3). We think the main cause of postoperative chronic pain is nerve damage which can happen during dissection of the hernia sac from the cord elements in the Bassini technique and during mesh fixation in standard or self-adhesive mesh repairs. However, as standard mesh repairs require more sutures, nerve damage can be more frequent in those techniques. Likewise, this study found a significant difference between standard and self-adhesive mesh repairs in terms of developing treatment-resistant pain. As self-adhesive mesh repairs require fewer sutures and have a lower risk of pain due to nerve damage, self-adhesive mesh repairs had a lower complication rate. Chronic pain results were also similar.

Self-adhesive mesh, like other materials with self-fixation and semi-absorption properties, is minimally invasive towards abdominal tissues and has satisfactory results in both open and laparoscopic repairs (1, 11). This is consistent with our study results and explains the lower complication rates.

Chronic post-herniorrhaphy pain can have a major impact on both quality of life and medical costs. No treatment has shown effective results for this condition, so it is essential to make a detailed analysis of potential risk factors and outcomes of different surgical techniques for prevention (12, 13).

The Bassini technique for herniorrhaphy was widely used in some Europe countries, including at Maastricht University Medical centre in the Netherlands as the standard procedure during the 1990s (14). Although this technique is rarely used today, we have a surgeon in our clinic who graduated in the 1990s and still uses it. We reviewed the results of his surgeries and compared the results with the techniques used by middle- and new-generation surgeons. The comparison revealed no significant difference between the Bassini technique and standard mesh repair and the Bassini technique and self-adhesive mesh repair terms of developing treatment-resistant pain. A study by Bay-Nielsen et al. of 2612 patients reported no significant difference in pain between standard mesh repair and tensioned repair without mesh (13). Modern guidelines do not recommend using larger volume mesh in herniorrhaphy due to risks of erosion and chronic pain (15). This can be considered an advantage of the Bassini technique over mesh repair in terms of chronic pain development risk, which was observed in the current study.

The VAS was used for pain severity assessment after one month. When paired comparisons were made, there were significant differences in all of the following: Bassini repair vs TAPP repair, Bassini repair vs self-adhesive mesh repair, self-adhesive mesh repair vs standard mesh repair, self-adhesive repair vs TAPP repair, and standard mesh repair vs TAPP repair. The only comparison with no significant difference was standard mesh repair vs Bassini repair. Pain and analgesic use were reportedly lower during the early postoperative period in laparoscopic repairs compared to Bassini repair (14, 18). Although most mesh supporters argue that tension-free repair causes less pain during the acute postoperative period, there is no clear evidence that supports this claim (19). In accordance with our study results, a study from 2016 reported less postoperative pain with ProGrip mesh repair than with standard mesh repair (18). However, laparoscopy requires the patient to be under general anaesthesia. Most conventional hernia repair methods can be performed under local or regional (epidural or spinal) anaesthesia (16). We believe the main reason for the increased VAS pain scores on the first day of surgery is its Bassini repair characteristic. The surgeon who chooses Bassini repair has no laparoscopic experience, and even though he can use standard mesh techniques, he prefers the Bassini method. It is highly likely that he is very well-versed in this technique and can carry it out with ease. Since there is no gold standard for herniorrhaphy, it is reasonable to use this technique. The VAS was used to assess pain severity at the end of the first month. The results showed that pain subsided over time in all groups, regardless of the technique used, which is consistent with the literature (7, 19). There was no significant difference in first-month VAS scores for Bassini repair vs standard mesh and self-adhesive repair vs standard mesh repair. Similarly, a study by Bay-Nielsen et al. reported no significant difference in first month results between repairs with or without mesh (13). Another study found no significant difference in VAS scores between Bassini and laparoscopic repairs six weeks after surgery (14).

The gold standard for hernia repair cannot be determined until a technique that prevents recurrence is clearly defined. In this study, the recurrence rates were 13% for Bassini repair, 8% for TAPP surgery, 3% for Self-adhesive mesh repair, and 2% for standard mesh repair. The reason why these recurrence rates are relatively higher when compared to the literature is that we followed up our patients for complications for more than two years. The literature reports that a three-year follow-up period is required to make a clear assessment of recurrences (13). There was a significant difference between the four groups when recurrence rates were compared. Paired comparisons between the groups found a significant difference for Bassini repair vs self-adhesive mesh repair and standard mesh repair in terms of recurrence rates. Standard mesh repair is more common than Bassini repair because of its lower recurrence rate. Self-adhesive mesh causes less fibrosis formation, so it may potentially cause higher recurrence rates. However, prospective and meta-analysis studies comparing ProGrip with conventional mesh techniques cannot confirm different recurrence rates (20-21). In our study, we were also unable to find a statistically significant difference for ProGrip vs standard mesh repair, with the recurrence rate being slightly higher with the ProGrip technique, which is consistent with the literature. We reported a recurrence rate of 13% for the Bassini technique, which is similar to the literature (22).

Another curious finding in our study is that, there was no statistically significant difference in recurrence rates between Bassini and laparoscopic repairs. These results might be explained by the fact that the laparoscopic surgeon had just finished his surgical training. No matter which technique is used, our study once again demonstrated the importance of experience in hernia repair.

CONCLUSIONS

Laparoscopic repair requires an experienced surgeon in order to reduce herniorrhaphy-related complications, which include pain and recurrence. However, other hernia repair techniques are still used in clinical practice today, and there is no clear gold standard for herniorrhaphy types. The combined approach of the Bassini and Lichtenstein techniques shows promising results in terms of reducing recurrences (**23**). Our study results that compare four techniques used by three surgeons from different eras could be beneficial in choosing a hernia repair type for reducing pain and recurrence complications.

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee. This study was approved by Yüksek İhtisas University Ethics Committee (dated 21.08.2020 and numbered 2020/08/03)

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REFERENCES

1. Nikkolo C, Vaasna T, Murruste M, Suumann J, Kirsimägi Ü, Seepter H, Tein A, et al. Three-year results of a randomized study comparing self-gripping mesh with sutured mesh in open inguinal hernia repair. J Surg Res. 2017 Mar;209:139-144.
2. Khalid W, Ahmed HH, Jamaluddin M. Amyand's Hernia - A Rare Intraoperative Finding in Inguinal Hernias. J Coll Physicians Surg Pak. 2020 Feb;30(2):226.
3. Percalli L, Pricolo R, Passalia L, Riccò M. Comparison between self-gripping, semi re-absorbable meshes with polyethylene meshes in Lichtenstein, tension-free hernia repair: preliminary results from a single center. Acta Biomed. 2018 Mar 27;89(1):72-78.
4. Treede RD, Rief W, Barke A, Aziz Q, Bennett MI, Benoliel R, et al. A classification of chronic pain for ICD-11. Pain. 2015 Jun;156(6):1003-7.
5. Nienhuijs S, Staal E, Strobbe L, Rosman C, Groenewoud H, Bleichrodt R. Chronic pain after mesh repair of inguinal hernia: a systematic review. Am J Surg. 2007 Sep;194(3):394-400.
6. Simons MP, Aufenacker T, Bay-Nielsen M, Bouillot JL, Campanelli G, Conze J, et al. European Hernia Society guidelines on the treatment of inguinal hernia in adult patients. Hernia. 2009 Aug;13(4):343-403.
7. Zwaans WAR, Verhagen T, Wouters L, Loos MJA, Roumen RMH, Scheltinga MRM. Groin Pain Characteristics and Recurrence Rates: Three-year Results of a Randomized Controlled Trial Comparing Self-gripping Progrip Mesh and Sutured Polypropylene Mesh for Open Inguinal Hernia Repair. Ann Surg. 2018 Jun;267(6):1028-1033.
8. Poobalan AS, Bruce J, Smith WC, King PM, Krukowski ZH, Chambers WA. A review of chronic pain after inguinal herniorrhaphy. Clin J Pain. 2003 Jan-Feb;19(1):48-54.
9. O'Dwyer PJ, Alani A, McConnachie A. Groin hernia repair: postherniorrhaphy pain. World J Surg. 2005 Aug;29(8):1062-5..
10. Nguyen DK, Amid PK, Chen DC. Groin Pain After Inguinal Hernia Repair. Adv Surg. 2016 Sep;50(1):203-20.
11. Kosai N, Sutton PA, Evans J, Varghese J. Laparoscopic preperitoneal mesh repair using a novel self-adhesive mesh. J Minim Access Surg. 2011 Jul;7(3):192-4.
12. Xie J, Zheng X, Gu C. Experience Management of Intraoperative Hemorrhage in Laparoscopic Inguinal Hernia Repair. J Coll Physicians Surg Pak. 2020 Nov;30(11):1232-1233
13. Bay-Nielsen M, Nilsson E, Nordin P, Kehlet H; Swedish Hernia Data Base the Danish Hernia Data Base. Chronic pain after open mesh and sutured repair of indirect inguinal hernia in young males. Br J Surg. 2004 Oct;91(10):1372-6.
14. Dirksen CD, Beets GL, Go PM, Geisler FE, Baeten CG, Kootstra G. Bassini repair compared with laparoscopic repair for primary inguinal hernia: a randomised controlled trial. Eur J Surg. 1998 Jun;164(6):439-47.
15. HerniaSurge Group. International guidelines for groin hernia management. Hernia. 2018 Feb;22(1):1-165. doi: 10.1007/s10029-017-1668-x. Epub 2018 Jan 12.
16. Stoker DL, Spiegelhalter DJ, Singh R, Wellwood JM. Laparoscopic versus open inguinal hernia repair: randomised prospective trial. Lancet. 1994 May 21;343(8908):1243-5
17. EU Hernia Trialists Collaboration. Mesh compared with non-mesh methods of open groin hernia repair: systematic review of randomized controlled trials. Br J Surg. 2000 Jul;87(7):854-9.
18. Verhagen T, Zwaans WA, Loos MJ, Charbon JA, Scheltinga MR, Roumen RM. Randomized clinical trial comparing self-gripping mesh with a standard polypropylene mesh for open inguinal hernia repair. Br J Surg. 2016 Jun;103(7):812-8.
19. Grant AM, Scott NW, O'Dwyer PJ; MRC Laparoscopic Groin Hernia Trial Group. Five-year follow-up of a randomized trial to assess pain and numbness after laparoscopic or open repair of groin hernia. Br J Surg. 2004 Dec;91(12):1570-4.
20. Changazi SH, Fatimah N, Naseer A, Wadood A, Ahmad QA, Ayyaz M. Neurectomy versus Nerve Sparing in Open Inguinal Hernia Repair: A Randomised Controlled Trial. J Coll Physicians Surg Pak. 2020 Sep;30(9):917-920.
21. Köhler G, Lechner M, Mayer F, Köckerling F, Schrittwieser R, Fortelny RH, Adolf D, Emmanuel K. Self-Gripping Meshes for Lichtenstein Repair. Do We Need Additional Suture Fixation? World J Surg. 2016 Feb;40(2):298-308.
22. Hosseini M, Ahmadian H, Farazmand B, Vosough F, Negahi A. Comparison of inguinal hernia recurrence rate after mesh plus Bassini repair and mesh repair alone. J Adv Pharm Educ Res 2019;9(S2):117–120.
23. Kumar B, Habibullah Ansari M, Kumar S, Asjad Karim Bakhteyar M, Yadav C, Yadav T. Comparison of outcome of inguinal hernia repair by combined method of repair (Lichtenstein and Bassini repair) and its comparison with older techniques. Int Surg J 2019;6(10):3698–3700.

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Brain volumetric MRI study in healthy adolescent and young person's using automated method

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ABSTRACT

Objective: Adolescence is a critical period for the maturation of neurobiological processes that underlie higher cognitive functions and social and emotional behaviour. However, there are limited studies that investigated brain volumes in healthy adolescents and young persons. The aim of this study was to compare the Grey Matter (GM), White Matter (WM) and some specific brain subcortical volumes such as hippocampus and amygdala between healthy adolescents and young groups by using VolBrain.

Material and Methods: Magnetic resonance imaging brain scans were retrospectively obtained from 20 healthy adolescent and young subjects. The mean ages of the adolescent and young persons were 13±1 and 24±2, respectively. Brain parenchyma (BP), GM, WM and asymmetry features were calculated using VolBrain, and the GM and WM volumes of each subjects were compared with those of the both groups. The current study to examine whether regional gray matter (GM), white matter (WM), cerebrospinal fluid (CSF), some brain subcortical structures volumes differed between healthy adolescent and young groups. Also, of the whole brain, hemispheres, and hippocampus, amigdala of adolescent and young subject volumes were measured with an automated method.

Results: We have observed that the young group was found to have a 4 % less in volume of GM, when compared with adolescent groups.

Conclusion: Our data indicate that quantitative structural Magnetic Resonance Imaging (MRI) data of the adolescent brain is important in understanding the age-related human morphological changes.

Keywords: Adolescence, Brain volume, Gray Matter (GM), White Matter (WM), VolBrain

INTRODUCTION

Adolescence is a critical period for psychological and social development. Second decade of life includes concurrent pubertal changes and sex-based vulnerabilities (**1, 2**). This is a period of physical and cognitive development that finds their identity and learns the mechanisms of adult personal relationships to cope with various problem behaviours. However, adolescence is a critical period for maturation of neurobiological processes that underlie higher cognitive functions and social and emotional behaviour (**3**) making these individuals under the risk of the development of major depressive disorder (MDD) that is a leading cause of inability worldwide with the peak period of onset occurring during adolescence (**4**).

In the early postnatal period the number of synapses in the brain, axonal and dendritic branches, together with myelination increase due to an extreme upsurge of brain volume. Studies have already revealed that brain volume shows a roughly linear increase in the White Matter (WM) volumes during the first 25 years of life. In this regards, the development of WM and Grey Matter (GM) is associated with new connections among neurons, glial cells, and myelin (**5**). During adolescence, brain development characteristically shows significant reductions of cortical gray matter, together with an increase in white matter (**6-8**). For instance, Giedd et al. found an increase in white matter is linearly throughout the development, while gray matter surges at pre-adolescence peak in the frontal cortex during adolescence, and continues to decrease all through post-adolescence (**7**).

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It is known that cortical and global volumes reduce in a linear manner together with age, while the volume of cerebrospinal fluid (CSF) increases. Furthermore, it is recognized that mean total cerebral volume was approximately 10% larger in adult male subjects compared with adult females (9). During the adolescence period, hormonal and neurodevelopmental changes are mediated by the growth of neural processes, remodeling of synaptic connections, increased myelination in prefrontal areas, and maturation of connecting subcortical regions (10).

To the best of our knowledge, there are limited brain volumetric studies in adolescents that have only included some mood disorders (10-12). These studies have shown that, there are critical differences in hippocampus and amygdala volumes in different neurological and psychiatric disorders in adolescence, including schizophrenia, temporal lobe epilepsy, depression, and bipolar disorder (13). Considering all of these findings, we aimed to compare the GM, WM, and some specific brain subcortical volumes such as hippocampus and amygdala between healthy adolescents and young groups by using VolBrain.

MATERIALS and METHODS

Study Population and Design

Patients

A total of 20 healthy female and male patients were included in this study. The average age of the male (n=5) and female (n =5) in the adolescent grouping was 13±1.1 and 14 ±1.5, respectively. The average ages of the male (n=5) and female (n=5) of the young group were 25±1.8 and 24.4 ± 2.5 years, respectively. All subjects were right-handed and healthy without known neurological or psychological disorders. No significant age variations between males and females were present in either of the groups.

This study was performed at the Radiology and Anatomy Departments in Alanya Alaaddin Keykubat University, Alanya Training and Research Hospital. Informed consent form (written) were supplied by their surrogates' for all patients. This study was approved by the local ethics committee of scientific researches of Alanya Alaaddin Keykubat University Faculty of Medicine (Ethics committee decision number: 2018/3)

Neuroimaging

Neuroimaging was performed using a 1.5 T MRI device (GE, SIGNA Explorer, General Electric, Mildwaukee, US). Structural images were acquired using 3D T1 fast spoiled gradient recalled acquisition in the steady state (FSPGR) sequence in the sagittal plane, using these parameters: TE= 1,7 msec, TR=5,95 msec, flip angle=12º, acquisition matrix= 256 x 256, FOV=256 mm2, number of slices=170 and slice thickness=1.0 mm.

VolBrain (https://volbrain.upv.es/ is a web based calculation of volume aiming to provide automatic analysis of MRI brain data. VolBrain operates as a black box solution obtaining anonymized MRI brain volumes in NIFTI format and then produces a report in pdf format including the volumes of the main IntraCranial Cavity (ICC) tissues (i.e., CSF, WM and GM), also providing volume data of macroscopic areas including cerebellum, brain hemisphere, and brainstem. Furthermore, automatic subcortical structure segmentation is achieved, and the associated label maps and volumes are delivered. The whole process takes average 12 min. But, the scan time can vary due to the amount of jobs lined up on the web-server. The following figure briefly outlines the process (14). The results can downloadable as PDF file (**Figure 1, Figure 2**). We calculated volumes of CSF, WM, GM, brain hemispheres, cerebellum, and brainstem using volBrain pipeline, **Figure 1 and Figure 2**.

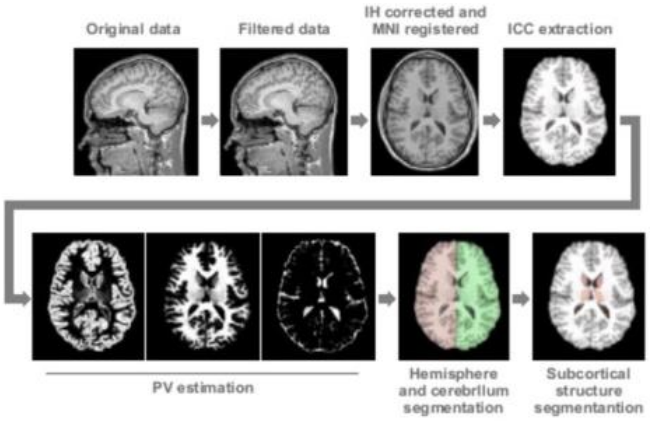
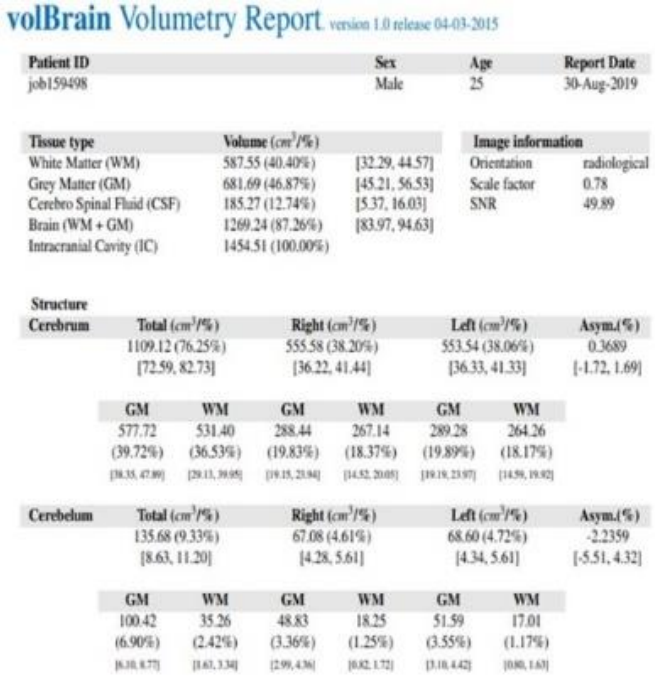


Figure 1: TIV, WM, GM, right and left side of the cerebral cortex in a healthy participant.


volBrain Volumetry Report version 1.0 release 04-03-2015

Patient ID	Sex	Age	Report Date
job159498	Male	25	30-Aug-2019

Tissue type	Volume (cm^3/%)		Image information	
White Matter (WM)	587.55 (40.40%)	[32.29, 44.57]	Orientation	radiological
Grey Matter (GM)	681.69 (46.87%)	[45.21, 56.53]	Scale factor	0.78
Cerebro Spinal Fluid (CSF)	185.27 (12.74%)	[5.37, 16.03]	SNR	49.89
Brain (WM + GM)	1269.24 (87.26%)	[83.97, 94.63]		
Intracranial Cavity (IC)	1454.51 (100.00%)			

Structure						
Cerebrum	Total (cm^3/%)		Right (cm^3/%)		Left (cm^3/%)	Asym.(%)
	1109.12 (76.25%)		555.58 (38.20%)		553.54 (38.06%)	0.3689
	[72.59, 82.73]		[36.22, 41.44]		[36.33, 41.33]	[-1.72, 1.69]
GM	WM	GM	WM	GM	WM	
577.72	531.40	288.44	267.14	289.28	264.26	
(39.72%)	(36.53%)	(19.83%)	(18.37%)	(19.89%)	(18.17%)	
[38.35, 47.89]	[29.13, 39.95]	[19.15, 23.84]	[14.52, 20.05]	[19.19, 23.97]	[14.59, 19.92]	
Cerebelum	Total (cm^3/%)		Right (cm^3/%)		Left (cm^3/%)	Asym.(%)
	135.68 (9.33%)		67.08 (4.61%)		68.60 (4.72%)	-2.2359
	[8.63, 11.20]		[4.28, 5.61]		[4.34, 5.61]	[-5.51, 4.32]
GM	WM	GM	WM	GM	WM	
100.42	35.26	48.83	18.25	51.59	17.01	
(6.90%)	(2.42%)	(3.36%)	(1.25%)	(3.55%)	(1.17%)	
[6.10, 8.77]	[1.63, 3.34]	[2.99, 4.36]	[0.82, 1.72]	[3.18, 4.42]	[0.80, 1.63]	

Figure 2: VolBrain PDF result.

