JOURNAL OF SCIENTIFIC PERSPECTIVES

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ABOUT THE JOURNAL

Journal of Scientific Perspectives (JSP) is a **scholarly** and **international peer-reviewed journal**. It is published quarterly in *January, April, July* and *October,* in the fields of **basic sciences, engineering, natural sciences** and **health sciences**. All articles submitted for publication are evaluated by the editor-in-chief, field editor, editorial board and referees. The original research papers, technical notes, letter to the editor, debates, case presentations and reviews, only in *English,* are published in the journal. Thus, it aims to bring together the views and studies of academicians, researchers and professionals working in the fields mentioned above.

Journal of Scientific Perspectives is an *open access electronic journal*. All articles published in the journal, are assigned the *DOI number*. Article submission and follow-up process are carried out by the manuscript submission system.

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THE AIM AND SCOPE OF THE JOURNAL

Journal of Scientific Perspectives provides open access to its content, embracing the principle of increasing the global sharing of information on free scientific research. This journal is a material in which academic studies are included and so, it provides a social service for the benefit of institutions and individuals engaged in scientific research as. In this context, it is aimed at providing readers with a common platform to share and improve the quality of recent research advancements in the fields of **basic sciences, engineering, natural and health sciences.** Thus, It is aimed at promoting research worldwide and publishes basic and advanced research work from the fields above.

The journal accepts only original works of quality which are products of a new solution approach or give a new view of an existing knowledge. In this context, it is open to any kind of constructive, creative and institutionalized knowledge providing that they contribute to universal science and technology. Thus, it is aimed to index the journal with various international indexes.

The study fields covered by the journal are

Basic Sciences and Engineering

- Chemical Engineering
- Computer Engineering and Informatics
- Constructional Engineering
- Environmental Engineering
- Electrical and Electronic Engineering
- Food Engineering
- Geology Engineering
- Industrial Engineering
- Mechanical Engineering
- Mining Engineering
- Physical Engineering
- Textile Engineering
- Other Engineering Fields
- Chemistry
- Physics
- Mathematics
- Statistics
- Materials Sciences
  (Material and Metallurgy Engineering, Topographical Engineering etc.)
- Space Sciences
- Earth Sciences
- Architecture
- Urban and Regional Planning
- Astronomy and Astrophysics

Health Sciences

- Medical Sciences (Surgery, International Medicine, Basic Medical Sciences)
- Dentistry
- Pharmacology and Pharmaceutics
- Nursing
- Nutrition and Dietary
- Veterinary Medicine

Natural Sciences

- Biology
- Environmental Sciences
- Food Science and Technology
- Animal Husbandary
- Forestry
- Marine, Aquatic Sciences and Fisheries
- Agricultural Science

There are no limits to the fields in which the study will be accepted to the journal. The journal is open to all works aimed at contributing to the national and international developments of the professional organizations and individuals who follow the developments in the field of health, science and engineering and to create a resource in these fields.

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1. *Journal of Scientific Perspectives* has begun publication in July 2017. It is an internationally peer-reviewed and periodical journal published regularly in four issues per year in **January, April, July and October**, in the fields of **basic sciences, engineering, natural sciences and health sciences.** All articles submitted for publication are evaluated by the editor in chief, field editor, editorial board and referees.
2. Journal only accepts the studies written in **English.** Original research papers, technical notes, letters to the editor, discussions, case reports and compilations are published in our journal.
3. Only the original scientific researches are included. It is essential that the information created in scientific study needs to be new, suggest new method or give a new dimension to an existing information
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9. Required changes must be made by the author within **15 days** after the decision of "Correction required" given in article acceptance decision.
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18. Articles submitted for evaluation must not exceed 25 pages after they are prepared according to the specified template. Article summary should not exceed 300 words and minimum 3 and maximum 7 keywords should be written.

JOURNAL OF SCIENTIFIC PERSPECTIVES JSP

ETHICAL GUIDELINES

Journal of Scientific Perspectives (JSP) is committed to meeting and upholding standards of ethical behaviour at all stages of the publication process. It strictly follows the general ethical guidelines provided by the Committee on Publication Ethics (COPE) and the Open Access Scholarly Publishers Association (OASPA). Depending on these principles and general publication requirements, editors, peer reviewers, and authors must take the following responsibilities in accordance to professional ethic and norms. The proper and ethical process of publishing is dependent on fulfilling these resposibilities.

1.The Responsibilities of Editors

1.1. The General Responsibilities

- Editors should be accountable for everything published in their journals.
- The editor should make the efforts to improve the quality of and contribute to the development of the journal.
- The editor should support authors' freedom of expression.