We downloaded MR T1 data from the scanner, transferred and processed using different softwares. We saved MR images as niftii format. For this purpose, we used personal computer on a 32-bit Dell PC, running Windows 10 operating system. We used volBrain to calculate volume. Using the volBrain (http://volbrain.upv.es/) pipeline does not require any installation, configuration or training. The volBrain volumetric analysis system works remotely through a web interface using a SaaS (Software as a Service) model to automatically provide a report containing volumetric information from any submitted case. Data analysis focused on volume (cm^3) in some regions in the brain: GM and WM

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of the cerebral cortex and total brain. The Asymmetry Index is calculated as the difference between right and left volumes divided by their mean (in percent) (**15, 16**).

Asymmetry index is calculated by

(left volumes-right volumes)/mean (left volumes, right volumes) × 100% Eq.1

Statistical Analysis

All statistical analyses were performed with the SPSS software package. The whole-brain volume analysis was performed with two-way analysis of variance (ANOVA) where, the two factors was sex and age.

The coefficient variation (CV) for the whole-brain volume, right and left hemisphere, in both adolescent and young groups, were calculated as the standard deviation of the volume and then divided by mean volume. Statistical significance tests were evaluated at a level of significance of 0.05.

RESULTS

The average brain volume was 1356,02±106,04 cm^3 for young female, 1290,20±100,54 cm3 for young male, 1276,17±106,44 cm^3 for adolescence female, and 1390,50±57,784 cm^3 for adolescence male.

Table 2 and 3 presents adult and adolescents' comparisons for average and relative volume of WM, GM, and CSF. Volumetric analyses showed that the young group had less GM volume than the adolescence group.

According to average GM volume measurements, approximately 4% volume loss from adolescent to young participants has been observed (**Table 2**). No significant variance was found between sex and age for brain volume.

Figure 3 displays the change in volume in the right and left hemispheres for adolescence and young female, and male participants (**Figure 3**).

Combining the data from the female and male subjects, young male subjects were found to have 1% volume forfeiture in the right hemisphere and 2% volume forfeiture in the left hemisphere for GM. Moreover, male subjects showed higher left or right hemisphere volume, than females in adolescence and young participants.

Right and left hemisphere volume was similar in young and adolescent participants. Detailed hemisphere volume and relative values are provided in **Table 3, 4**.

We found that there was a significant group–sex effect on WM volume using two-way ANOVA. This indicates that group-related brain volume depended on sex. But, no significant group–sex effect on GM volume using two-way ANOVA was found, indicating that group brain volumes were not dependent on sex (**Table 5, 6**). CV volume values in adolescent and young groups were between 2 and 12%.

Table 7 shows the descriptive statistics for adolescent and young groups in subcortical structures asymmetry. Although, the Amigdala has negative asymmetry among the age related groups , the statistically important difference was not found.

We found that the young group had slightly less caudate, thalamus and globus pallidus volumes than adolescence group. But, there were no statistically important differences between groups for subcortical structures volumes (p>0.05) (**Table 8**).

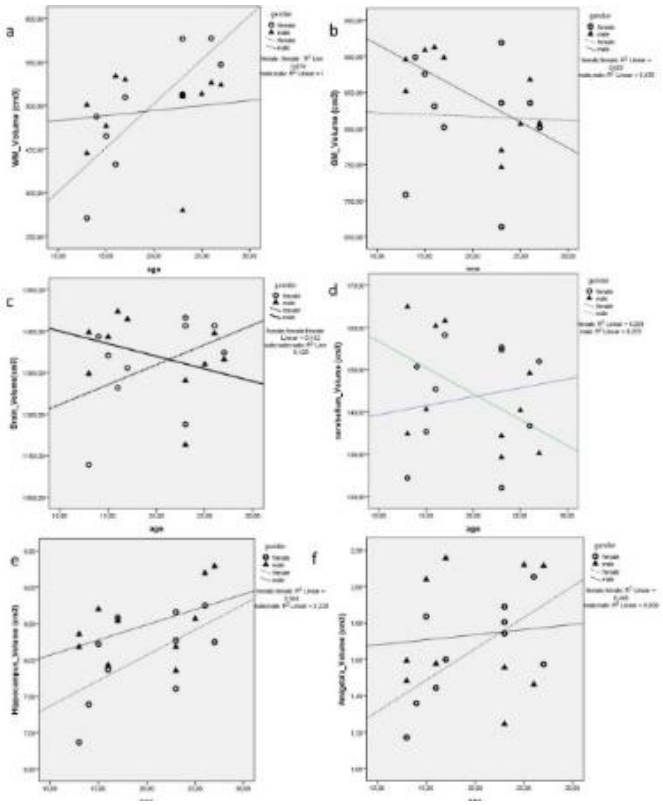

Figure 3: Scatterplots of 20 healthy subjects with absolute WM, GM, brain, cerebral asymmetry, cerebellum, hippocampus, and amygdala volumes shown as a function of age (a-f).

Table 1. Average volume of Brain, GM, WM, CSF (cm^3).

	WM	GM	CSF	Brain
Adolesence				
Female	453.19±48.41	822.98±66.73	150.12±31.36	1276.17±106.44
Male	497.38±37.24	893.11±24.31	136.58±44.26	1390.50±57.784
Young				
Female	545.16±32.47	810.86±93.16	132.24±28.17	1356.02±106.04
Male	490.76±62.43	799.43±46.12	189.12±63.72	1290.20±100.54

Note: Value represents means ± standard deviations.

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Table 2. The relative volume of Brain, GM, WM, CSF (%)

	WM	GM	Brain Tissue
Adolesence			
female	31.75±2.00	57.72±1.46	10.52±1.93
male	32.56±0.72	58.62±2.61	8.82±2.40
Young			
female	36.78±2.80	54.39±2.32	8.83±1.34
male	33.14±2.66	54.23±2.80	12.63±3.36

Value represents means ± standard deviations

Table 3. Hemisphere volume values (cm^3)

	Cerebrum Right	Cerebrum Right GM	Cerebrum Right WM	Cerebrum Left	Cerebrum Left GM	Cerebrum Left WM
Adolescence						
Female	556.57±48.4	350.67±30.8	205.90±21.9	554.37±48.3	350.82±31.5	203.54±21.3
Male	607.52±24.7	381.83±10.6	225.69±16.9	606.68±21.3	382.63±9.60	224.05±16.3
Young						
Female	593.98±45.6	346.16±39.76	247.82±15.5	593.37±47.1	347.65±39.625	245.72±16.14
Male	565.23±47.5	342.82±21.5	222.41±28.7	565.31±46	342.07±19.44	223.23±30.4

Table 4. The relative volume of hemisphere (%)

	Cerebrum Right	Cerebrum R GM	Cerebrum R WM	Cerebrum Left	Cerebrum L GM	Cerebrum L WM
Adolesence						
Female	39.01±0.75	24.58±0.7	14.42±0.87	38.86±0.8	24.60±0.86	14.26±0.84
Male	39.84±1.12	25.07±1.2	14.77±0.25	39.80±1.3	25.13±1.4	14.67±0.36
Young						
Female	39.94±0.43	23.22±0.9	16.72±1.31	39.89±0.3	23.32±0.9	16.57±1.21
Male	38.25±1.32	23.24±0.9	15.01±1.18	38.27±1.5	23.20±1.1	15.07±1.42

Table 5. Two way ANOVA of WM volume

	Df	SS	MS	F	P
Group	1	9104.58	9104.68	3.924	0.065
Sex	1	129.978	129.978	0.056	0.816
Group X sex	1	12149.87	12149.87	5.243	0.036*
Residual	16	37076.29	2317.26		
Total	19	58460.82	3076.88		

Table 6. Two way ANOVA of GM volume

	Df	SS	MS	F	p
Group	1	13988.35	13988.35	3.298	0.088
Sex	1	430.07	430.07	1.016	0.329
GroupXsex	1	8314.51	8314.51	1.960	0.181
Residual	16	678.5991	4241.25		
Total	19	94470.17	4972.11		

Table 7. Subcortical structures asymmetry for Adolescent and Young groups

	Adolescence				Young			
	Female		Male		Female		Male	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Caudate	-0,41	1,90	3,85	3,29	3,51	2,98	-0,74	3,65
Putamen	-2,50	1,83	-0,66	2,67	-3,31	1,22	-2,12	2,06
Globus Pallidus	3,40	2,53	3,43	5,46	0,67	6,60	0,46	9,11
Thalamus	-2,35	3,38	-0,14	5,07	-0,68	3,32	1,51	5,11
Hippocampus	-5,58	7,41	-0,83	4,69	1,95	5,01	-2,89	3,34
Amigdala	-8,79	10,17	0,60	17,93	1,58	5,64	-11,69	19,43

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Tablo 8. Average subcortical structures (cm^3).

	Caudate		Thalamus		Globus Pallidus		Hipocampus		Amygdala	
Subject	**A**	**Y**	**A**	**Y**	**A**	**Y**	**A**	**Y**	**A**	**Y**
1	9,55	8,40	14,03	14,04	2,54	2,53	7,39	8,27	1,36	1,80
2	6,51	8,30	11,76	13,34	2,02	2,71	6,87	8,75	1,17	2,05
3	8,88	7,90	11,41	11,70	2,29	2,46	7,87	7,60	1,44	1,89
4	8,45	9,74	12,83	13,53	2,67	2,52	8,22	8,66	1,83	1,74
5	8,37	7,10	14,49	12,92	3,02	2,28	8,58	8,24	1,60	1,57
6	8,00	7,71	12,03	11,78	2,18	2,03	8,70	7,85	2,04	1,24
7	8,06	7,62	13,00	11,22	2,59	2,56	8,35	9,19	1,59	1,46
8	8,70	8,76	14,24	12,41	2,61	2,44	8,54	8,56	2,16	2,12
9	9,59	7,48	13,83	12,59	2,89	2,31	8,18	8,18	1,48	1,55
10	9,22	9,00	13,31	12,33	2,71	2,73	7,93	9,29	1,58	2,11
Art.Ort	**8,53**	**8,20**	**13,09**	**12,59**	**2,55**	**2,46**	**8,06**	**8,46**	**1,63**	**1,76**
St:Dev	**0,91**	**0,80**	**1,08**	**0,89**	**0,31**	**0,21**	**0,57**	**0,54**	**0,30**	**0,30**

A: Adolescence, Y: Young

DISCUSSION

Advances in neuroimaging techniques have enabled us to study the human brain in new ways. Particularly, magnetic resonance imaging (MRI) could provide unparalleled investigation of brain structure and function (**17, 18**). Most of the studies on functional or structural changes in the brain of adolescents with psychiatric disorders, are based on magnetic resonance imaging (**10**). However, no consistent results have been obtained from the few MRI studies of these brain regions in children and adolescents. Previous neuroimaging studies have performed into the development of GM, WM and frontal lobe in children and adolescents (**19, 20**). The adolescent brain also remains under development during this time. Adolescents often engage in increased risk-taking behaviours and experience heightened emotions during the puberty; this may be due to the fact that their frontal lobes—which are responsible for judgment, impulse control, and planning—are still maturing until the early adulthood (**21**).

Hariri et al. have shown that amplified prefrontal activity is related to heightened modulation of the amygdala response to angry and fearful faces stimuli (**22, 23**).

Accordingly, adolescents who continued their aggressive affective behaviour for a longer duration during a conflict episode with their parents were observed to have enlarged amygdala volumes (**24, 25**).

This is consistent with previous evidence suggesting that the amygdala plays a key role in aggressive behaviour and angering processing.

Also, an increased baseline of amygdala activity was observed in aggressive adult populations, while physical amygdala irregularities in adult psychopathologies were manifested by aggressive behaviour and impulsivity (**24, 25**). Hare et al. (**26**) suggested that an increased volume of the amygdala in adolescence may indicate to a predisposition towards a sustained experience of negative affect that could impair behavioural and cognitive regulation and thereby lead to aggressive behaviour. In line with this, Groen et al. (**27**) found that the right amygdala and left hippocampus were significantly enlarged in the autism compared with the control group while no significant correlations were observed between age and amygdala or the hippocampus volume.

These findings were confirmed by Blumberg et al. (**28**), which revealed that the amygdala and hippocampus were significantly smaller in the bipolar disorder compared with the control group. However, many of these studies had small sample sizes, potentially causing some inconsistencies in results (**29**). Indeed, through adolescence, the frontal gray matter volume visualized by a structural MRI was observed to shrink while white matter steadily rises (**10**).

The progression of white and gray matter in adolescent schizophrenia is retarded from adolescent controls and gradually deviates from normal control patients to follow a similar pattern to the irregular development of neuropathology in adult-onset schizophrenia (**30**). These studies finally indicated that longitudinal research is needed to establish whether a larger amygdala volume during early adolescence could produce a risk for the development of psychopathology and aggressive behaviour in adulthood (**25**). In our study, we found that adolescence group had slightly less amygdala volume than the young group. But, there were no statistically significant differences between the groups. It is difficult to estimate what caused to this inconsistency. However, it can be hypothesized that increases in the white matter might reflect, in part, increased myelination, which might lead to relative decreases in gray matter volumes. Accordingly, brain size during adolescence typically shows significant decreases in cortical gray matter and increases in white matter (**8, 31, 32**). Despite these findings, Giedd et al. (**19**) stated that frontal and temporal gray matter volumes peak between 11 to 16 years in girls and boys. Also, the dorsal lateral prefrontal cortex which is the latest brain region, start to mature in early of 20s. Thus, structural volumetric neuroimaging studies have stated that, even though global brain volume is established by early school-age (33), the transformation of white and grey matter happens during adolescence and remains open until early-adulthood (34). For instance, the hippocampus plays a very important role in cognitive development in children and adolescents (13), and white matter volume steadily increases during the adolescence (**19**). Also there have been many external factors (i.e., stroke, trauma) shown to increase the risk of the development of mood disorders connected with critical brain regions such as the hippocampus (**35-40**). Accordingly,

increased gray matter density of the hippocampus, amygdala, and the posterior temporal cortex have been already reported among the adolescence (**41, 42**). We found that there was approximately 4% volume loss in GM from adolescent to young participants volume while there was significant group–sex effect on WM volume by using two-way ANOVA, which could mean that group-related brain volume changes were sex-dependent. However, our results indicated that there were no statistically differences between groups for subcortical structures volumes. It has already been shown that there is total volume forfeiture with age, and cortical volumes reduce in a linear manner with age as the CSF volumes rise (**20**). Our results have the context of the following study limitations; first, our sample size is relatively small, and further investigations with a larger sample size might be needed to endorse the findings in this study. Second, we used automated segmentation procedures, and we did not compare manual tracing method although studies have shown that there are no significant differences between automated segmentation method and manual tracing of the brain subcortical volume in the literature (**43**).

CONCLUSIONS

As a conclusion, our present results suggest that quantitative structural MR data of the adolescent brain is vital in determining age-related human brain functioning, which could help in refining the clinical diagnosis of various psychiatric disorders characterized with brain volume loss.

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee.

Conflict of interest: The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Asato MR, Terwilliger R, Woo J, Luna B. White matter development in adolescence: a DTI study. Cereb Cortex. 2010;20(9):2122-31.
2. Meeus W. Adolescent psychosocial development: A review of longitudinal models and research. Dev Psychol. 2016;52(12):1969-93.
3. Spear LP. The adolescent brain and age-related behavioural manifestations. Neurosci Biobehav Rev. 2000;24(4):417-63.
4. Avenevoli S, Swendsen J, He JP, Burstein M, Merikangas KR. Major depression in the national comorbidity survey-adolescent supplement: prevalence, correlates, and treatment. J Am Acad Child Adolesc Psychiatry. 2015;54(1):37-44 e2.
5. Giedd JN, Lalonde FM, Celano MJ, White SL, Wallace GL, Lee NR, et al. Anatomical brain magnetic resonance imaging of typically developing children and adolescents. J Am Acad Child Adolesc Psychiatry. 2009;48(5):465-70.
6. Giedd JN, Rumsey JM, Castellanos FX, Rajapakse JC, Kaysen D, Vaituzis AC, et al. A quantitative MRI study of the corpus callosum in children and adolescents. Brain Res Dev Brain Res. 1996;91(2):274-80.
7. Giedd JN, Blumenthal J, Jeffries NO, Rajapakse JC, Vaituzis AC, Liu H, et al. Development of the human corpus callosum during childhood and adolescence: a longitudinal MRI study. Prog Neuropsychopharmacol Biol Psychiatry. 1999;23(4):571-88.
8. Yurgelun-Todd DA, Killgore WD, Young AD. Sex differences in cerebral tissue volume and cognitive performance during adolescence. Psychol Rep. 2002;91(3 Pt 1):743-57.
9. Goldstein JM, Seidman LJ, Horton NJ, Makris N, Kennedy DN, Caviness VS, Jr., et al. Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. Cereb Cortex. 2001;11(6):490-7.
10. Miguel-Hidalgo JJ. Brain structural and functional changes in adolescents with psychiatric disorders. Int J Adolesc Med Health. 2013;25(3):245-56.
11. Wilke M, Kowatch RA, DelBello MP, Mills NP, Holland SK. Voxel-based morphometry in adolescents with bipolar disorder: first results. Psychiatry Res. 2004;131(1):57-69.
12. Janssen J, Reig S, Parellada M, Moreno D, Graell M, Fraguas D, et al. Regional gray matter volume deficits in adolescents with first-episode psychosis. J Am Acad Child Adolesc Psychiatry. 2008;47(11):1311-20.
13. Herten A, Konrad K, Krinzinger H, Seitz J, von Polier GG. Accuracy and bias of automatic hippocampal segmentation in children and adolescents. Brain structure & function. 2019;224(2):795-810.
14. Huhtaniska S, Jaaskelainen E, Heikka T, Moilanen JS, Lehtiniemi H, Tohka J, et al. Long-term antipsychotic and benzodiazepine use and brain volume changes in schizophrenia: The Northern Finland Birth Cohort 1966 study. Psychiatry Res Neuroimaging. 2017;266:73-82.
15. Manjon JV, Coupe P. volBrain: An Online MRI Brain Volumetry System. Frontiers in neuroinformatics. 2016;10:30.
16. Wang Y, Xu Q, Li S, Li G, Zuo C, Liao S, et al. Gender differences in anomalous subcortical morphology for children with ADHD. Neuroscience letters. 2018;665:176-81.
17. Acer N, Bastepe-Gray S, Sagiroglu A, Gumus KZ, Degirmencioglu L, Zararsiz G, et al. Diffusion tensor and volumetric magnetic resonance imaging findings in the brains of professional musicians. J Chem Neuroanat. 2018;88:33-40.
18. Cantou P, Platel H, Desgranges B, Groussard M. How motor, cognitive and musical expertise shapes the brain: Focus on fMRI and EEG resting-state functional connectivity. J Chem Neuroanat. 2018;89:60-8.
19. Giedd JN. Structural magnetic resonance imaging of the adolescent brain. Ann N Y Acad Sci. 2004;1021:77-85.
20. Shan ZY, Liu JZ, Sahgal V, Wang B, Yue GH. Selective atrophy of left hemisphere and frontal lobe of the brain in old men. The journals of gerontology Series A, Biological sciences and medical sciences. 2005;60(2):165-74.
21. Casey BJ, Tottenham N, Liston C, Durston S. Imaging the developing brain: what have we learned about cognitive development? Trends in cognitive sciences. 2005;9(3):104-10.
22. Hariri AR, Bookheimer SY, Mazziotta JC. Modulating emotional responses: effects of a neocortical network on the limbic system. Neuroreport. 2000;11(1):43-8.

23. Hariri AR, Mattay VS, Tessitore A, Fera F, Weinberger DR. Neocortical modulation of the amygdala response to fearful stimuli. Biol Psychiatry. 2003;53(6):494-501.

24. Tebartz van Elst L, Hesslinger B, Thiel T, Geiger E, Haegele K, Lemieux L, et al. Frontolimbic brain abnormalities in patients with borderline personality disorder: a volumetric magnetic resonance imaging study. Biol Psychiatry. 2003;54(2):163-71.

25. Whittle S, Yap MB, Yucel M, Fornito A, Simmons JG, Barrett A, et al. Prefrontal and amygdala volumes are related to adolescents' affective behaviours during parent-adolescent interactions. Proc Natl Acad Sci U S A. 2008;105(9):3652-7.

26. Hare TA, Tottenham N, Davidson MC, Glover GH, Casey BJ. Contributions of amygdala and striatal activity in emotion regulation. Biol Psychiatry. 2005;57(6):624-32.

27. Groen W, Teluij M, Buitelaar J, Tendolkar I. Amygdala and hippocampus enlargement during adolescence in autism. J Am Acad Child Adolesc Psychiatry. 2010;49(6):552-60.

28. Blumberg HP, Kaufman J, Martin A, Whiteman R, Zhang JH, Gore JC, et al. Amygdala and hippocampal volumes in adolescents and adults with bipolar disorder. Archives of general psychiatry. 2003;60(12):1201-8.

29. Merz EC, He X, Noble KG, Pediatric Imaging N, Genetics S. Anxiety, depression, impulsivity, and brain structure in children and adolescents. Neuroimage Clin. 2018;20:243-51.

30. Douaud G, Mackay C, Andersson J, James S, Quested D, Ray MK, et al. Schizophrenia delays and alters maturation of the brain in adolescence. Brain : a journal of neurology. 2009;132(Pt 9):2437-48.

31. Jernigan TL, Trauner DA, Hesselink JR, Tallal PA. Maturation of human cerebrum observed in vivo during adolescence. Brain : a journal of neurology. 1991;114 (Pt 5):2037-49.

32. Pfefferbaum A, Mathalon DH, Sullivan EV, Rawles JM, Zipursky RB, Lim KO. A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. Archives of neurology. 1994;51(9):874-87.

33. Reiss AL, Abrams MT, Singer HS, Ross JL, Denckla MB. Brain development, gender and IQ in children. A volumetric imaging study. Brain : a journal of neurology. 1996;119 (Pt 5):1763-74.

34. Durston S, Hulshoff Pol HE, Casey BJ, Giedd JN, Buitelaar JK, van Engeland H. Anatomical MRI of the developing human brain: what have we learned? J Am Acad Child Adolesc Psychiatry. 2001;40(9):1012-20.

35. Caglayan B, Kilic E, Dalay A, Altunay S, Tuzcu M, Erten F, et al. Allyl isothiocyanate attenuates oxidative stress and inflammation by modulating Nrf2/HO-1 and NF-κB pathways in traumatic brain injury in mice. Molecular biology reports. 2019;46(1):241-50.

36. Cankaya S, Cankaya B, Kilic U, Kilic E, Yulug B. The therapeutic role of minocycline in Parkinson's disease. Drugs in context. 2019;8:212553.

37. Paul A. Lapchak, Zhang JH. Neuroprotective Therapy for Stroke and Ischemic Disease: Springer International Publishing; 2017.

38. Yulug B. Neuroprotective treatment strategies for poststroke mood disorders: A minireview on atypical neuroleptic drugs and selective serotonin re-uptake inhibitors. Brain research bulletin. 2009;80(3):95-9.

39. Yulug B, Hanoglu L, Khanmammadov E, Duz OA, Polat B, Hanoglu T, et al. Beyond The Therapeutic Effect of rTMS in Alzheimer's Disease: A Possible Neuroprotective Role of Hippocampal BDNF? : A Minireview. Mini reviews in medicinal chemistry. 2018;18(17):1479-85.

40. Yuluğ B, Ozan E, Kilic E. Brain-derived neurotrophic factor polymorphism as a genetic risk for depression? A short review of the literature. The Journal of neuropsychiatry and clinical neurosciences. 2010;22(1):123.e5-6.

41. Gogtay N, Thompson PM. Mapping gray matter development: implications for typical development and vulnerability to psychopathology. Brain and cognition. 2010;72(1):6-15.

42. Giedd JN, Castellanos FX, Rajapakse JC, Vaituzis AC, Rapoport JL. Sexual dimorphism of the developing human brain. Prog Neuropsychopharmacol Biol Psychiatry. 1997;21(8):1185-201.

43. Akudjedu TN, Nabulsi L, Makelyte M, Scanlon C, Hehir S, Casey H, et al. A comparative study of segmentation techniques for the quantification of brain subcortical volume. Brain imaging and behaviour. 2018;12(6):1678-95.

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Evaluation of Respiratory Functions with Spirometry in Patients with SARS-CoV-2 Pneumonia

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ABSTRACT

Objective: This study aims to evaluate respiratory functions in patients Coronavirus disease-2019 (COVID-19) with and without pneumonia.

Material and Methods: This single-center, prospective study included a total of 72 patients who were diagnosed with COVID-19 infection as confirmed by real-time reverse transcriptase polymerase chain reaction (rtPCR). The patients were divided into two groups according to the physical and thoracic computed tomography (CT) findings as mild symptomatic patients without COVID-19 pneumonia (n=26) and symptomatic cases with COVID-19 pneumonia (n=46). Respiratory functions were evaluated by spirometry in the second and fourth months of the disease onset.

Results: The average age of 72 patients, 41 of whom were men, was 40.5±12.27 years. Thoracic CT revealed infiltrations compatible with COVID-19 pneumonia in 46 (63.9%) patients. Hypertension (12.5%) and diabetes (5.6%) were the most common comorbidities. When the results of the patients with and without pneumonia at the second and fourth months were compared, there was no significant difference between the forced expiratory volume in the first second (FEV1) (p1=0,975, p2=0,291), forced vital capacity (FVC) (p1=0,668, p2=0,481) and FEV1/FVC ratio (FER) (p1=0,378, p2=0,980) values. When the repeated Anova test was used in the comparison of the two visit differences between the groups, it was seen that there was no difference in any heading (FVC: p=0.077; FEV1: p=0.150; FER: p=0.355).

Conclusions: Our study results show no significant difference in the pulmonary function tests of patients with mild and moderate COVID-19 pneumonia at two and four months, compared to those without pneumonia However, additional studies are needed for severe and critical cases.

Key Words: COVID-19, pulmonary function tests, SARS-CoV-2, pneumonia, spirometry

INTRODUCTION

In December 2019, several cases of pneumonia of unknown causes were found in Wuhan, Hubei province of China, which were later identified as novel coronavirus-2019 (2019-nCoV), a novel beta-coronavirus belonging to subgenus (**1**). As its genome is phylogenetically similar to that of the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome, it is termed as SARS-coronavirus-2 (SARS-CoV-2). The World Health Organization (WHO) later named the virus as the novel coronavirus-2019 (COVID-19) (**2**). Although COVID-19 involves many tissues in the human body, the lungs are the main organs affected by the virus. Previous studies have shown that survivors of SARS and MERS have persistent lung impairment for months or even years (**3-5**). The SARS-CoV-2 enters the pulmonary epithelial cells by binding to angiotensin-converting enzyme 2 (ACE2) receptors and induces viral replication, leading to apoptosis of alveolar type 2 epithelial cells. In addition to its direct cytopathic effect, the presence of inflammation and elevated cytokine levels cause diffuse alveolar damage and the formation of fibrin-rich exudates (i.e., hyalin membranes). At the end of this pathological process, recovery occurs with scarring in the lung epithelium and fibrosis in the lung parenchyma (**6**).

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Hariri et al. (7) reported that histopathological changes in asymptomatic cases were less severe than in symptomatic cases. Theoretically, it is not unexpected that survivors may have impaired pulmonary functions.

Histopathological findings of lungs are often based on post-mortem studies in COVID-19. These are already severe and critical cases. There is a limited number of evidence regarding the histopathological lung findings of mild and non-critical COVID-19. The disease course may mild-to-moderate in the majority of cases worldwide and future studies would shed light into the lung functions of recovered patients, which would be helpful to decide treatment and follow-up. Therefore, in the present study, we included mild-to-moderate COVID-19 cases.

MATERIALS and METHODS

Study design and study population

This single-center, prospective study was conducted at Department of Chest Diseases of a tertiary care center between June 24th, 2020 and December 15th, 2020. Prior to the study and all diagnostic and therapeutic procedures, all participants were informed in detail, and a written informed consent form was obtained. The study protocol was approved by the local Ethics Committee (No: 2020/0407-Date: 24.06.2020). The study was conducted in accordance with the principles of the Declaration of Helsinki.

All patients aged between 18 and 65 years with a confirmed diagnosis of COVID-19 by real-time reverse transcriptase polymerase chain reaction (RT-PCR) were screened. A total of 72 patients who had positive RT-PCR from nasopharyngeal swab samples. As the pulmonary function tests (PFTs) are aerosol-producing procedures and entail a risk of infection for both patients and healthcare workers (HCWs), these tests were avoided in our daily practice during the COVID-19 pandemic. Only the patients who met the inclusion criteria of the study underwent PFTs. Those having myocardial infarction within the last week, stroke within the past month, decompensated heart failure, malignant hypertension, undergoing thoracic, abdominal, ear, or eye operations within the past month, pregnancy, an active respiratory infection, having difficulties in cooperation with the HCWs, and those having anatomical chest deformities were excluded from the study. All patients underwent thoracic computed tomography (CT). According to the physical and imaging examination findings, the patients were divided into two groups as mild symptomatic patients without COVID-19 pneumonia on CT (n=26) and symptomatic cases with COVID-19 pneumonia on CT not requiring oxygen support (n=46). Patients with severe pneumonia defined as the radiographic evidence of pneumonia, a respiratory rate of $\geqslant$30 breaths/min, oxygen saturation of $\leqslant$93% without severe dyspnea at rest and with >50% increase in the lung lesions within the last 24 to 48 hours; critically ill patients (i.e., septic shock, requiring non-invasive or invasive mechanical ventilation, multiple organ failure, and requiring intensive care) were also excluded from the study.

The CT images were quantitatively evaluated according to the involvement due to the inflammatory lesions of the total lung parenchyma and scored as follows: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%).

Study procedures

Demographic and clinical characteristics and comorbidities of all patients were recorded. Prior to PFTs, body temperature was measured, and symptoms were questioned for all patients. Those who tested negative for two consecutive RT-PCR within the past 48 to 72 hours underwent PFTs. During the measurement, a disposable bacterial and viral filters were used for each patient. The technician who performed the PFTs complied with the donning/doffing procedures of the personal protective equipment (PPE). The onset of the disease was considered the date of the first symptom onset. The PFTs were repeated at minimum of 60 days and 120 days after the disease onset, respectively.

All CT images were acquired at the end of inhalation using a 16-slice CT scanner (SOMATOM Scope Power; Siemens Healthineers, Forchheim, Germany). The PFTs were performed by technicians in the PFT laboratory using a spirometer (SpiroLab III®; MIR Medical International Research, Rome, Italy). All PFTs were carried out in accordance with the 2019 American Thoracic Society (ATS) / European Respiratory Society (ERS) technical statement (8). The spirometry was performed in accordance with the prespecified national spirometry and laboratory standards and repeatability and precision criteria (9). The spirometer was calibrated on a regular basis. All PFT results were expressed in percentage of the predicted normal values.

Statistical Analysis

Statistical analysis was performed using the SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed as mean ± standard deviation (SD), median (min-max), or number and frequency, where applicable. The Student's t-test was used to compare quantitative variables between the groups. The repeated measures analysis of variance (ANOVA) was performed to analyse the difference between the measurements at two-time points. The chi-square ($\chi 2$) test was used to compare categorical variables. The Pearson correlation coefficient was used to examine the relationship between the quantitative variables. A two-tailed p-value of <0.05 was considered statistically significant.

RESULTS

Of the patients, 41 (56.9%) were males, and 31 (43.1%) were females with an overall mean age of 40.5±12.27 (range, 18 to 64) years. There was no significant difference in the sex of the patients with and without COVID-19 pneumonia ($\chi 2$=1.93, p=0.164). However, the mean age was significantly higher in the patients with COVID-19 pneumonia compared to those without (44.91±10.90 years vs. 32.69±10.71 years, respectively; t=4.60, p<0.001). Thoracic CT revealed normal findings in 36.1% (n=26) of the patients, while it showed lung infiltrations compatible with SARS-CoV-2 pneumonia in 63.9% (n=46). The overall mean bodyweight of the patients was 79.51±15.11 kg, and the mean body mass index (BMI) was 27.96±5.09 kg/m^2. There was a significant correlation between body weight and BMI and CT positivity (t=3.52, p<0.001). The baseline demographic and clinical characteristics of the patients are shown in **Table1**.

Comorbidities of the patients are summarized in **Table 2**. The most common comorbidities included hypertension (n=9,

12.5%), diabetes (n=4, 5.6%), and coronary artery disease (n=4, 5.6%). Only three patients (4.2%) had a previous history of asthma.

Eleven (15.1%) of the patients were smokers. There was no significant correlation between smoking and CT positivity (p=0.735). None of the patients required non-invasive or invasive mechanical ventilation. The most common symptoms were fever (n=61, 84.7%) and dry cough (n=38, 52.8%), followed by fatigue (40.3%), myalgia (33.3%), dyspnea (22.2%), and loss of taste and smell (6.9%). We found a significant correlation between dyspnea and CT positivity ($\chi 2$=7.95, p=0.005), while there was no significant relationship between the other symptoms and CT positivity (p>0.05).

There was no significant difference in the forced vital capacity (FVC) of the patients with mild and moderate pneumonia at two and four months, compared to those without pneumonia (Visit 1: t=-0.431, p=0.668; Visit 2: t=0.709, p=0.481). In addition, there was no significant difference in the forced expiratory volume in one second (FEV1) of the patients with mild and moderate pneumonia at two and four months, compared to those without pneumonia (Visit 1: t=-0.032, p=0.975; Visit 2: t=1.063, p=0.291). No significant difference in the FEV1/FVC ratio (FER) of the patients with mild and moderate pneumonia at two and four months, compared to those without pneumonia (Visit 1: t=0.888, p=0.378; Visit 2: t=0.025, p=0.980) (**Table 3**). The FEV1 (>80%), FVC (>80%), and FER (>70%) values were found to be normal in three patients with a previous history of asthma at two and four months. There was no significant difference in the delta-FVC, delta-FEV1, and delta-FER values between the patients with and without pneumonia (t=1.794, p=0.077; t=1.455, p=0.150; t=-0.931, p=0.355, respectively) (**Table 3**).

According to the repeated measures ANOVA test, there was no significant difference in the other variables between the measurements at two-time points (FVC: F=3.218, p=0.077; FEV1: F=2.118, p=0.150; FER: F=0.867, p=0.355, respectively) (**Table 4**).

In the multivariate linear regression analysis, independent variables such as age, sex, duration, and the first visit measurements and dependent variables with delta differences were analysed, and no significant difference was observed.

According to the radiological scoring of all patients, 0 was assigned to 36% (n=26), 1 to 37.5% (n=27), 2 to 22.2% (n=16), 3 to 4.2% (n=3), and 4 to 0.0% (n=0) of the patients. Based on the radiological scoring in an ordinal scale, no statistically significant correlation between any of the variables including delta differences was observed (non-parametric Spearman correlation coefficient, p>0.05).

In addition, there was no statistically significant correlation between the radiological scores (0-1-2-3) and FEV1, FVC, FEV1/FVC, peak expiratory flow (PEF), and forced expiratory flow (FEF)25-75 at two- and four-month measurements (r=0.08, p=0.947; r=-0.015, p=0.901; r=0.038, p=0.749; r=-0.044, p=0.716; r=-0.089, p=0.457; r=0.062, p=0.606; r=-0.054, p=0.650; r=0.065, p=0.587; r=-0.129, p=0.278; r=0.041, p=0.732, respectively). Similarly, we found no significant correlation between the radiological scores (0-1-2-3) and delta-different FEV12-1 (r=-0.067, p=0.576), delta-different FVC2-1 (r=-0.132, p=0.269), delta-different PEF2-1 (r=0.163, p=0.173), and delta-different FEF25-75(2-1) (r=0.043, p=0.718).

Of 46 patients with COVID-19 pneumonia on CT, there were minimal, but persistent radiographic abnormalities in only three patients (6.5%). The high-resolution CT revealed normal findings in the remaining patients. The PFTs yielded no obstructive or restrictive pattern in the patients with minimal radiographic sequelae (p>0.05).

Table 1. Baseline demographic and clinical characteristics of patients

	Total	CT group	n	Min	Max	Mean	SD	*p value*
Age (year)	n=72 Mean=40,5 ±12,27 range;18-19	CT (-) CT (+)	26 46	18 22	50 64	32,69 44,91	10,71 10,90	p<0,001 t=-4,60
Sex	Male (n=41); 59.9% Female (n=31) 43.1%	CT (-) CT (+)	Female: n=14 (53,8%) Female: n=17 (36,9%)		Male: n=12 (46,2%) Male: n=29 (63,1%)			p=0,164 χ^2=1,93
	Total	**CT group**	**n**	**Min**	**Max**	**Mean**	**SD**	***p value***
Weight (kg)	Mean=79,51 ±15,11	CT (-) CT (+)	26 46	48 54	100 117	71,42 84,09	15,087 13,212	p<0,001 t=-3,71
Height (cm)	Mean=168,68 ±9,16	CT (-) CT (+)	26 46	145 153	187 195	167,96 169,09	8,973 9,342	p=0,620 t=-0,49
BMI (kg/m^2)	Mean=27,96 ±5,09	CT (-) CT (+)	26 46	17,01 21,09	41,38 39,54	25,35 29,43	5,41 4,30	p=0,001 t=-3,52
SBP (mmHg)	Mean=125,76±13,41	CT (-) CT (+)	26 46	100 100	150 180	122,69 127,50	8,48 15,34	p=0,91 t=-1,71
DBP (mmHg)	Mean=76,65 ±8,85	CT (-) CT (+)	26 46	68 64	100 100	75,08 77,54	7,205 9,610	p=0,259 t=-1,14

$\chi 2$=Pearson chi-square, t=t-test for equality of means. CT=computed tomography; Min=minimum, max=maximum, SD=standard deviation, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure.