1.2. Relations with Readers

- Readers should be informed about who has funded research or other scholarly work and whether the funders had any role in the research and its publication and, if so, what this was.
- The editor should ensure that the non-peer-reviewed sections of the journal (letters, essays, announcements of conferences etc.) are clearly identified.
- The editor should make efforts to ensure that the articles published align with the knowledge and skills of the readers.

1.3. Relations with Reviewers

- The editor should match the knowledge and expertise of the reviewers with the manuscripts submitted to them to be reviewed ensuring that the manuscripts are adequately reviewed by qualified reviewers.
- The editor should require reviewers to disclose any potential competing interests before agreeing to review a submission.
- The editor should provide necessary information about the review process to the reviewers about what is expected of them.
- The editor must ensure that the review process is double blind and never reveal the identities of the authors to the reviewers or vice versa.
- The editör encourage reviewers to evaluate manuscripts in an objective, scientific and objective language.

- The editor should develop a database of suitable reviewers and update it on the basis of reviewer performance and timing
- In the reviewer database; It should be attentive to scientists who evaluate the manuscripts objectively, perform the review process on time, evaluate the manuscirpt with constructive criticism and act in accordance with ethical rules.

1.4. Relations with Authors

- The editor should provide clear publication guidelines and an author guidelines of what is expected of them to the authors and continuously review the guidelines and templates.
- The editor should review the manuscript submitted in terms of guidelines of the journal, importance of the study, and originality and if the decision to reject the manuscript is made editor should explain it to the authors with clear and unbiased way. If the decision is made that the manuscript should be revised by the authors in terms of written language, punctuation, and/or rules in the guidelines (spacing, proper referencing, etc.) the authors should be notified and given time to do the corrections accordingly.
- The authors should be provided with necessary information about the process of their review (at which stage is the manuscript at etc.) complying with the rules of double blind review.
- In the case of an editor change, the new editor should not change a decision taken by the previous editor unless it is an important situation.

1.5. Relations with Editorial Board Members

- Editor should provide publication policies and guidelines to the editorial board members and explain what is expected of them.
- Editor should ensure that the editorial board members have the recently updated publication guidelines and policies.
- Editor should review the editorial board members and include members who can actively contribute to the journal's development.
- Editorial board members should be informed about their roles and responsibilities such as
    - Supporting development of the journal
    - Accepting to write reviews in their expertise when asked
    - Reviewing publication guidelinesand improving them consistently
    - Taking responsibility in journal's operation

2.The Ethical Responsibilities of Reviewers

- The reviewers must only agree to review manuscripts which align with their expertise.
- The reviewers must make the evaluation in neutrality and confidentiality. In accordance with this principle, they should destroy the manuscripts they examine after the evaluation process, but use them only after they are published. Nationality, gender, religious belief, political belief and commercial concerns should not disrupt the neutrality of the assessment.

- The reviewers must only review manuscripts which they do not have any conflict of interests. If they notice any conflict of interest they should inform the editor about it and decline to be a reviewer to the related manuscript.
- Reviewers must include the Manuscript Evaluation Form for the manuscripts they evaluate without indicating their names to protect the blind review process. And they should include their final decision about the manuscript whether or not it should be published and why.
- The suggestions and tone of the reviews should be polite, courteous and scientific. The reviewers should avoid including hostile, disrespectful, and subjective personal comments. When these comments are detected they could be reviewed and returned to the reviewer to be revised by the editor or editorial board.
- The reviewers should respond in time when a manuscript is submitted to them to be reviewed and they should adhere to the ethical responsibilities declared hereby.

3.The Ethical Responsibilities of Author(s)

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- The potential conflicts of interest of the author(s) should be stated and the reason should be explained
- The bibliography list is complete and should be prepared correctly and the cited sources must be specified.
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- The author(s) should contact the editor to provide information, correction or withdrawal when they notice the error regarding the manuscirpt in the evaluation and early view phase or published electronically.
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All manuscripts submitted to Journal of Scientific Perspectives (JSP) are reviewed through iThenticate software before publishing. The maximum similarity rate accepted is **30%**. Manuscripts which exceed these limits are analyzed in detail and if deemed necessary returned to the authors for revision or correction, if not they could be rejected to be published if any plagiarism or unethical behavior is detected.

Following are some of the behaviors which are accepted as unethical:

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- Salami slicing, producing more than one article from a single study.
- Not declaring conflicting interests or relations in the manuscripts submitted.
- Unveiling double blind process.