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Table 2. Comorbidities of patients

	Total n	Total %	CT_group	n	%	*p value*
Fever	61	84.7	CT (-) CT (+)	22 39	84.6 84.8	p=1,000 (Fisher's)
Dry cough	38	52.8	CT (-) CT (+)	10 28	38.5 60.9	p=0,067 χ^2=3,35
Dyspnea	16	22.2	CT (-) CT (+)	1 15	3.8 32.6	p=0,005 χ^2=7,95
Myalgia	24	33.3	CT (-) CT (+)	10 14	38.5 30.4	p=0,488 χ^2=0,48
Fatigue	29	40.3	CT (-) CT (+)	9 20	34.6 43.5	p=0,461 χ^2=0,54
Loss of taste and smell	5	6.9	CT (-) CT (+)	1 4	3.8 8.7	p=0,647 (Fisher's)
Hypertension	9	12.5	CT (-) CT (+)	1 8	3.8 17.4	p=0,143 (Fisher's)
Diabetes	4	5.6	CT (-) CT (+)	0 4	0 8.7	p=0,289 (Fisher's)
Coronary artery disease	4	5.6	CT (-) CT (+)	1 3	3.8 6.5	p=1,000 (Fisher's)
Asthma	3	4.2	CT (-) CT (+)	1 2	3.8 4.3	p=1,000 (Fisher's)
Smoker	11	15.3	CT (-) CT (+)	3 9	11.5 17.4	p=0,735 (Fisher's)

CT= Computed Tomography.

Table 3. PFT results of patients with and without CT positivity

	CT group	n	Mean %	SD	t-test for equality of means t	*p* value
FEV1 Visit 1	CT (-) CT (+)	26 46	96,92 97,02	13,79 11,81	-0,032	0,975
FEV1 Visit 2	CT (-) CT (+)	26 46	98,50 95,35	14,59 10,43	1,063	0,291
FVC Visit 1	CT (-) CT (+)	26 46	94,81 96,11	13,25 11,73	-0,431	0,668
FVC Visit 2	CT (-) CT (+)	26 46	95,69 93,57	13,83 11,24	0,709	0,481
FER Visit 1	CT (-) CT (+)	26 46	102,08 100,91	4,93 5,56	0,888	0,378
FER Visit 2	CT (-) CT (+)	26 46	101,88 101,85	5,40 6,24	0,025	0,980
PEF Visit 1	CT (-) CT (+)	26 46	90,92 91,26	20,97 19,19	-0,069	0,945
PEF Visit 2	CT (-) CT (+)	26 46	90,04 95,26	19,09 20,84	-1,052	0,296
FEF_{25-75} Visit 1	CT (-) CT (+)	26 46	102,35 96,26	20,86 22,15	1,143	0,257
FEF_{25-75} Visit 2	CT (-) CT (+)	26 46	103,58 100,61	24,34 23,40	0,510	0,612
Delta_diff_FVC_2_1	CT (-) CT (+)	26 46	0,88 -2,54	7,15 8,12	1,794	0,077
Delta_diff_FEV_2_1	CT (-) CT (+)	26 46	1,58 -1,67	8,05 9,64	1,455	0,150
Delta_diff FEV1/FVC_2_1	CT (-) CT (+)	26 46	-0,19 0,93	5,35 4,68	-0,931	0,355
Delta_diff_PEF_2_1	CT (-) CT (+)	26 46	-0,88 4,00	16,59 15,51	-1,252	0,215
Delta_diff_FEF_{25_75}_2_1	CT (-) CT (+)	26 46	1,23 4,35	18,95 23,39	-0,564	0,564

t= t-test for equality of means. CT= computed tomography; SD= standard deviation, FEV1=forced expiratory volume in one second, FVC=forced vital capacity, FER= forced expiratory volume in one second/forced vital capacity ratio, PEF=peak expiratory flow, FEF=forced expiratory flow, FEF25-75=forced expiratory flow at 25-75% of the pulmonary volume.

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Table 4. Frequency of abnormal PFT results and relationship between groups

		CT group		Total	*p* value (Fisher's exact test)
		CT (-)	CT (+)		
FVC Visit 1 <80%pred	N	3	3	6	0,661
	% within CT group	11.5%	6.5%	8.3%	
FVC Visit 2 <80%pred	N	2	3	5	1,000
	% within CT group	7.7%	6.5%	6.9%	
FEV1 Visit 1 <80%pred	N	2	3	5	1,000
	% within CT group	7.7%	6.5%	6.9%	
FEV1 Visit 2 <80%pred	N	1	3	4	1,000
	% within CT group	3.8%	6.5%	5.6%	
FER Visit 1 <70%pred	N	0	0	0	
	% within CT group	0	0	0	
FER Visit 2 <70%pred	N	0	0	0	
	% within CT group	0	0	0	
PEF Visit 1 <65%pred	N	3	5	8	1,000
	% within CT group	11.5%	10.9%	11.1%	
PEF Visit 2 <65%pred	N	1	3	4	1,000
	% within CT group	3.8%	6.5%	5.6%	
FEF_{25-75} Visit 1 <65%pred	N	1	3	4	1,000
	% within CT group	3.8%	6.5%	5.6%	
FEF_{25-75} Visit 2 <65%pred	N	0	2	2	0,532
	% within CT group	0.0%	4.3%	2.8%	
Repeated measures ANOVA (multivariate analysis)				F	*p* value
FVC	Visit 1*2			0,754	0,388
	Visits* CT group			3,218	0,077
FEV1	Visit 1*2			0,002	0,965
	Visits CT group			2,118	0,150
FER	Visit 1*2 (Pillai's Trace)			0,376	0,542
	Visits *CT group (Pillai's Trace)			0,867	0,355
PEF	Visit 1*2			0,637	0,427
	Visits *CT group			1,567	0,215
FEF_{25-75}	Visit 1*2			1,077	0,303
	Visits *CT group			0,336	0,564

CT= computed tomography; SD= standard deviation, FEV1= forced expiratory volume in one second, FVC= forced vital capacity, FER= forced expiratory volume in one second/forced vital capacity ratio, PEF= peak expiratory flow, FEF= forced expiratory flow, FEF25-75= forced expiratory flow at 25-75% of the pulmonary volume.

DISCUSSION

From the beginning of the declaration of COVID-19 pandemic by the WHO on March 11th, 2020, a total of 28,637,952 positive cases were identified with 917,417 deaths until September 14th, 2020 (10). Patients may present with a wide range of symptoms from asymptomatic or mild disease to septic shock and multiple organ dysfunction. The disease is mainly classified into four types: mild, moderate, severe, and critical (11). The diagnosis of COVID-19 is made based on clinical findings, as well as laboratory and imaging test results; however, it is not always possible to establish the definitive diagnosis due to non-specific nature of the clinical and imaging signs of COVID-19.

On the molecular basis, the diagnosis is confirmed using RT-PCR which can qualitatively detect the nucleic acid from the nasopharyngeal/oropharyngeal swabs (12). The sensitivity of RT-PCR is 36% for oropharyngeal swabs and up to 63% for nasopharyngeal swabs (13). However, a single negative swab test alone does not rule out SARS-CoV-2 infection and there is still no ideal specimen for the definitive diagnosis of COVID-19 (14). In repeated negative test results, serologic testing (i.e., IgM and IgG antibodies) can guide the diagnosis (15).

The PFTs are useful, non-invasive tests for screening, diagnosis, and follow-up of respiratory track diseases. Spirometry is the most common type of PFTs and is a physiological test that measures the inhalation and exhalation flow/volumes of air as a function of time (16).

The most common parameters measured in spirometry during forced breathing maneuvers include vital capacity (VC), FVC, FEV, forced expiratory flow (FEF), and peak expiratory flow (PEF).

Previous studies have demonstrated that SARS-CoV-2 infection can cause a variety of symptoms ranging from mild infiltration to acute respiratory distress syndrome (ARDS). In a postmortem biopsy study, Xu et al. (17) reported a case of COVID-19 who died from ARDS. The histological examination showed diffuse alveolar damage with cellular fibromyxoid exudates and evident desquamation of pneumocytes and hyaline membrane formation with diffuse alveolar damage, indicating ARDS. In another postmortem study, Hanley et al. (18) showed diffuse alveolar damage and hyaline membrane formation in a COVID-19 case. In addition, Pan et al. (19) examined the imaging characteristics of the COVID-19 pneumonia in 63 confirmed cases and reported fibrous stripes in 11 (17.5%) patients as assessed by CT imaging. Recent autopsy studies also revealed that the lungs of the COVID-19 non-survivors were filled with clear liquid jelly containing probably hyaluronan, which has a high water-absorption capability (17). Elevated inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α) are potent inducers of hyaluronan synthesis, which are seen in the lungs of COVID-19 cases (20). In these patients, both the direct cytopathic effect of the virus and exaggerated inflammatory response caused by elevated proinflammatory cytokines such as IL-1, IL-6, and

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TNF-α result in damage in the alveolar epithelial cells and endothelial cells. Consequently, the connection between the cells is disrupted, leaking into the interstitial and alveolar spaces, and ARDS develops (10). Elevated cytokine expression has been shown to induce fibroblast migration and proliferation, thereby, resulting in lung fibrosis (21). The ACE2, itself, acts as a protective protein against the fibroblast cascade and reduced ACE2 in COVID-19 with increased angiotensin I and II contributes to the development of lung fibrosis. This theory can explain the higher mortality rates in patients with obesity, hypertension, and cardiovascular diseases in which baseline ACE2 levels are lower (10).

Incidental histopathological changes were found in the pathological examinations of 14 patients who were asymptomatic in terms of COVID-19 and were found to have new coronavirus infection after lung nodule resection (7). Of these, Kuang et al. explained that they detected changes such as interstitial pneumonia and hyaline membrane related to the new coronavirus in their lung cancer tissue sampling (22). The majority of cases have been reported proteinaceous exudate, pneumocyte hyperplasia, irregular chronic inflammation and focal edema with multinucleated pneumocytes (23, 24). Hariri et al. reported that histopathological changes in asymptomatic cases were less severe than in symptomatic cases (7). In their series of seven cases by Chai et al., they stated that only one patient had changes compatible with interstitial inflammation, while there were no changes associated with SARS-CoV-2 in the other six patients (25). However, in this study, there is no clear information regarding the pre-operative presence of COVID-19 infection in six of the seven cases. These studies have shown that asymptomatic patients may have mild histopathological changes. Histopathological changes in severe and critical cases were revealed by postmortem studies. However, we do not have enough information about what kind of histopathological changes occur in symptomatic mild and moderate cases. On the other hand, it is still unclear whether these pathological alterations in the lung parenchyma lead to sequelae in the long-term or how they affect the pulmonary functions in the mid- and long-term.

In a study, Zha et al. (26) reported two COVID-19 cases who developed severe ARDS. During three-month follow-up, although most of the ground-glass opacities resolved, there were fibrotic changes in bilateral lungs on thoracic CT with worse lung ventilation compatible with the restrictive pulmonary disease (FVC: 62.3%, FER: 80.1%). In another study investigating long-term pulmonary function and physiological features of 55 COVID-19 survivors, Zhao et al. (27) excluded critical cases. There were still radiological and physiological abnormalities in three-fourth of the patients three months after discharge. Similarly, Mo et al. (28) found impaired diffusion capacity to be the most frequent abnormality of lung function in discharged COVID-19 survivors. However, there was no significant difference in the other ventilatory defects including FEV1, FVC, and FER among the survivors with different severity of disease. In a randomized-controlled study, Liu et al. (29) examined the effect of respiratory rehabilitation training in elderly patients with COVID-19. The authors reported that this patient population had different degrees of disorders in respiratory function after discharge, possibly due to residual fibrotic lesions and reduced respiratory muscle strength and that respiratory rehabilitation could significantly improve the lung function. Furthermore, Frija-Masson et al. (30) evaluated functional characteristics of 50 patients with COVID-19 pneumonia one month after infection and reported impaired lung function with a mix of restrictive and low diffusion patterns in more than half of the patients, indicating no association with the severity of the disease. In our study, we found no significant difference in the FVC of the patients with mild and moderate pneumonia at two and four months, compared to those without pneumonia (Visit 1: t=-0.431, p=0.668; Visit 2: t=0.709, p=0.481). In addition, there was no significant difference in the FEV1 pneumonia (Visit 1: t=-0.032, p=0.975; Visit 2: t=1.063, p=0.291) and FER (Visit 1: t=0.888, p=0.378; Visit 2: t=0.025, p=0.980) of the patients with mild and moderate pneumonia at two and four months, compared to those without pneumonia. In addition, the repeated measures ANOVA test revealed no significant difference in the other variables between the measurements at two time points (FVC: F=3.218, p=0.077; FEV1: F=2.118, p=0.150; FER: F=0.867, p=0.355, respectively). These results are consistent with the findings of Mo et al. (28) At the time of the first visit, abnormalities were observed in FVC (68 to 78%pred) in six patients (8.3%) and in FEV1 (71 to 78%pred) in five patients (6.9%), while, the FER value was normal (>70%pred) in all patients. This finding indicates mild restrictive spirometric patterns. In four patients (5.5%), the FEF25-75 was abnormal (46 to 64% pred), compatible with small airway obstruction. At the time of the second visit, abnormalities were seen in FVC (66 to 76%pred) in five patients (6.9%) and in FEV1 (69 to 76%pred) in four patients (5.5%); however, the FER value was normal in all patients. In only two patients (2.8%), the FEF25-75 was abnormal (55 to 62%pred), compatible with small airway obstruction. On the other hand, there was no statistically significant difference between the patient groups at two different time points (p>0.05). We also found no significant difference in the delta-FVC, delta-FEV1, and delta-FER values between the patients with and without pneumonia (p=0.077, p=0.150, and p=0.355, respectively).

Based on the radiological scoring in an ordinal scale, no statistically significant correlation between any of the variables including delta differences was observed (non-parametric Spearman correlation coefficient, p>0.05).

Although there was no significant difference in the sex between the patients with and without COVID-19 pneumonia (p=0.164), the mean age was significantly higher in those with pneumonia (p<0.001). This can be attributed to the fact that viruses have the ability to penetrate into the alveolar epithelial cells easily due to decreased mucociliary activity in advanced age, thereby, leading to the reduced regenerative capacity of the alveolar epithelial cells (6).

In a large-scale meta-analysis, the most common symptoms of COVID-19 were fever (81.2%), dry cough (62.9%), dyspnea (26.9%), and loss of taste (25.4%) (31). In our study, the most frequent symptoms were fever (84.7%), dry cough (52.8%), fatigue (40.3%), myalgia (33.3%), dyspnea (22.2%), and loss of taste and smell (6.9%). Although we observed no significant difference in the fever, dry cough, myalgia, and fatigue between the patients with and without COVID-19 pneumonia, we found a significant correlation between dyspnea and CT positivity (p=0.005). This finding is also

consistent with one of our previous reports including 206 RT-PCR-confirmed COVID-19 cases and showing a link between critical illness and CT positivity (**32**).

Limitations

The main limitation of the present study is the lack of homogeneous distribution of the patients between the groups and the relatively small sample size in the mild symptomatic patient group without COVID-19 pneumonia. In addition, the pulmonary functions of the patients before COVID-19 infection are not fully known, which may have led to incomplete interpretation of the measured values during the study. The unequal number of patients in each radiological scoring group is also another limitation which may have led to bias in the statistical calculation. Further prospective studies are warranted to gain a better understanding of the respiratory functions in severe and critical cases with SARS-CoV-2 pneumonia including those having 3-4 radiological scores.

CONCLUSION

In conclusion, our study results showed no significant difference in the PFT results of the patients with confirmed mild and moderate COVID-19 pneumonia at two and four months, compared to those without pneumonia. No obstructive or restrictive spirometric patterns were observed. However, further large-scale studies are needed in severe and critically ill pneumonia cases.

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REFERENCES

1. Chen X, Tian J, Li G, Li G. Initiation of a new infection control system for the COVID-19 outbreak. The Lancet Infectious diseases. 2020;20(4):397-8.
2. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, et al. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). International journal of surgery (London, England). 2020;76:71-6.
3. Hui DS, Joynt GM, Wong KT, Gomersall CD, Li TS, Antonio G, et al. Impact of severe acute respiratory syndrome (SARS) on pulmonary function, functional capacity and quality of life in a cohort of survivors. Thorax. 2005;60(5):401-9.
4. Ngai JC, Ko FW, Ng SS, To KW, Tong M, Hui DS. The long-term impact of severe acute respiratory syndrome on pulmonary function, exercise capacity and health status. Respirology (Carlton, Vic). 2010;15(3):543-50.
5. Park WB, Jun KI, Kim G, Choi JP, Rhee JY, Cheon S, et al. Correlation between Pneumonia Severity and Pulmonary Complications in Middle East Respiratory Syndrome. Journal of Korean medical science. 2018;33(24):e169.
6. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. The European respiratory journal. 2020;55(4).
7. Hariri LP, North CM, Shih AR, Israel RA, Maley JH, Villalba JA, et al. Lung Histopathology in Coronavirus Disease 2019 as Compared With Severe Acute Respiratory Sydrome and H1N1 Influenza: A Systematic Review. Chest. 2021;159(1):73-84.
8. Townsend MC. The American Thoracic Society/European Respiratory Society 2019 Spirometry Statement and Occupational Spirometry Testing in the United States. American journal of respiratory and critical care medicine. 2020;201(8):1010-1.
9. Ulubay G, Köktürk N, Görek Dilektaşlı A, Şen E, Çiftci F, Demir T, et al. [Turkish Thoracic Society national spirometry and laboratory standards]. Tuberkuloz ve toraks. 2017;65(2):117-30.
10. Pollard CA, Morran MP, Nestor-Kalinoski AL. The COVID-19 pandemic: a global health crisis. Physiological genomics. 2020;52(11):549-57.
11. Hassan SA, Sheikh FN, Jamal S, Ezeh JK, Akhtar A. Coronavirus (COVID-19): A Review of Clinical Features, Diagnosis, and Treatment. Cureus. 2020;12(3):e7355.
12. Zheng Q, Lu Y, Lure F, Jaeger S, Lu P. Clinical and radiological features of novel coronavirus pneumonia. Journal of X-ray science and technology. 2020;28(3):391-404.
13. Zitek T. The Appropriate Use of Testing for COVID-19. The western journal of emergency medicine. 2020;21(3):470-2.
14. Winichakoon P, Chaiwarith R, Liwsrisakun C, Salee P, Goonna A, Limsukon A, et al. Negative Nasopharyngeal and Oropharyngeal Swabs Do Not Rule Out COVID-19. Journal of clinical microbiology. 2020;58(5).
15. Xie J, Ding C, Li J, Wang Y, Guo H, Lu Z, et al. Characteristics of patients with coronavirus disease (COVID-19) confirmed using an IgM-IgG antibody test. Journal of medical virology. 2020;92(10):2004-10.
16. Ulubay G, Dilektaşlı AG, Börekçi Ş, Yıldız Ö, Kıyan E, Gemicioğlu B, et al. Turkish Thoracic Society Consensus Report: Interpretation of Spirometry. Turkish thoracic journal. 2019;20(1):69-89.
17. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet Respiratory medicine. 2020;8(4):420-2.
18. Hanley B, Lucas SB, Youd E, Swift B, Osborn M. Autopsy in suspected COVID-19 cases. Journal of clinical pathology. 2020;73(5):239-42.
19. Pan Y, Guan H, Zhou S, Wang Y, Li Q, Zhu T, et al. Initial CT findings and temporal changes in patients with the novel coronavirus pneumonia (2019-nCoV): a study of 63 patients in Wuhan, China. European radiology. 2020;30(6):3306-9.
20. Bell TJ, Brand OJ, Morgan DJ, Salek-Ardakani S, Jagger C, Fujimori T, et al. Defective lung function following influenza virus is due to prolonged, reversible hyaluronan synthesis. Matrix biology : journal of the International Society for Matrix Biology. 2019;80:14-28.
21. Venkataraman T, Frieman MB. The role of epidermal growth factor receptor (EGFR) signaling in SARS coronavirus-induced pulmonary fibrosis. Antiviral research. 2017;143:142-50.

22. Kuang D, Xu SP, Hu Y, Liu C, Duan YQ, Wang GP. [Pathological changes with novel coronavirus infection in lung cancer surgical specimen]. Zhonghua bing li xue za zhi = Chinese journal of pathology. 2020;49(5):471-3.

23. Pernazza A, Mancini M, Rullo E, Bassi M, De Giacomo T, Rocca CD, et al. Early histologic findings of pulmonary SARS-CoV-2 infection detected in a surgical specimen. Virchows Archiv : an international journal of pathology. 2020;477(5):743-8.

24. Tian S, Hu W, Niu L, Liu H, Xu H, Xiao SY. Pulmonary Pathology of Early-Phase 2019 Novel Coronavirus (COVID-19) Pneumonia in Two Patients With Lung Cancer. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2020;15(5):700-4.

25. Cai Y, Hao Z, Gao Y, Ping W, Wang Q, Peng S, et al. Coronavirus Disease 2019 in the Perioperative Period of Lung Resection: A Brief Report From a Single Thoracic Surgery Department in Wuhan, People's Republic of China. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2020;15(6):1065-72.

26. Zha L, Shen Y, Pan L, Han M, Yang G, Teng X, et al. Follow-up study on pulmonary function and radiological changes in critically ill patients with COVID-19. The Journal of infection. 2021;82(1):159-98.

27. Zhao YM, Shang YM, Song WB, Li QQ, Xie H, Xu QF, et al. Follow-up study of the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery. EClinicalMedicine. 2020;25:100463.

28. Mo X, Jian W, Su Z, Chen M, Peng H, Peng P, et al. Abnormal pulmonary function in COVID-19 patients at time of hospital discharge. The European respiratory journal. 2020;55(6).

29. Liu K, Zhang W, Yang Y, Zhang J, Li Y, Chen Y. Respiratory rehabilitation in elderly patients with COVID-19: A randomized controlled study. Complementary therapies in clinical practice. 2020;39:101166.

30. Frija-Masson J, Debray MP, Gilbert M, Lescure FX, Travert F, Borie R, et al. Functional characteristics of patients with SARS-CoV-2 pneumonia at 30 days post-infection. The European respiratory journal. 2020;56(2).

31. Bennett S, Tafuro J, Mayer J, Darlington D, Wong CW, Muntean EA, et al. Clinical features and outcomes of adults with coronavirus disease 2019: A systematic review and pooled analysis of the literature. International journal of clinical practice. 2020:e13725.

32. Karaaslan T, Karatoprak C, Karaaslan E, Sasak Kuzgun G, Gunduz M, Sekerci A, et al. Markers predicting critical illness and mortality in COVID-19 patients: A multi-centre retrospective study. Ann Clin Anal Med. 2021;12:159-65.

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Validation of the modified nutric score on critically ill patients with acute exacerbations of chronic obstructive pulmonary disease: A retrospective study

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ABSTRACT

Objective: In critical care patients, the nutritional status is related to many factors such as existing co-morbidities, nutritional history, and the current disease. It is crucial to apply a comprehensive nutritional assessment and to start nutritional support as soon as possible in intensive care unit(ICU) where malnutrition is common. There are many studies on the association between modified Nutritional Risk in Critical Patients (mNUTRIC) score and outcome in ICU patients but the effectiveness of tools for risk assessment is still remains unclear. We aimed to define the correlation between the mNUTRIC score and 28-day mortality in patients with chronic obstructive pulmonary disease (COPD) in ICU.

Materials and Methods: The admission of COPD patients to the respiratory ICU in 2018 were determined retrospectively. Demographic data of all patients, body mass index (BMI), mNUTRIC scores, Acute Physiology and Chronic Health Assessment II (APACHE II), Sequential Organ Failure Assessment (SOFA) scores, Charlson Comorbidity Index (CCI), time from patient ward to ICU admission, sepsis parameters including C-reactive protein (CRP) and procalcitonin, ICU length of stay (LOS ICU), vasopressor use, and 28-day mortality were recorded.

Results: 159 COPD patients were involved in the study. Age, CCI, day from patient ward to ICU admission, SOFA score, APACHE II score and 28-day mortality were detected to be statistically higher in patients with mNUTRIC $\geqslant 5$ ($p < 0.05$).

Conclusion: The mNUTRIC score could be an proper method for nutritional risk to predict prognosis in critically ill COPD patients.

Keywords: Modified NUTRIC score, Chronic Obstructive Pulmonary Disease, Nutritional risk, Intensive care unit, 28-Day mortality

INTRODUCTION

Malnutrition in critical patients adversely affects the course of the intensive care unit (ICU) patients and also related with poor outcomes (1, 2). In patients ICU admission, the nutritional status is related to many factors such as existing co-morbidities, nutritional history, and the current disease requiring ICU. This is associated with a 5-25% loss of lean body mass, depending on the severity of the current clinical condition, within 10 days after admission to ICU (2, 3).

It is crucial to apply a comprehensive nutritional assessment and to start nutritional support as soon as possible in ICU where malnutrition is common. Although many nutritional assessment tools are practiced in clinical setting, the effectiveness of these tools is still controversial (2, 4, 5).

Various nutritional risk assessment tools such as Nutritional Risk in Critical Patients (NUTRIC) score, malnutrition universal screening tool (MUST), Nutritional risk assessment (NRS-2002) have been employed in critical patients (2, 3). MUST score is comprehended body mass index (BMI), in past six months percentage of weight loss, and disease effect. The NUTRIC score, first enhanced particularly for patients in ICU; to recognize who would advantage from aggressive nutrition by correlating starvations, inflammation, and consequences (6).

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Initially, the parameters forming the NUTRIC score included IL6 level, but due to the difficulty and high cost of studying this parameter in the clinical setting, the modified NUTRIC (mNUTRIC) score was determined by removing IL6. The NUTRIC score consists of five parameters including age, comorbidities, length of stay ICU, Acute Physiology and Chronic Health Assessment II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores.

Many studies investigate the association between mNUTRIC score and outcome in heterogenous ICU patients. Chronic Obstructive Pulmonary Disease (COPD) is one of the most important chronic diseases which cause significant mortality and morbidity worldwide. Poor nutritional status is very common in COPD, and it affects the course of disease in negative way. Admission to ICU is quite common in COPD patients due to acute attack, and this situation, when combined with poor nutritional status, pessimistically affects the outcomes of the patients.

In this study, we aimed to define the correlation between the mNUTRIC score and 28-day mortality in COPD patients who have ICU admission due to acute exacerbations of COPD (AECOPD). Secondary aim of this study was to evaluate the effect of MUST, NRS-2002, and other severity risk scoring system commonly used in ICU in these patients.

MATERIALS and METHODS

After ethical committee approval (04/19/2019-624) this study was conducted with the data analysis of critically ill AECOPD patients admitted to the respiratory ICU in 2018. We obtained informed consent from the patient or the legally responsible relatives. The data were collected from the medical records of the patients.

Inclusion criteria determined as; patients with a diagnosis of COPD, and admitted to ICU due to AECOPD. Patients who were hospitalized from another center or transferred to another center for any reason, which had multiple comorbidities like malignancy, who had multiple admission to ICU, and who received mechanical ventilation (MV) less than 24 hours were excluded from the study (**Figure 1**).

Demographic data of all patients, Charlson Comorbidity Index (CCI), time from patient ward to ICU admission, sepsis parameters including procalcitonin and C-reactive protein (CRP), body mass index (BMI), parameters used in mNUTRIC score, ICU length of stay (LOS ICU), vasopressor use, and 28-day mortality were recorded. We also determined the MUST, NRS-2002, and mNUTRIC scores.

Physicians calculated the mNUTRIC and MUST score for all patients and mNUTRIC score of above and below 5 were standardized. Malnutrition risk was considered high in patients with mNUTRIC score $\geqslant 5$.

Statistical Analysis

Data analyses were performed by using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variables was normal or not was determined by Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean ± standard deviation for normal distributions, and median (minimum - maximum value) for skewed distributions. Categorical data were described as number of cases (%).

Statistical analysis differences in normally distributed variables between two independent groups were compared by Student's t test, and Mann Whitney U test were applied for comparisons of the not normally distributed data. Categorical variables were compared using Pearson's Chi-Square test or Fisher's exact test.

First of all it was used univariate logistic regression with risk factors that is thought to be related with mortality. Risk factors that has p-value < 0.25 one variable logistic regression was included to model on multivariable logistic regression. Whether every independent variables were significant on the model was analysed with Wald statistic. It was evaluated with Nagelkerke R2 how much independent variable explained dependent variable. Besides, it was evaluated model adaptation of estimates with Hosmer and Lemoshow model adaptation test. Receiver operating characteristic (ROC) curve analysis was used to determine the cut-off points. It was accepted p value < 0.05 as a significant level on all statistical analyses.

RESULTS

This study was conducted with the data analysis of 351 critical ill patients in respiratory ICU in 2018. The 235 of these had history of COPD. The 76 of these patients did not meet the inclusion criteria and were excluded from the study. The 100 (62.9%) males and 59 (37.1%) females were involved in the study, and the mean age was 70.92 ± 11.11 years.

Age, CCI, day from patient ward to ICU admission, SOFA score, APACHE II score, MUST score, NRS-2002 and 28-day mortality were found to be statistically significantly higher in those with mNUTRIC $\geqslant 5$ ($p<0.05$) , (**Table 1**).

When patients evaluated in terms of 28-day mortality, the mNUTRIC score ($p < 0.002$), MUST ($p < 0.001$), SOFA score ($p<0.001$), APACHE II ($p<0.001$) score, CRP ($p < 0.002$), and procalcitonin ($p< 0.020$) was found statistically significantly higher (**Figure 2**), (**Table 2**). The MV day was not statistically significant in terms of 28-day mortality ($p > 0.072$), (**Table 2**).

The logistic regression (LR) analysis was utilized to evaluate the factors affecting 28-day mortality. Variables with $p < 0.25$ as a result of univariate analysis were applied to the multivariate analysis. Backward LR method was used in multivariate analysis. The values indicated in the Table 3 belong to the sixth step, which is the last step. Here the interpretation is made according to the results of multiple analyzes, $p < 0.05$ are considered significant. The Nagelkerke R2 is desired to be between 0.20 and 0.40, because it is in this range, it is understood that the model established is meaningful, and $p > 0.05$ in the Hosmer and Lemoshow test, and the model has a good fit with the data. SOFA score and CRP value appear to have an effect on mortality. Increasing the SOFA score by one unit increases the risk of mortality 2.469 times. One unit increase in CRP increases the risk of mortality by 1,058 times.

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The ROC analysis for mortality, the area under the process characteristic curve (AUC) in terms of mNUTRIC score was calculated as 0.741, and the mNUTRIC score was statistically significant in determining mortality in cases. In order to answer the question of which value should be taken as the cut-off value for this test, each sensitivity and specificity values given as a result of the analysis were examined and the optimum point was chosen. The cut-off value was calculated as 5.5 with a sensitivity of 75.5% and a specificity of 65.1%. It shows that the risk of mortality was higher in patients with mNUTRIC score above 5.5 (sensitivity %75.5 and specificity %65.1) (**Figure 3**).

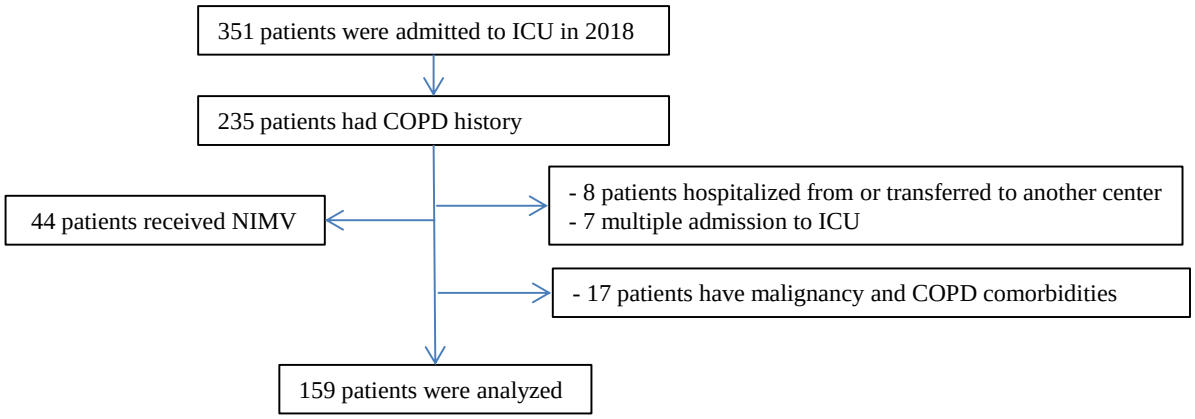

Figure 1: Flow chart of the patients

Table 1: Demographic and clinical characteristic of patients

		mNUTRIC Score		P
		<5 (n:46)	≥5 (n:113)	
Age (years)		63.43 ± 7.76	73.97 ± 10.85	**< 0.001**
BMI(kg/m^2)		24.5(13.3 - 40.6)	26.0(13.8- 49.9)	0.149
Gender	**Male n(%)**	30 (65.2%)	70 (61.9%)	0.699
	Female n(%)	16 (34.8%)	43 (38.1%)	
CCI		4 (2 - 7)	6 (3 - 13)	**< 0.001**
ICU LOS(day)		4 (2 - 20)	5 (2 - 50)	0.246
Days from ward to ICU		2.72 ± 4.01	4.60 ± 6.90	0.034
Vasopressor use		7 (15.2%)	33 (29.2%)	0.065
MV(day)		4 (1 - 21)	5 (1 - 50)	0.298
CRP (mg/L)		3.10(0.08-33.6)	3.74 (0.01-34)	0.770
Procalcitonin(ng/ml)		0.16 (0.01-14.5)	0.26 (0.01-97)	0.266
SOFA Score		5 (4 - 7)	6 (4 - 12)	**< 0.001**
APACHE II Score		17 (10 - 27)	23 (12 - 43)	**< 0.001**
MUST Score		10 (21.7%)	83 (73.5%)	**< 0.001**
NRS -2002		4 (2 - 6)	5 (3 - 6)	**0.004**
28-day mortality		7 (15.2%)	46 (40.7%)	**0.002**

mNUTRIC: Modified Nutritional Risk in Critical Patients. **BMI:** Body mass index**, CCI:** Charlson Comorbidite Index**, ICU:** Intensive care unit**, LOS:** lenght of stay**, MV:**mechanical ventilation**, CRP:** C-reactive protein**, SOFA:** Sequential Organ Failure Assessment**, APACHE II:** Acute Physiology and Chronic Health Assessment II**, MUST:** Malnutrition universal screening tool**, NRS- 2002:** Nutrition risk screening

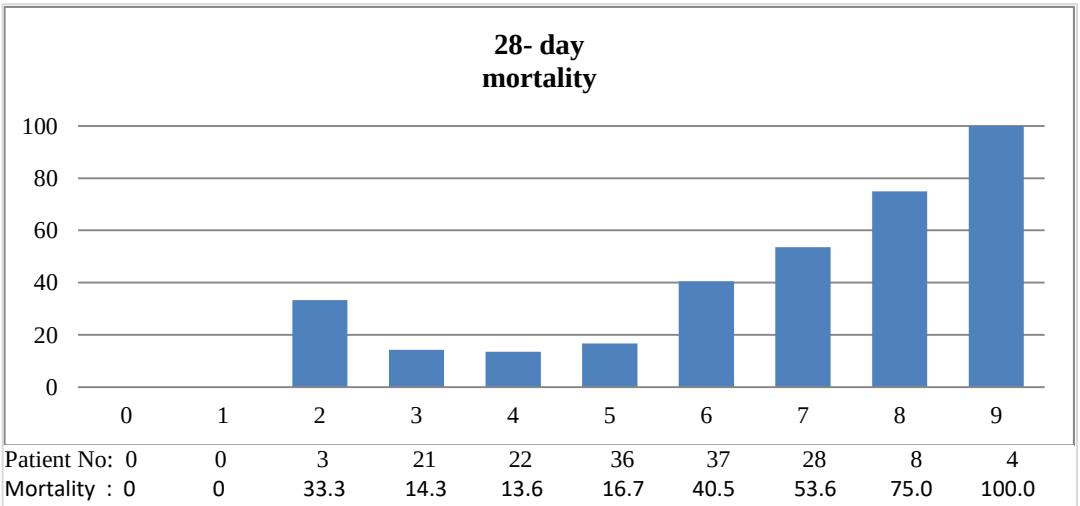

Figure 2: Correlation between mNUTRIC score and 28-day mortality

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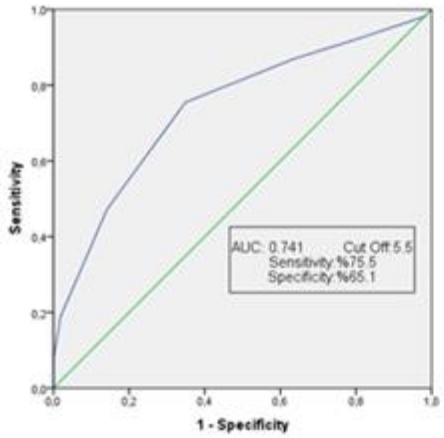
Table 2: Correlation between 28-day mortality and scoring systems, MV day, CRP, and procalcitonin

	28 Day Mortality		
	No Mortality	**Mortality**	**P**
mNUTRIC Score	67 (63.2%)	46 (86.8%)	**0.002**
MUST Score	52 (49.1%)	41 (77.4%)	**0.001**
SOFA Score	5 (4 - 9)	7 (4 - 12)	**<0.001**
APACHE II Score	20.05 ± 4.79	25.40 ± 6.90	**<0.001**
MV(day)	4 (1 - 50)	5 (2 - 34)	0.072
CRP(mg/L)	2.38(0.01-33.60)	5.05 (0.05-34)	**0.002**
Procalcitonin(ng/ml)	0.20 (0.01-14.50	0.30 (0.01-97)	**0.020**

mNUTRIC: Modified Nutritional Risk in Critical Patients, **MUST**: Malnutrition universal screening tool, **SOFA**: Sequential Organ Failure Assessment, **APACHE II:** Acute Physiology and Chronic Health Assessment II, **MV**: mechanical ventilation, **CRP**: C-reactive protein



Area	Std. Error	p	Confidence Interval %95	Cut off	Sensitivity	Specificity
0.741	0.043	<0.001	0.656 – 0.826	5.5	%75.5	%65.1

Figure 3: The ROC analysis for mortality, the area under the process characteristic curve (AUC) in terms of mNUTRIC score

DISCUSSION

In this study, we defined that the mNUTRIC score could be a risk assessment tool for critically ill AECOPD patients to predict mortality. We also found that MUST, SOFA, APACHE II scores, CRP, and procalcitonin affect the 28-day mortality like mNUTRIC score.

Undernourished status is quite common in COPD patients, and this situation affects approximately one-third of patients which are associated with poor outcomes (7). Therefore, many critically ill AECOPD patients are undernourished in ICU or on the ward. In such cases, that is important to be able to identify who would benefit from adequate nutritional support. Clinicians should decide early whether the patient needs nutritional support. Even if different nutritional estimation tools have been used in clinical practice, the mNUTRIC score is an important scoring system that can use to evaluate the risk of malnutrition recently. Furthermore, it is a useful prewarning marker (2, 4, 6). Ozbilgin et al. determined that the mNUTRIC score was a good predictor of both mortality and morbidity in the postoperative acute care unit (8).