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AUTHOR GUIDELINES

INSTRUCTION FOR AUTHORS

The authors are cordially invited to submit significant new findings of their research work papers in word and pdf formats to the journal office via online submission or e-mail: jsp@ratingacademy.com.tr along with a JSP cover letter. The journal will cover the topics related to the fields of **basic sciences, engineering, natural sciences and health sciences.** All articles submitted for publication are evaluated by the editor-in-chief, field editor, editorial board and referees. The original research papers, technical notes, letter to the editor, debates, case presentations and reviews only in *English* are published in the journal.

The editorial board of JSP welcomes original novel contributions and reviews in word format. By submission of a manuscript an author certifies that the work is original and is not being considered simultaneously by other journals. All articles are subjected to critical reviews by referees.

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The cover letter should be prepared and sent to the Editor-in-Chief via e-mail.

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- Regular paper should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly. The total length of any manuscript submitted must not exceed **25 pages.**
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- Manuscripts should be prepared in English using a word processor. MS Word for Windows and docfiles are preferred. Manuscripts may be prepared with other software provided that the full document (with figures, schemes and tables inserted into the text) is exported to a MS Word format for submission.
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MERCER, P.A. and SMITH, G., 1993, *Private Viewdata in the UK*, 2

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EVANS, W.A., 1994, Approaches to Intelligent Information Retrieval, *Information Processing and Management*, 7 (2), 147-168.

Conferences:

SURNAME, NAME, Publication Year, Name of Report, *Name of Conference Bulletin*, Date and Conference Place, Place of Publication: Publishing, Page Numbers

SILVER, K., 1991, Electronic Mail: The New Way to Communicate, *9th International Online Information Meeting*, 3-5 December 1990, London, Oxford: Learned Information, 323-330.

Thesis:

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Maps:

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MASON, James, 1832, Map of The Countries Lying Between Spain and India, 1:8.000.000, London: Ordnance Survey.

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HOLLAND, M., 2002, Guide to Citing Internet Sources [online], Poole, Bournemouth University, http://www.bournemouth.ac.uk/library/using/guide_to_citing_internet_sourc.html, [Date Accessed: 4 November 2002].

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Brief checklist for submission.

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4. Have you provided Keywords?
5. Are your Tables denoted by Arabic numerals, and are they in order as cited in the text?
6. Are your Figures denoted by Arabic numerals, and are they in order as cited in the text?
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ABSTRACTING & INDEXING

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JOURNAL OF SCIENTIFIC PERSPECTIVES JSP

EDITORIAL

Dear colleagues,

Journal of Scientific Perspectives, which started its activity in July 2017, has been accepting articles from three different areas. The journal, which is recognized for its high quality publications in three main areas such as Basic Sciences and Engineering, Health Science, and Natural Science, has decided to make changes in terms of its management and content.

At the outset, an editor change was made in January 2021. Dr. Hasan Erbay preceded by Prof. Dr. Özlem Yayıntaş (2018 - 2021) is appointed the new editor in chief. Henceforth, the editorial board members and referees will be updated in accordance with the content of the journal.

As of April 2021, the name and ISSN of the journal will change. The journal which has been operating under the name Journal of Scientific Perspectives since July 2017, will continue its publishing activity with its new name Health Sciences Quarterly from April 2021 onwards.

The aforementioned changes include not only changes in management and name but also in content of the journal. Our journal, which used to accept articles from Basic Sciences and Engineering, Health Science, and Natural Science until January 2021, will accept articles in the field of Health Sciences starting from the next issue.

The changes will also affect the design and typesetting processes. As of the next issue, the design and the website of the journal will be restructured in accordance with its content. Hence, the publishing policies and spelling rules will also be updated.

We strongly anticipate that the changes will make the efforts made so far more significant and enhance the quality of the journal.

Best of luck to the scientific community.

Prof. Dr. Mehmet ŞAHİN

Rating Academy Founder and CEO

JOURNAL OF SCIENTIFIC PERSPECTIVES JSP

International Peer-Reviewed Journal

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Research Article

A Study On the Sums of Squares of Generalized Tribonacci Numbers: Closed Form Formulas of $\sum_{k=0}^{n} kx^k W_k^2$

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Abstract. In this paper, closed forms of the sum formulas $\sum_{k=0}^{n} kx^k W_k^2$, $\sum_{k=0}^{n} kx^k W_{k+2}W_k$ and $\sum_{k=0}^{n} kx^k W_{k+1}W_k$ for the squares of generalized Tribonacci numbers are presented. Here, $\{W_m\}_{m\in\mathbb{Z}}$ is the generalized Tribonacci sequence, n is a non-negative integer and x is a real or complex number. As special cases, we give summation formulas of Tribonacci, Tribonacci-Lucas, Padovan, Perrin numbers and the other third order recurrence relations.