Although studies on mNUTRIC were conducted in heterogeneous patient groups, we also observed that the mNUTRIC score was an effective parameter in predicting mortality in our study involving AECOPD patients.

Since the severity of the disease in the ICU also negatively affected nutrition, those with a mNUTRIC score of 5 and above had higher ICU severity scores including APACHE II and SOFA. The mNUTRIC score can be a useful tool for optimizing clinical nutrition practices in the ICU setting and evaluating patients' response to nutritional support.

The LOS ICU and duration of MV had been studied by the researchers (9-11). Mendes et al. (9) found that, patients with high mNUTRIC scores had a long LOS ICU and high mortality. Moretti et al. (10) also found similar results in a study they conducted. Rahman et al (11) suggested; patients with high mNUTRIC scores had longer LOS ICU and the mortality rate was 31% in this group. In our study, in addition to these parameters, we also evaluated the duration of ward to ICU. Mortality rate and duration of the ward to ICU were higher in patients mNUTRIC score $\geqslant$ 5, but LOS ICU was

similar in both groups. Ward to ICU time was also evaluated in our patients in order to assess the ongoing poor nutritional status of COPD patients. Loss of muscle and fat mass in COPD patients is a natural consequence of chronic long-term illness. In addition to poor nutritional status, particularly in COPD patients, adequate nutritional support might be underestimated during hospitalization. As seen in our results, we observed that patients with high mNUTRIC scores also had longer hospital ward stay. This suggests that, especially in COPD patients who admitted to ICU, it would be appropriate to consider the LOS hospital when nutritional support is determined.

This study showed that MUST, mNUTRIC, APACHE II, SOFA scores, CRP, and procalcitonin value influenced the mortality. Already, the mNUTRIC scoring system includes the SOFA score, which is used to determine the risk of organ dysfunction and death in ICU patients (**12**). The study by Coltman suggested that ICU severity scores (APACHE II and SOFA) were important factors like mNUTRIC score contributing to LOS ICU (**4**). Therefore, the correct identification of malnourished patients using the mNUTRIC score provides a more appropriate application of nutritional support and can thus reduce LOS.

Ping Zhang et al. (**13**) accomplished a study on Coronavirus Disease 2019 (COVID-19) patients, and they found that 28-day mortality was higher in patients with a high nutritional risk score in ICU admission. Kalaiselvan and colleagues (**14**) studied on ICU patients who need MV and they found that nearly half of MV patients are at nutritional risk, and high mNUTRIC scores increases LOS ICU and mortality. Our study indicated that, there is a high nutritional risk in COPD patients admitted to the ICU and higher mNUTRIC scores increase 28-day mortality.

We have several limitations in this study. First this study was retrospective study and the representation of the groups with high and low mNUTRIC scores had a limited number. In addition, mNUTRIC score calculation was based solely on the clinically specified by the physicians, and a large group of COPD patients receiving non-invasive MV was excluded.

CONCLUSION

The intensive care severity scores and 28-day mortality rates increase in critically COPD patients with high mNUTRIC score. Malnutrition due to sepsis affects critically ill patients even more negatively. As a result, the mNUTRIC score may be an appropriate tool for nutritional risk assessment and prognosis prediction in critically ill AECOPD patients.

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REFERENCES

1. Heyland DK, Cahill N, Day AG. Optimal amount of calories for critically ill patients: depends on how you slice the cake! Crit Care Med. 2011 Dec; 39(12):2619-2626.
2. de Vries MC, Koekkoek WK, Opdam MH, van Blokland D, van Zanten AR. Nutritional assessment of critically ill patients: validation of the modified NUTRIC score. Eur J Clin Nutr. 2018 Mar;72(3):428-435.
3. Puthucheary ZA, Rawal J, McPhail M, Connolly B, Ratnayake G, Chan P, et al. Acute skeletal muscle wasting in critical illness. JAMA. 2013 Oct 16; 310(15):1591-1600.
4. Coltman A, Peterson S, Roehl K, Roosevelt H, Sowa D. Use of 3 tools to assess nutrition risk in the intensive care unit. JPEN J Parenter Enteral Nutr. 2015 Jan;39(1):28-33.
5. Lew CCH, Yandell R, Fraser RJL, Chua AP, Chong MFF, Miller M. Association Between Malnutrition and Clinical Outcomes in the Intensive Care Unit: A Systematic Review. JPEN J Parenter Enteral Nutr. 2017 Jul;41(5):744-758.
6. Heyland DK, Dhaliwal R, Jiang X, Day AG. Identifying critically ill patients who benefit the most from nutrition therapy: The development and initial validation of a novel risk assessment tool. Crit Care. 2011;15(6):1-11.
7. Holdoway A, J Banner, and B Bostock. Managing Malnutrition in COPD. BAPEN 2020.
8. Ozbilgin S, Hanci V,Omur D, Ozbilgin M, Tosun M, Yurtlu S, et al. Morbidity and mortality predictivity of nutritional assessment tools in the postoperative care unit. Medicine 2016, 95(40).
9. Mendes R, Policarpo S, Fortuna P, Alves M, Virella D, Heyland DK. Nutritional risk assessment and cultural validation of the modified NUTRIC score in critically ill patients-A multicenter prospective cohort study. J Crit Care 2017;37: 245-249.
10. Moretti D, Bagilet DH, Buncuga M, Settecase CJ, Quaglino MB, Quintana R. Study of two variants of nutritional risk score "NUTRIC" in ventilated critical patients.Nutr Hosp2014;29(1):166-172.
11. Rahman A, Hasan RM, Agarwala R, Martin C, Day AG, Heyland DK. Identifying critically ill patients who will benefit most from nutritional therapy: Further validation of the "modified NUTRIC" nutritional risk assessment tool. Clin Nutr 2016;35(1):158-62.
12. Medlej K. Calculated decisions: sequential organ failure assessment (SOFA) score. Emerg Med Pract 2018;20(Suppl 10):CD1-CD2 .
13. Ping Zhang, Zhigang He, Gang Yu, Dan Peng, Yikuan Feng, Jianmin Ling et al.The modified NUTRIC score can be used for nutritional risk assessment as well as prognosis prediction in critically ill COVID-19 patients. Clinical Nutrition 2021, 40(2): 534-541.
14. Kalaiselvan MS, Renuka MK, Arunkumar AS. Use of nutrition risk in critically ill (NUTRIC) score to assess nutritional risk in mechanically ventilated patients: a prospective observational study. Indian J Crit Care Med 2017;21(5):253.

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The effect of vitamin K1 on VEGF levels in chick embryos with type 1 diabetes and Diabetic Retinopathy induced by streptozotocin

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ABSTRACT

Objective: Hyperglycemia caused by Diabetes Mellitus (DM) is associated with long-term dysfunction such as diabetic retinopathy (DRP). The most effective growth factor in the development of DRP is the vascular endothelial growth factor (VEGF). Vitamin K1 reduces hyperglycemia and prevents the development of DM. In this study, we aimed to create streptozotocin (STZ) induced DM and DRP in chick embryos and to show whether vitamin K1 can prevent early-stage DRP by measuring VEGF levels.

Material and Methods: The 140 specific pathogen-free (SPF) fertilized chicken eggs were used in this study. Three different STZ doses were administered to 120 SPF eggs for an induced DM model. Three different vitamin K1 doses were administered in each STZ dose group. On the 12th day and 18th day the remaining 20 SPF eggs were separated as control groups. On the 18th-day, blood glucose, blood insulin and VEGF levels were measured.

Results: 0.45 mg/egg STZ dose (STZ3) was determined as the optimal/ideal dose for the DM model. When the group-administered STZ3 and vitamin K1 were evaluated among themselves; it was determined that there were significant changes in blood glucose, blood insulin, VEGF levels of the STZ3+K1-3 group compared to the STZ3+K1-1 and STZ3+K1-2 groups ($p<0.05$).

Conclusion: Vitamin K1 increased blood insulin levels and decreased blood glucose levels. When hyperglycemia reduced, the VEGF levels reduced. Vitamin K1 may protect from DRP by reducing VEGF levels.

Keywords: Chick Embryo, Type 1 Diabetes Mellitus, Vascular Endothelial Growth Factor, Vitamin K1

INTRODUCTION

Diabetic retinopathy (DRP) is the most common complication of diabetes mellitus (DM) (1). The two main changes seen in DRP due to glucose metabolism disorder are increased vascular permeability and micro vascular occlusion. Blocking blood flow due to capillary occlusion causes retinal hypoxia and ischemia. Ischemia in the retina causes abnormal new vessel formation (neovascularization). The new vessel formation seen in DRP is driven by growth factors released from hypoxic retinal tissue (2–5). The primary growth factor responsible for these vascular changes seen in DRP is the vascular endothelial growth factor (VEGF) (6). Hypoxia is the major stimulus for VEGF expression (7). Because of retinal hypoxia and ischemia, VEGF levels increase in retinal and ocular fluids in patients with DRP (8). VEGF increases vascular permeability against macromolecules, increases monocyte chemotaxis and tissue factor production, causing diabetic micro vascular complications and DRP (9). Vitamin K is a vitamin that has two biologically active forms, phylloquinone (vitamin K1) and menaquinone (vitamin K2) (10). In previous studies, it has been reported that vitamin K1 reduces hyperglycemia and insulin resistance, has a hypoglycemic effect and prevents the development of type 2 DM (11–14).

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The aim of this study was to create streptozotocin (STZ)-induced early onset DM and DRP in chick embryos and to show whether vitamin K1 can prevent early-stage DRP by measuring VEGF levels.

MATERIALS and METHODS

Ethics committee approval was obtained from Afyon Kocatepe University Animal Experiments Local Ethics Committee with the decision dated 07.10.2019 and numbered 49533702/122.. The experimental phase and morphological analysis of the study were carried out in Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Anatomy.

A total of 140 specific pathogen free (SPF) fertilized eggs were used in this study. After all the eggs were provided by the research coordinator, they were placed in the incubator in Anatomy laboratory. SPF eggs were kept in the incubators at 70% humidity and 37.5 ^{0}C. STZ (N-(Methyl Nitroso Carbamoil)-a-D-glucosamine, CAS Number: 18883-66-4, Sigma-Aldrich Chemie GmbH, Germany) was dissolved in saline and a stock STZ solution was prepared. STZ doses given to chick embryos were determined according to the literature (**15–18**). There were 14 groups in total, with 10 SPF fertilized eggs in each group. The first group was the control group, whose blood glucose and blood insulin levels were measured on the 12th day. There were three main groups in which only STZ was applied on the 12th day (STZ1, STZ2, STZ3). It was desired to determine the most appropriate dose of STZ by applying three different doses of STZ. STZ1 dose was 0.15 mg/egg, STZ2 dose was 0.30 mg/egg, STZ3 dose was 0.45 mg/egg.

Nine more groups were formed to administer three different doses of vitamin K1(Konakion 10 mg/ml, Roche) to each group, to which STZ1, STZ2, and STZ3 doses were to be administered. Vitamin K1-1 dose was 0.005 mg/egg (0.1 mg/kg), Vitamin K1-2 dose was 0.025 mg/egg (0.5 mg/kg), Vitamin K1-3 dose was 0.050 mg/egg (1 mg/kg). One more control group was formed to measure blood glucose and blood insulin levels on the 18th day.

On the 12th day, the control group SPF eggs were sterilized by rubbing 70% ethyl alcohol on the egg shell and opened from the upper part of the air sac. The inner shell membrane was carefully removed and at least 200 µl of chick embryo blood was taken from the thickest vessel under it with 30GX13 mm diameter mesotherapy needle attached to the tip of the insulin injector. Insulin levels were measured by means of the chick embryo insulin kit (INS ELISA KIT, BT-LAB/EA0012Ch) from these blood samples. Then, another thick vessel was found under the inner shell membrane and a blood glucose strip used for glucose measurement was placed under the vessel in a way that would not damage the structures. Afterwards, the vessel was gently lifted with a strip to allow blood circulation in the vessel. The amniotic fluid around the vessel was cleaned with the cotton part of the ear cleaner so as not to damage the vessel. The vessel on the strip was fragmented through the mesotherapy needle tip. From the blood, dispersed on the strip, glucose level from was measured as mg/dl with the Accu-check blood glucose meter.

On the 12th day, the egg shells of the groups to be made Type1 DM with three different STZ doses were sterilized by applying 70% ethyl alcohol. Then, 0.5 mm hole was drilled through the egg shell on the air sac, through which the Hamilton needle could pass, and STZ at a dose of 0.15 mg/egg (STZ1, 40 eggs) was administered to the first group, and 0.30mg/egg STZ (STZ2, 40 eggs) to the second group. STZ was administered to the third group at a dose of 0.45 mg/egg (STZ3, 40 eggs). After STZ injections, the hole opened in the egg shell was closed with tape so that the egg would not get air. Groups (10+10+10=30 eggs) that were administered only STZ1, STZ2, STZ3 doses were put back into the incubator to be opened on the 18th day.

The remaining 30 eggs in each STZ group (STZ1, STZ2, STZ3) were divided into 3 subgroups. In the STZ1, STZ2, STZ3 groups, vitamin K1-1 at a dose of 0.005 mg/egg for the first 10 eggs, vitamin K1-2 at a dose of 0.025 mg/egg for the second 10 eggs, and vitamin K1-3 at a dose of 0.050 mg/egg for the third 10 eggs, administered via insulin injector and it was closed with a tape so that no air could enter. The eggs, which were closed with tape, were placed back in the incubator. On the 18th day, control group, STZ and vitamin K1 applied groups were sterilized by rubbing 70% ethyl alcohol on the egg shell and opened from the upper part of the air sac. With the same blood sampling techniques applied to the control group on the 12th day; blood glucose levels, blood insulin levels and VEGF levels were measured of STZ and vitamin K1 applied groups.

On the 18th day, the eyes of the chick embryos were centrifuged at 1500 rpm for 10 minutes in the Biochemistry department. Chicken vascular endothelial cell Growth Factor, VEGF ELISA Kit (BT-LAB/E0223Ch) was used to measure VEGF levels and studied with ELISA method.

Statistical analysis

Statistical analysis was performed with the IBM Statistical Package for the Social Sciences program. Kolmogorov Smirnov test was used to determine the normal distribution of data. Kruskal-Wallis test was used to compare the groups since the data were not normally distributed and $n \leqslant 30$. Dunn test were employed as post-hoc tests and $p<0.05$ were considered significant. Pearson's correlation analysis was used to determine whether there is a linear relationship between blood glucose and blood insulin measurements, and if so, the direction and severity of this relationship. Mean statistical values were expressed as Mean±Standard Deviation (Mean±SD).

RESULTS

In this study, the effect of different doses of STZ on the development of DM were examined in the firstly. The most appropriate dose of STZ for the DM model in chick embryos was tried to be determined. Then, three different doses of vitamin K1 were given to chick embryos and their effects on VEGF levels were examined.

Mean blood glucose levels of chick embryos opened on the 12th day were 124.70±12.3 mg/dL and blood insulin levels were 2.05±0.11 mIU/L. Mean blood glucose levels of the control group chick embryos opened on the 18th day were 133.90±9.12 mg/dL, and blood insulin levels were

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5.88±2.01mIU/l. The mean blood glucose and insulin levels of the groups that were administered only STZ and tried to be DM on the 18th day are given in Table 1. According to these results, 0.45 mg/egg STZ dose (STZ3) was determined as the optimal/ideal dose for the DM model in our study.

The mean blood glucose levels, blood insulin levels and VEGF levels of the groups given vitamin K1 to prevent DRP after STZ application are given in Table 2.

When the group administered STZ3 and vitamin K1 were evaluated among themselves; it was determined that there were significant changes in blood glucose, blood insulin, VEGF levels of the STZ3+K1-3 group compared to the STZ3+K1-1 ($p<0.001$, $p<0.001$, $p<0.001$, respectively) and STZ3+K1-2 ($p<0.05$, $p<0.05$, $p<0.05$, respectively) groups. There was no significant difference in blood glucose, blood insulin and VEGF levels between STZ3+K1-1 and STZ3+K1-2 groups ($p=0.061$, $p>0.05$, $p=0.089$, respectively).

DISCUSSION

In this study, we aimed to determine whether vitamin K1 has a DRP-reducing effect in chick embryos induced with STZ-induced Type 1DM by measuring VEGF levels. In literature, there are few chick embryo DM models induced by STZ in the literature and they used different STZ doses and different incubation days (15–18). In literature, STZ application day changed by 12th day to 14th day. We determined to inject STZ on the 12th day. The purpose of injecting STZ on the 12th day of incubation is completing the development of the pancreas, which started on the 5th day, by the 12th day (16).

When we searched the literature, Yoshiyama et al. stated in their study that 0.3 mg/egg STZ dose is the ideal dose to increase blood glucose levels and decrease serum insulin levels (17). Sivajothi et al., like the study of Yoshiyama et al., created DM model with dose of 0.3 mg/egg STZ (16). In the study of Shi et al., three different doses of STZ ranging from 250 to 300 mg/kg/egg were used (15).

Therefore, we used different doses of STZ on the 12th day of incubation to create a DM model. As in our previous DM model study (18), STZ3 dose statistically significantly increased both blood glucose levels and decreased blood insulin levels compared to the other STZ1 and STZ2 doses. We determined that the STZ3 dose was the most applicable dose to create a DM model in chick embryos.

In addition, serum insulin levels could not be measured until the 12th day of chick embryo development in the literature (16). We think that this is due to the small, fragile chick embryo vessels and insulin kit. However, we easily measured blood insulin levels. We determined that by using Atay et al.'s blood sampling technique (19) and chick embryo insulin kit (INS ELISA KIT, BT-LAB/EA0012Ch) blood insulin levels can be measured.

Vitamin K1 is the primary circulating form of Vitamin K and has been successfully measured worldwide in various population-based and clinical-based studies to assess circulating Vitamin K status. In previous studies, it has been shown that dietary vitamin K1 reduces hyperglycemia and reduces the risk of type 2 DM (20). In the study of Zwakenberg et al., higher circulating vitamin K1 levels were found to be causally associated with a lower risk of type 2 DM, emphasizing the importance of adequate vitamin K1 in the human diet (21).

In the study of Dihinga et al. in type 2 DM mice; they divided the mice with type 2 DM into two groups and gave only olive oil to the first group and olive oil and Vitamin K1 to the other group. They found that vitamin K1 decreased body weight, basal glucose and insulin levels, glycated hemoglobin A1c (HbA1C) and homeostasis model assessment-estimated insulin resistance (HOMA-IR) levels dose-dependently in the group receiving vitamin K1 compared to the control group (20). In the study of Ibarrola-Jurado et al., it was shown that higher dose vitamin K1 intake was associated with a decrease in the risk of new onset type 2 DM.

Table 1. Control and STZ groups' mean blood glucose and insulin levels

Groups	Mean blood glucose levels (mg/dL)	Mean blood insulin levels (mIU/L)
18th day control	133.90±9.12	5.88±2.01
18th day STZ1	151.30±9.15	5.34±2.11
18th day STZ2	178.80±9.89[a,b]	4.83±1.62
18th day STZ3	191.56±17.74[a,b]	3.21±1.18[a]

aThere was a statistically significant difference with the control group. $p < 0.05$, Kruskal-Wallis test, Dunn test as post-hoc test.
bThere was a statistically significant difference with ASM 1 group. $p < 0.05$, Kruskal-Wallis test, Dunn test as post-hoc test.

Table 2. After vitamin K1, mean blood glucose, insulin and VEGF levels of STZ groups

Groups	Mean blood glucose levels (mg/dL)	Mean blood insulin levels (mIU/L)	VEGF levels
18th day control	133.90±9.12	5.88±2.01	41.3
STZ1+Vitamin K1-1	150.90±8.65	5.36±1.95	48.37
STZ1+Vitamin K1-2	136.76±9.08	5.81±1.76	40.33
STZ1+Vitamin K1-3	130.89±7.56*	5.91±1.54*	38.27*
STZ2+Vitamin K1-1	179.90±13.15	4.81±1.1	54.27
STZ2+Vitamin K1-2	165.12±8.92	5.21±0.9	48.12
STZ2+Vitamin K1-3	134.90±7.15*	5.75±0.8*	39.59*
STZ3+Vitamin K1-1	189.21±12.36	3.69±2.32	65.76
STZ3+Vitamin K1-2	165.00±12.57	4.86±3.35	58.28
STZ3+Vitamin K1-3	141.01±5.23*	5.59±1.31*	41.1*

*In each STZ group, according to the dose of vitamin K1; there is a significant difference in blood glucose, insulin and VEGF levels. $p < 0.05$

Moreover, in this study, increased intake of vitamin K1 during follow-up was associated with a 51% lower risk of diabetes in elderly patients at high cardiovascular risk, after a median follow-up of 5.5 years (13). Varsha et al administered STZ to male Wistar rats for three days. Then, they administered vitamin K1 (5 mg/kg, twice a week) to DM treated rats for 2.5 months. At the end of their experiment, the pancreas of the rats was dissected and HbA1C, plasma insulin and islet areas were determined. Varsha et al. found that the treatment of vitamin K1 saved the endocrine pancreas cell from death caused by STZ, and vitamin K1 stimulated islet cell proliferation/regeneration. In addition, they determined that Vitamin K1 caused increased insulin secretion and normal blood glucose and HbA1c levels in diabetic rats. The main findings of this study demonstrated the anti-diabetic mechanism of vitamin K1 (22).

In this study, we applied vitamin K1 at doses of 0.005 mg/egg (0.1 mg/kg), 0.025 mg/egg (0.5 mg/kg), 0.050 mg/egg (1 mg/kg) to chick embryos treated with STZ. Like the literature, we found that vitamin K1 increased blood insulin levels and decreased blood glucose levels. However, in our study, we found that vitamin K1-3 (0.050 mg/egg (1 mg/kg)) was the most effective dose in reducing VEGF, blood glucose levels and increasing blood insulin levels.

We think that vitamin K1 does this by repairing or regenerating pancreatic islet cells. However, we think that larger experimental studies are needed to prove this. In addition, we think that experimental animals larger than chick embryos will need to be used to easily examine pancreatic histology. When hyperglycemia reduces with the effect of vitamin K1, vascular occlusion and hypoxia in the retina reduces. VEGF levels and neovascularization are reduced. So, vitamin K1 protects from new vessel formation and DRP by reducing VEGF levels.

CONCLUSION

These results show that Vitamin K1 increased blood insulin levels and accordingly caused a decrease in blood glucose levels. It also shows that VEGF levels, one of the first indicators of DRP due to hyperglycemia, decreased with Vitamin K1 treatment. In this context, a sufficient dose of Vitamin K1 may prevent DRP by providing glucose homeostasis..

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REFERENCES

1. Wang W, Lo A. Diabetic retinopathy: pathophysiology and treatments. Int J Mol Sci. 2018;19(6):1816.
2. Zhang D, Lv FL, Wang GH. Effects of HIF-1α on diabetic retinopathy angiogenesis and VEGF expression. Eur Rev Med Pharmacol Sci. 2018;22(16):5071–5076.
3. Cheung N, Wong IY, Wong TY. Ocular Anti-VEGF therapy for diabetic retinopathy: overview of clinical efficacy and evolving applications. Diabetes Care. 2014;37(4):900–905.
4. Song SJ, Han K, Choi KS, Ko S-H, Rhee E, Park C-Y, et al. Trends in diabetic retinopathy and related medical practices among type 2 diabetes patients: Results from the National Insurance Service Survey 2006-2013. J Diabetes Investig. 2018;9(1):173–178.
5. Rasier R, Artunay Ö, Yüzbaşioğlu E, Şengül A, Görmüş U, Öncel M, et al. The levels of IL-8 and TNF-A in vitreous samples from patients with diabetic retinopathy. Retina-Vitreus. 2011;19(3):190–193.
6. Kohner EM, Henkind P. Correlation of Fluorescein Angiogram and Retinal Digest in Diabetic Retinopathy. Am J Ophthalmol. 1970;69(3):403–414.
7. Reynolds LE, Wyder L, Lively JC, Taverna D, Robinson SD, Huang X, et al. Enhanced pathological angiogenesis in mice lacking β3 integrin or β3 and β5 integrins. Nat Med. 2002;8(1):27–34.
8. Kijlstra A. The role of cytokines in ocular inflammation. Br J Ophthalmol. 1994;78(12):885–886.
9. Cha DR, Kang YS, Han SY, Jee YH, Han KH, Han JY, et al. Vascular endothelial growth factor is increased during early stage of diabetic nephropathy in type II diabetic rats. J Endocrinol. 2004;183(1):183–194.
10. Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food. Pathophysiol Haemost Thromb. 2000;30(6):298–307.
11. Sai Varsha MKN, Raman T, Manikandan R, Dhanasekaran G. Hypoglycemic action of vitamin K1 protects against early-onset diabetic nephropathy in streptozotocin-induced rats. Nutrition. 2015;31(10):1284–1292.
12. Ohsaki Y, Shirakawa H, Hiwatashi K, Furukawa Y, Mizutani T, Komai M. Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat. Biosci Biotechnol Biochem. 2006;70(4):926–932.
13. Ibarrola-Jurado N, Salas-Salvadó J, Martínez-González MA, Bulló M. Dietary phylloquinone intake and risk of type 2 diabetes in elderly subjects at high risk of cardiovascular disease. Am J Clin Nutr. 2012;96(5):1113–1118.
14. Yoshida M, Booth SL, Meigs JB, Saltzman E, Jacques PF. Phylloquinone intake, insulin sensitivity, and glycemic status in men and women. Am J Clin Nutr. 2008;88(1):210–215.
15. Shi L, Ko ML, Huang CCY, Park SY, Hong MP, Wu C, et al. Chicken embryos as a potential new model for early onset type 1 diabetes. J Diabetes Res. 2014;1–10.
16. Sivajothi V, Dakappa SS. In vitro and in silico antidiabetic activity of pyran ester derivative isolated from Tragia cannabina. Asian Pac J Trop Biomed. 2014;4:455–459.
17. Yoshiyama Y, Sugiyama T, Kanke M. Experimental diabetes model in chick embryos treated with streptozotocin. Biol Pharm Bull. 2005;28(10):1986–1988.
18. Bozkurt E, Atay E, Bilir A, Ertekin A, Koca HB, Sabaner MC. A novel model of early type 1 diabetes mellitus: The chick embryo air sack model. Saudi J Biol Sci. 2021;28(10):5583-5546

19. Atay E, Bozkurt E, Bilir A, Ertekin A, Vurmaz A. Blood glucose analysis with different blood sampling techniques in chick embryos. Int J Acad Med Pharm. 2021;3(3):224–228.

20. Dihingia A, Ozah D, Ghosh S, Sarkar A, Baruah PK, Kalita J, et al. Vitamin K1 inversely correlates with glycemia and insulin resistance in patients with type 2 diabetes (T2D) and positively regulates SIRT1/AMPK pathway of glucose metabolism in liver of T2D mice and hepatocytes cultured in high glucose. J Nutr Biochem. 2018;52:103–114.

21. Zwakenberg SR, Remmelzwaal S, Beulens JWJ, Booth SL, Burgess S, Dashti HS, et al. Circulating phylloquinone concentrations and risk of type 2 diabetes: a mendelian randomization study. Diabetes. 2019;68(1):220–225.

22. Varsha MKNS, Thiagarajan R, Manikandan R, Dhanasekaran G. Vitamin K1 alleviates streptozotocin-induced type 1 diabetes by mitigating free radical stress, as well as inhibiting NF-κB activation and iNOS expression in rat pancreas. Nutrition. 2015;31(1):214–222.

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Histological investigation of the effects of western diet on pressure wound healing period

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ABSTRACT

Objective: The stages of sKin wound healing are a dynamic process and it is thought to be related to nutrition. Carbohydrates, proteins and fats have particular importance in different periods of recovery process. Our study has aimed to examine the effects of a western diet with high protein, fat, and carbohydrate content on pressure ulcer healing.

Material and Methods: In this study, we used 22 healthy male Sprague Dawley rats weighing 100-185 g. We randomly divided the rats into two groups. The rats were fed according to the indicated diets (standard diet and western diet). On the first day of the fourth week, ischemia sKin by histopathological examinations of the wound tissue samples on the 7th and 14th days of the wound healing period.

Results: Statistically significant differences were observed in histological and immunohistochemical parameters in the tissue samples on the 7th and 14th days. On the 7th day, there were re-epithelialization (P=0.003), granulation cell density (P=0.004), inflammation (P=0.004), and angiogenesis (P= 0.003). We found re-epithelialization (P=0.001), granulation cell density (P=0.002), inflammation (P=0.002), and angiogenesis (P=0.001) on the 14th day. On the 7th and 14th days, we found the p-value between Ki-67 immunohistochemical staining percentages as P= 0.003 and the p-value for VEGF as P=0.002.

Conclusion: We determined that in short-term wound healing, the western type diet was more effective on pressure wound healing than the standard diet.

Keywords: decubitus ulcer, western diet, wound healing

INTRODUCTION

A decubitus ulcer is defined as the skin or tissue damage generally on the pointed parts of the bones caused by both pressure and compression or only by force (1). In addition to the more frequent observations of decubitus ulcers generally on the pointed parts of the bones, they could also be caused by the medical equipment, which leads to compression, pressure or friction such as orthopaedic casts, cartridges, catheters, and compression machines (2). Nearly 4 % of decubitus ulcer cases need hospital care to treat thoroughly. This treatment also requires a substantial amount of money (3).

The dermal wound healing is an active process. It includes the phases of inflammation, proliferation, and the reformation of the skin into its old state. In the inflammation phase, cell migrations, cytokines, and growth factors play an essential role. This is sovereign in the stimulation of the vascular proliferation; thus, the tissues' reformation occurs (1). The studies conveyed indicate that wound healing connects with nutrition by nature. In wound healing and other cases requiring treatment, insufficient nourishment affects the recovery period negatively. This situation, which is derived from especially the lack of protein and energy, is also corroborated by the clinical results (4).

The most energy-requiring phase in wound healing is the collagen synthesis phase. In the wound healing period, if there isn't enough carbohydrate intake, other energy sources such as proteins can be used as the primary source, leading to the limitation of wound healing (1).

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Insufficient carbohydrate intake may substantially set wound healing back by causing the inflammation phase to drag out. It is thought that increasing the protein intake in wound healing or sickness period may ensure a shorter reparation of the tissue damage (2). The role of fats in wound healing has not been studied in detail (3, 5). Nevertheless, it is known that there has been a rising need for essential oils during traumatization.

The Western diet is a diet with low consumption of vegetables and beverages with high protein content (mainly processed meats), saturated fats, refined grains, sugar, alcohol, salt, and corn syrup (6). Living conditions in daily life have allowed western type nutrition to be preferred widely, especially among the working population. In such a context, we aim to study the effects of the western diet with high protein, fat, and carbohydrate content on pressure ulcers the decubitus ulcer wound healing.

MATERIALS and METHODS

This study has been conducted by Sakarya University Animal Testing Local Ethics Committee approval in Sakarya University Experimental Research and Medical Centre (Dated 05/08/2020 and numbered 46). The procedures on the rats are humane, and the standards of the study are pertinent to the standards of the existing ethical animal testing procedures.

Experimental Animals

In this study, 22 healthy male Sprague Dawley rats weighing between 100-185 grams were used as experimental animals. This experiment has been conducted in Sakarya University Experimental Research and Medical Center (Sakarya, TURKEY). The rats were fed with water and ad libitum according to the determined diets (standard diet and western diet). Rats were kept at 21±1 °C in well-ventilated places within 12- hour day-night cycle. Animal diets, both the standard and the western type, were produced by the experts at a private corporation (Arden Research& Experiment, Ankara, TURKEY).

Experimental Protocol

The 22 rats were randomly divided into two groups, 11 each in the control and western diet groups, which would be statistically significant. Rats were fed with a Standard diet (calories 77.3% carbohydrate, 2.7% fat, and 20% protein) and a western diet (calories 39.70% carbohydrate, 39.51% fat, 19.53% protein, and other components 1.26%) according to the groups.

The decubitus ulcer model has been used by Stadler et al. (7). After feeding procedure, the rats were anesthetized by the Ketamine HCL 100 mg/kg IM (Ketasol% 10 10 ml) (Ketasol, Richterfarma, Austria) and then by Xylazine 10 mg on the first day of the fourth week. The hair between the two blade bones of the rats was shaved. The skins between the two blade bones of the rats in all groups were gently removed. Two neodymium magnets with 15x5 mm in diameter and 2000 Gauss in power (6.53g in weight) were implemented into both sides of the removed skin.

Compression was applied in 8-hour magnet fastening and 8-hour release (ischemia-reperfusion model), just as in the literature and the decubitus ulcers were developed after 72 hours (7). The rats were fed identical diets until the end of the study.

Histopathological Evaluation

Tissue samples were collected from wounds on the seventh and fourteenth days of the decubitus ulcers. The tissue samples were immobilized in the formaldehyde buffered 10% for 48 hours. The tissue sample was soaked in paraffin-embedded blocks after the tissue embedding processes. The 4–5-micron incisions taken by the Leica RM 2255 microtome were processed in hematoxylin-eosin staining. According to "The Wound Healing Points Evaluation Criteria", a histologist evaluated the histopathological examinations via a Nikon model light microscope according to "The Wound Healing Points Evaluation Criteria". A score of 0–3 was given to each section according to the presence of inflammatory cells and the levels of angiogenesis and epithelialization as previously described by Sedighi et al., 2016 (8) with minimal modifications (**Table1**).

Immunohistochemical Staining Method

The tissue samples, which were cut in 4 microns from the paraffin-embedded blocks, were deparaffinised and got through decreasing alcohol series. The preparations in citrate buffers went under heat treatment for 20 minutes in the microwave. After that, all incisions were blocked in 3% of H2O2 by endogen peroxidase- activity. Primary antibodies were VEGF (Genetex), and Ki-67 (Genetex), which were used in a 1/300 ratio, and then secondary antibodies (Ultra Vision Large Volume Detection System Anti-rabbit by Lab Vision, HRP) were used. Each step was implemented by the producing company's procedures. Diaminobenzidine (DAB) was used to make the paint visible. Mayer's hematoxylin was used for contrast colouring. The preparation was covered by the mounting medium (Aqueous Mounting Medium by Scy Tek). As a result of Ki-67 and VEGF staining, the preparations were scored in randomly chosen five staining areas and the area with the highest score was determined. In both groups, at least 100 cells were marked within each x40 magnifying area. In incisions, the percentage of the stained cells and staining level were the criteria to be chosen. For each incision, Immunohistochemical staining scoring was calculated according to H-SCORE, which is a scoring algorithm formulated as (I x PC), (I: the level of staining, PC: the percentage of stained cells in each level) (9).

Statistical Analysis

Statistical analyses were performed using the SPSS 24.0 package program (SPSS Inc. and Lead Tech. Inc. Chicago, USA). Shapiro Wilks test was used in compliance with normal distribution. Kruskal Wallis test was used for numerical data of the subgroups that did not show normal distribution. Intergroup evaluations for statistically different parameters were performed using the Mann-Whitney U test and comparing them in pairs. Results were given as mean ± standard deviation. For all statistical analyses, a two-tailed P-value <0.05 was considered statistically significant.

Table 1. Histological scoring parameter of epithelialization, angiogenesis, granulation tissue formation, and inflammatory cells.

Parameter/Score	0	1	2	3
Inflammatory cells	1–5 inflammatory cells per histological field	5–8 inflammatory cells per histological field	8–11 inflammatory cells per histological field	11–15 inflammatory cells per histological field
Reepithelialization	Absence of epithelial proliferation in ≥70% of tissue	Incomplete epidermal organization in ≥50% of tissue	Moderate epithelial proliferation in ≥60% of tissue	Complete epidermal remodeling in ≥80% of tissue
Angiogenesis	Absence of angiogenesis including congestion and hemorrhage	2–4 vessel per site, congestion and hemorrhage	4–6 vessel per site, slight congestion	7–8 vessel per site vertically disposed towards the epithelial surface
Granulation cell density	None, completely Disorganized and distorted	Minimal/immature thin	Mild/moderately mature granule layer	Evident/ Thick, ≥80% organized

RESULTS

Re- epithelialization

In standard and Western diet groups, when the tissue samples from the 7th and 14th days were compared in terms of re-epithelialization, statistically significant differences between the tissues of those two days were observed (P-value in order is p=0,003; p=0,001). When the tissue samples of standard diet groups from the seventh and fourteenth days were compared in terms of re-epithelialization, statistically significant differences between the tissues of those two days were observed (p=0,002). In the fourteenth day samples, better re-epithelialization was observed. In terms of re-epithelialization, statistically significant differences between the tissue's samples of the seventh and fourteenth days were observed in the Western diet group (p=0,001). In the fourteenth day samples, better re-epithelialization was observed (**Image 1**).

Granulation Cell Density

When the standard and Western diet groups were compared on the 7th and 14th days in terms of granulation cell density, there were statistically significant differences in terms of granulation cell density in favour of the western diet group on both the 7th and the 14th days (P-value in order was p=0,004; p=0,002). The granulation cell density in the standard diet tissue samples was observed to be better on the 14th day than on the 7th day (p= 0,001). Under the same conditions of the western group, statistically significant differences were recorded on the 14th day compared to the 7th day (p=0,000) (**Image 1**).

Inflammation

When the 7th day and the 14th-day results of standard and western diet results were compared in terms of inflammation cell density, on both dates, the inflammation cell density had statistically significant differences in favour of the diet of the west group. The P values on the 7th and the 14th days were respectively p= 0,004 and p=0,002. The tissue samples of the standard diet were observed to have statistically significant differences on the 14th day than on the 7th day (p= 0,001). The inflammation cell density of the Western diet group's tissue samples on the 14th day had a statistically significant difference compared to the 7th-day samples (p=0,000). Under the same conditions, it was in favour of the western group on the 14th day (p=0,000) (**Image 1**).

Angiogenesis

When the standard and Western diet groups were compared on the 7th and 14th days in terms of angiogenesis ratio, there were statistically significant differences in favour of the western diet group on both the 7th and the 14th days (P-value in order was p=0,003; p=0,001). The angiogenesis ratio in the tissue samples of the standard diet was observed to have statistically significant differences on the 14th day than on the 7th day (p= 0,002). The angiogenesis tissue samples of the western diet were honoured to have statistically significant differences on both the 7th day and the 14th day (p= 0,000)(**Image 1**).

Immunostaining Results

The tissue samples of standard and Western diet groups on the 7th and the 14th days were separately stained, and the results were evaluated.

Ki-67 Immunostaining

When the standard and Western diet groups were compared on the 7th, there was a statistically significant difference between the two groups (P= 0,000). The percentage of the Ki-67 immunostaining was observed to be relatively high in the western group. When the standard and Western diet groups' results were compared on the 14th, there was a statistically significant difference in the percentage of the Ki-67 immunostaining between the two groups (P= 0,000). The rate of the Ki-67 immunostaining in the Western group on the 14th day was observed to be relatively low (**Image 2**).

VEGF Immunostaining

When the standard and Western diet groups were compared on the 7th regarding the percentage of the VEGF immunostaining, there was a statistically significant difference between the two groups (P= 0,000). The rate of the VEGF immunostaining in the western group was observed to be relatively high. When the standard and Western diet groups' results were compared on the 14th, there were statistically significant differences in the percentage of the VEGF immunostaining between the two groups (P= 0,000). The rate of immunostaining in the Western group on the 14th day was observed to be lower (**Image 3**).

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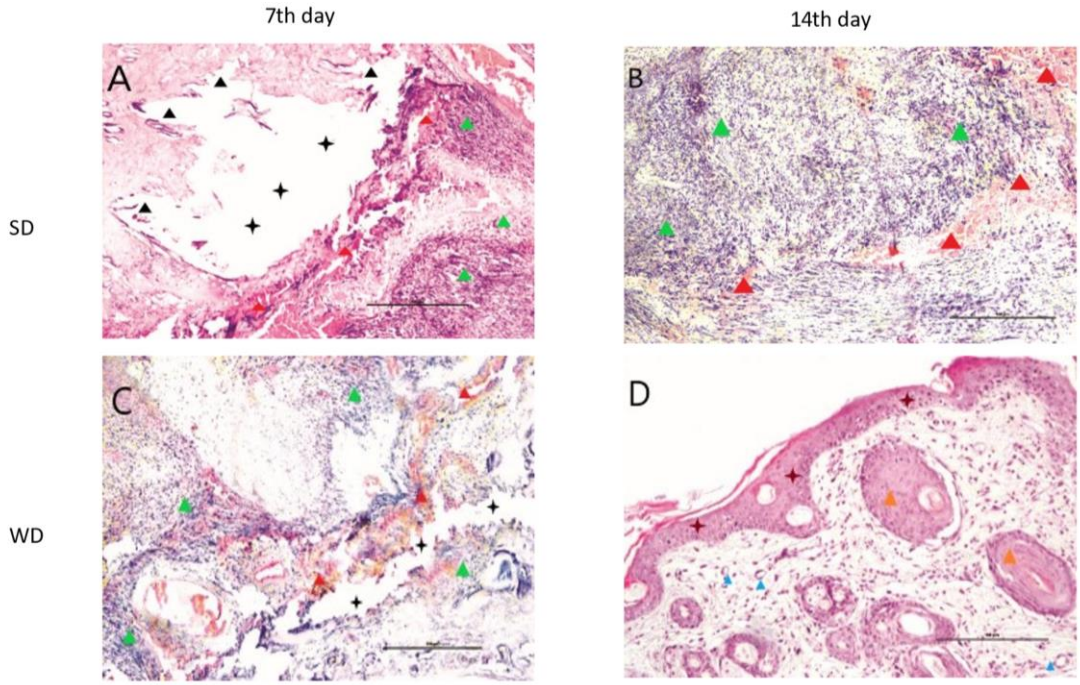

Image 1. 7th and 14th-day scar tissue samples from SD and WD nutrition groups. X100, 100 scale bar (Hematoxylin Eosin). Large wound areas were seen in the 7-day examples in the SD (A) group, and dense granulation areas were seen in the 14th-day samples (B). Small wound areas and bleeding areas were seen in the 7th-day models (C) of the WD group. It was observed that epithelialization was completed, and healing was completed in the 14th-day samples (D). SD: Standard diet group, WD: Western diet group, black star: wound area, red arrowhead: bleeding area, green arrowhead: granulation area, black arrowhead: damaged muscle area, blue arrowhead: blood vessel, orange arrowhead: hair root, burgundy arrowhead: epithelial layer.

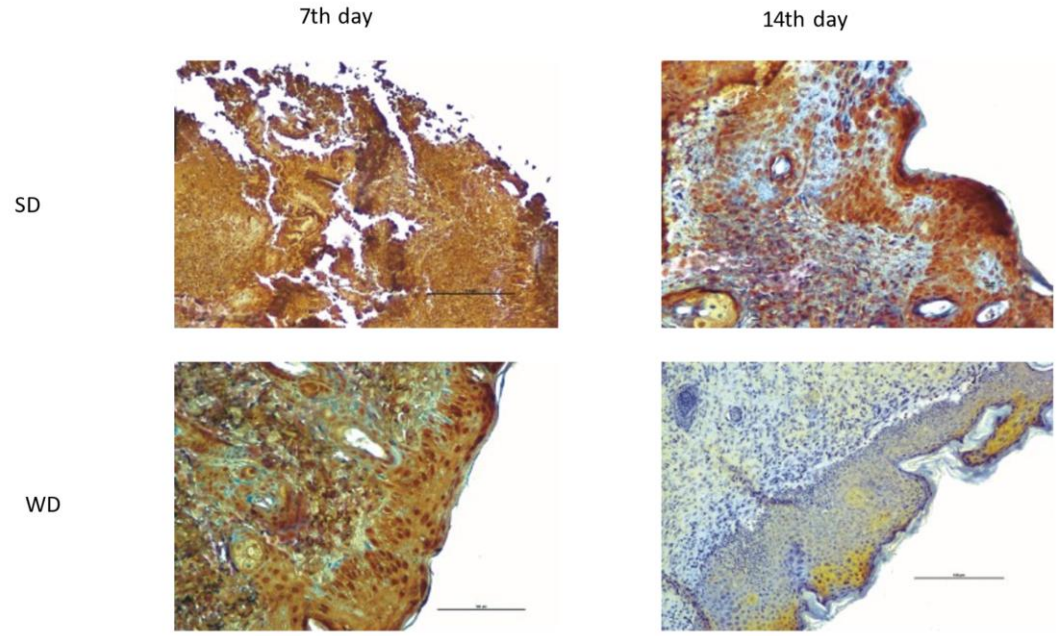

Image 2. Ki-67 immunoreactivity preparations in the wound tissue samples of the SD and WD groups on the 7th and 14th days. X100, 100 scale bar. It was seen that Ki-67 activity in the 14th-day models in the WD group was significantly lower than in the SD group. In the tissue samples on the 7th day, more intense Ki-67 immunoreactivity was observed in the SD group than in the WD group. The more intense Ki-67 immune reactivity in the SD group indicates that the healing process is slower in the acute period. SD: Standard diet group, WD: Western diet group.

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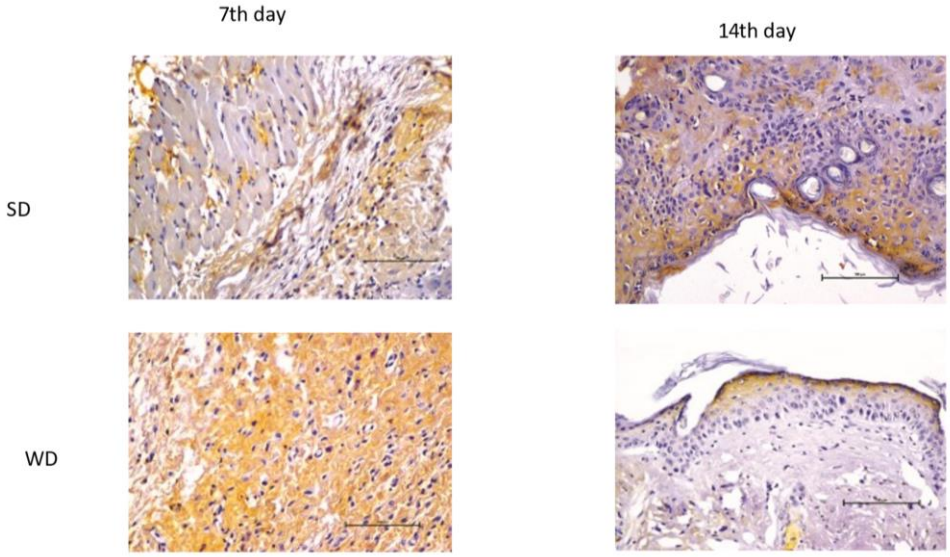

Image 3. VEGF immunoreactivity preparations in the wound tissue samples of the SD and WD groups on the 7th and 14th days. X100, 100 scale bar. It was observed that VEGF immune reactivity in the WD group was less intense in the samples on the 14th day compared to the SD group, and the wound healing process was completed at the end of the 14th day. SD: Standard diet group, WD: Western diet group.

DISCUSSION

Decubitus ulcers are related to ischemia-reperfusion injury (10, 11). The physical movements on the skin tissues lead to blood vessel pressure and, therefore they cause ischemia. When the pressure effect decrease or increase, and the decompression on the vessels, the re-oxygenation of the tissues may induce ischemia-reperfusion injury (**12**). For this reason, to understand the mechanisms in the formation and the rehabilitation of decubitus ulcers, the dynamics of blood vessel formation need to be researched. In our study, we have used the ischemia-reperfusion model of Stadler et al. (**7**). Vascular endothelial growth factor (VEGF) underlies vascularization (**13-15**). VEGF can be related to cell regeneration, granulation tissue formation, and reformation (**16, 17**). Ki-67 is an important nucleoprotein, which marks the proliferative cells defined as proliferation indicator (**18-21**). Our study used Ki-67 to show the cell formation density of the newly forming cells with its VEGF effect on the wound area since the beginning of neovascularization. In our study, the tissue samples were collected from the Western Diets, and Standard Diets applied rats' wound area on the 7th and 14th days. We have identified the density of VEGF and Ki-67 in our tissue samples as semiquantitative (H-score). In addition to this, we have used the histological markers, which indicate the stages of wound healing such as inflammatory cell density, granulation tissue, angiogenesis and re-epithelialization while examining the preparation stained with H.E. on the 7th and 14th days.

The formation of granulation tissue is the most significant indicator of wound healing (**22**). In the appearance of the wound, the fibroblasts, the collagen sarcostyles and the capillary vessels between the wound lips colligate the wound lips by becoming parallel to the wound surface (**23**). In the last phase of wound healing, the re-formation is characterized by the new Epithelialization and scar tissue formation (**24**).

In our study, dense blood vessels (angiogenesis) and granular cell accumulation in the tissue samples which were collected from the Western Diets group and stained by H.E. on the 7th day were observed more in comparison with the 7th-day tissue samples from Standard Diets group.

This observation leads to the idea of W.D.'s acceleration of the wound healing process. It was observed that the angiogenesis and the inflammatory cell accumulation in the preparation of the tissue samples taken from the rats fed on W.D. lowered significantly in comparison to the S.D. group on the 14th day. It was observed that there were more intense spots of fibroblast in the WD-14th-day samples than 7th-day samples. Concordantly, it was observed that the fast-healing process continued, and the formation of folliculitis and epithelialization started to be seen in the Western Diets group. When microscopically examined, the vascularization, granulation, and collagenases spots were much slower in the standard group than in the western group.

In this study, the percentage of Ki-67 and VEGF immune expression was higher in WD than SD in the 7th-day samples. Also, it was observed that the percentage of Ki-67 and VEGF immune expression was higher in SD than WD in the 14th-day samples. It is remarkable that the intensities of Ki-67 and VEGF immune expression show an increase and decrease in line with the preparation, which indicates the wound healing stages in the HE stained samples. Thereby, in our study, we have observed that WD can affect the completion of cellular regeneration in shorter periods than SD due to its cell proliferation in the wound healing areas.

There are studies in literature stating that a high fat diet impedes wound healing on the dermis (**25-27**). In those studies, the importance of the diet combination used in the healing process on wound healing is emphasized.

Until nowadays, the studies examining especially the effect of WD on decubitus wounds' healing process frequently have been found. The studies on high-fat and sugar diets are conducted more frequently. In one study, it is reported that high-fat diets are effective on collagen production and wound repair and this effect is due to its contribution to the wound healing process rather by changing the nitrogen balance (**28**). In the mentioned study, they got similar results to our study. There are also other studies with different results from our study in literature. Paulino (2011) reported that the animals fed on a high-fat diet have a delayed wound closure due to long term inflammation after seven days from the formation of the wound (**26**).

Vascularization is an important factor in wound healing owing to the fact that it provides oxygen and nutrition for cell metabolism (**29-31**). In a different study from ours, they reported that in mice, a high-fat diet caused a decrease in VEGF expression and, therefore, decreased tissue vascularization in wound healing (**27, 32**).

CONCLUSION

Frequently consumed WD diet causes renal failure, obesity, high blood pressure, the aggravation of colitis symptoms, the shortening of the colon, the increase in tumorigenesis, the increase in insulin resistance, the development of hepatosteatosis (**33**). In the wound healing stage, it is suggested that approximately 20-25 % of the total calorie should be given as protein (**34**). In general, it is advised that the suggested amount of protein in wound healing stages or other wound stages should be given more than the amount needed (**35**). For that reason, we think that if WD becomes a continuous preference, it can lead to a severe health problem; however, it is suitable for short-term use in the early periods of the wound healing stage due to the need for high protein intake.

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Conflict of interest: The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Rümeysa Yeniçağ NR. Pressure Ulcers and Nutritional Treatment in Older Adults. Sakarya Medical Journal. 2019;2019;9(3).
2. Jackson D, SarKi AM, Betteridge R, Brooke J. Medical device-related pressure ulcers: A systematic review and meta-analysis. International journal of nursing studies. 2019;92:109-20.
3. Dealey C, Posnett J, Walker A. The cost of pressure ulcers in the United Kingdom. Journal of wound care. 2012;21(6):261-2, 4, 6.
4. Ojeh N, Pastar I, Tomic-Canic M, Stojadinovic O. Stem Cells in SKin Regeneration, Wound Healing, and Their Clinical Applications. International journal of molecular sciences. 2015;16(10):25476-501.
5. Medlin S. Nutrition for wound healing. British Journal of Nursing. 2012;21(Sup12):S11-S5.
6. Varlamov O. Western-style diet, sex steroids and metabolism. Biochimica et biophysica acta Molecular basis of disease. 2017;1863(5):1147-55.
7. Stadler I, Zhang RY, Oskoui P, Whittaker MS, Lanzafame RJ. Development of a simple, noninvasive, clinically relevant model of pressure ulcers in the mouse. Journal of investigative surgery : the official journal of the Academy of Surgical Research. 2004;17(4):221-7.
8. Anahita Sedighi DM, Reza Shirazi. Histopathological evaluation of the healing effects of human amniotic membrane transplantation in third-degree burn wound injuries. Comparative Clinical Pathology. 2016 >> 25 (2) 381-5.
9. Ulloa-Padilla JP, Ghassibi MP, Dubovy SR, Kerr DA. Clinicopathologic Correlation of Kaposi Sarcoma Involving the Ocular Adnexa: Immunophenotyping of Diagnostic and Therapeutic Targets. Ophthalmic plastic and reconstructive surgery. 2020;36(2):185-90.
10. Peirce SM, Skalak TC, Rodeheaver GT. Ischemia-reperfusion injury in chronic pressure ulcer formation: a sKin model in the rat. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 2000;8(1):68-76.
11. Reid RR, Sull AC, Mogford JE, Roy N, Mustoe TA. A novel murine model of cyclical cutaneous ischemia-reperfusion injury. The Journal of surgical research. 2004;116(1):172-80.
12. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. Science translational medicine. 2014;6(265):265sr6.
13. Bates DO, Jones ROP. The Role of Vascular Endothelial Growth Factor in Wound Healing. The International Journal of Lower Extremity Wounds. 2003;2(2):107-20.
14. Brown LF, Yeo KT, Berse B, Yeo TK, Senger DR, Dvorak HF, et al. Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. The Journal of experimental medicine. 1992;176(5):1375-9.
15. Johnson KE, Wilgus TA. Vascular Endothelial Growth Factor and Angiogenesis in the Regulation of Cutaneous Wound Repair. Advances in wound care. 2014;3(10):647-61.
16. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science (New York, NY). 1989;246(4935):1306-9.
17. Pufe T, Paulsen F, Petersen W, Mentlein R, Tsokos M. The angiogenic peptide vascular endothelial growth factor (VEGF) is expressed in chronic sacral pressure ulcers. The Journal of pathology. 2003;200(1):130-6.
18. Delahunt B, Bethwaite PB, Thornton A, Ribas JL. Proliferation of renal cell carcinoma assessed by fixation-resistant polyclonal Ki-67 antibody labeling. Correlation with clinical outcome. Cancer. 1995;75(11):2714-9.
19. Key G, Petersen JL, Becker M, Duchrow M, Schlüter C, Askaa J, et al. New antiserum against Ki-67 antigen suitable for double immunostaining of paraffin wax sections. Journal of clinical pathology. 1993;46(12):1080-4.
20. Sato H, Abe Y, Noguchi M, Kurokawa K, Sakai H. Inhibitory effect of thyrotropic hormone on apoptosis induced by actinomycin D in a functioning rat thyroid cell line. Endocrine journal. 1999;46(2):309-15.

21. Sayar I, Gelincik I, Bozkurt A, Bayram I. Significance of Ki-67, Bcl-2 and C-erbB2 markers in benign, premalign and malign prostatic lesions/ benign, premalign ve malign prostat lezyonlarinda Ki-67, Bcl-2 ve C-erbB2 belirteclerinin onemi. The Journal of Kartal Training and Research Hospital. 2012;23:123+.

22. Hopf HW, Gibson JJ, Angeles AP, Constant JS, Feng JJ, Rollins MD, et al. Hyperoxia and angiogenesis. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 2005;13(6):558-64.

23. Güran Ş, Fen T, Tunca Y. Anjiyogenezis ve antianjiyogenik ilaçların kanser tedavisindeKi rolü. T Klin Tıp Bilimleri. 2004;24:380-2.

24. Ramasastry SS. Acute wounds. Clinics in plastic surgery. 2005;32(2):195-208.

25. Nascimento A, Monte Alto Costa A. Both obesity-prone and obesity-resistant rats present delayed cutaneous wound healing. The British journal of nutrition. 2011;106:603-11.

26. Paulino do Nascimento A, Monte-Alto-Costa A. Both obesity-prone and obesity-resistant rats present delayed cutaneous wound healing. The British journal of nutrition. 2011;106(4):603-11.

27. Seitz O, Schürmann C, Hermes N, Müller E, Pfeilschifter J, Frank S, et al. Wound healing in mice with high-fat diet- or ob gene-induced diabetes-obesity syndromes: a comparative study. Experimental diabetes research. 2010;2010:476969.

28. ÖZKORKMAZ EG, Yusuf Ö. Yara iyileşmesi ve yara iyileşmesinde kullanılan bazı bitKiler. Türk Bilimsel Derlemeler Dergisi. 2009;2(2):63-7.

29. Flegg JA, Menon SN, Maini PK, McElwain DLS. On the mathematical modeling of wound healing angiogenesis in sKin as a reaction-transport process. Frontiers in Physiology. 2015;6(262).

30. Radomska-Leśniewska DM, SkopińsKi P, Niemcewicz M, ZdanowsKi R, LewicKi S, Kocik J, et al. The Effect of Anti-Inflammatory and Antimicrobial Herbal Remedy PADMA 28 on Immunological Angiogenesis and Granulocytes Activity in Mice. Mediators of Inflammation. 2013;2013:853475.

31. Su WH, Cheng MH, Lee WL, Tsou TS, Chang WH, Chen CS, et al. Nonsteroidal anti-inflammatory drugs for wounds: pain relief or excessive scar formation? Mediators of inflammation. 2010;2010:413238.

32. Rosa DF, Sarandy MM, Novaes RD, Freitas MB, do Carmo Gouveia Pelúzio M, Gonçalves RV. High-Fat Diet and Alcohol Intake Promotes Inflammation and Impairs SKin Wound Healing in Wistar Rats. Mediators of Inflammation. 2018;2018:4658583.

33. Baena M, Sangüesa G, Hutter N, Beltrán JM, Sánchez RM, Roglans N, et al. Liquid fructose in Western-diet-fed mice impairs liver insulin signaling and causes cholesterol and triglyceride loading without changing calorie intake and body weight. The Journal of nutritional biochemistry. 2017;40:105-15.

34. Mayes T, Gottschlich M, Scanlon J, Warden GD. Four-year review of burns as an etiologic factor in the development of long bone fractures in pediatric patients. The Journal of burn care & rehabilitation. 2003;24(5):279-84.

35. Gottschlich M. Nutrition in the burned pediatric patient. Handbook of Pediatric Nutrition ASPEN. 1999:193-511.

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