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Keywords. Sums of squares, third order recurrence, generalized Tribonacci numbers, Padovan numbers, Perrin numbers, Narayana numbers.

1. Introduction

The generalized Tribonacci sequence $\{W_n(W_0, W_1, W_2; r, s, t)\}_{n\geq 0}$ (or shortly $\{W_n\}_{n\geq 0}$) is defined as follows:

$$W_n = rW_{n-1} + sW_{n-2} + tW_{n-3}, \qquad W_0 = a, W_1 = b, W_2 = c, \quad n \geq 3 \tag{1.1}$$

where W_0, W_1, W_2 are arbitrary complex numbers and r, s, t are real numbers. The generalized Tribonacci sequence has been studied by many authors, see for example [1,2,6,7,13,14,19,20,21,23,35,36,37,38].

The sequence $\{W_n\}_{n\geq 0}$ can be extended to negative subscripts by defining

$$W_{-n} = -\frac{s}{t}W_{-(n-1)} - \frac{r}{t}W_{-(n-2)} + \frac{1}{t}W_{-(n-3)}$$

for $n = 1, 2, 3, ...$ when $t \neq 0$. Therefore, recurrence (1.1) holds for all integer n.

In literature, for example, the following names and notations (see Table 1) are used for the special case of r, s, t and initial values.

Table 1 A few special case of generalized Tribonacci sequences.

Sequences (Numbers)	Notation	OEIS [22]
Tribonacci	$\{T_n\} = \{V_n(0,1,1;1,1,1)\}$	A000073, A057597
Tribonacci-Lucas	$\{K_n\} = \{V_n(3,1,3;1,1,1)\}$	A001644, A073145
third order Pell	$\{P_n^{(3)}\} = \{V_n(0,1,2;2,1,1)\}$	A077939, A077978
third order Pell-Lucas	$\{Q_n^{(3)}\} = \{V_n(3,2,6;2,1,1)\}$	A276225, A276228
third order modified Pell	$\{E_n^{(3)}\} = \{V_n(0,1,1;2,1,1)\}$	A077997, A078049
Padovan (Cordonnier)	$\{P_n\} = \{V_n(1,1,1;0,1,1)\}$	A000931
Perrin (Padovan-Lucas)	$\{E_n\} = \{V_n(3,0,2;0,1,1)\}$	A001608, A078712
Padovan-Perrin	$\{S_n\} = \{V_n(0,0,1;0,1,1)\}$	A000931, A176971
Pell-Padovan	$\{R_n\} = \{V_n(1,1,1;0,2,1)\}$	A066983, A128587
Pell-Perrin	$\{C_n\} = \{V_n(3,0,2;0,2,1)\}$	
Jacobsthal-Padovan	$\{Q_n\} = \{V_n(1,1,1;0,1,2)\}$	A159284
Jacobsthal-Perrin (-Lucas)	$\{L_n\} = \{V_n(3,0,2;0,1,2)\}$	A072328
Narayana	$\{N_n\} = \{V_n(0,1,1;1,0,1)\}$	A078012
Narayana-Lucas	$\{U_n\} = \{V_n(3,1,1;1,0,1)\}$	A001609
Narayana-Perrin	$\{H_n\} = \{V_n(3,0,2;1,0,1)\}$	
third order Jacobsthal	$\{J_n^{(3)}\} = \{V_n(0,1,1;1,1,2)\}$	A077947
third order Jacobsthal-Lucas	$\{j_n^{(3)}\} = \{V_n(2,1,5;1,1,2)\}$	A226308
3-primes	$\{G_n\} = \{V_n(0,1,2;2,3,5)\}$	
Lucas 3-primes	$\{H_n\} = \{V_n(3,2,10;2,3,5)\}$	
modified 3-primes	$\{E_n\} = \{V_n(0,1,1;2,3,5)\}$	

The evaluation of sums of powers of these sequences is a challenging issue. Two pretty examples are

$\sum_{k=0}^{n} k(-1)^k T_k^2 = \frac{1}{4}((-1)^n ((n+1)T_{n+3}^2 - (2n+1)T_{n+2}^2 + (3n+2)T_{n+1}^2 - 2(n+2)T_{n+1}T_{n+3} + 2T_{n+2}T_{n+1}) + 1)$

and

$\sum_{k=0}^{n} k(-1)^k N_k^2 = \frac{1}{9}((-1)^n ((3n+7)N_{n+3}^2 - (6n+5)N_{n+2}^2 + (6n-1)N_{n+1}^2 - 6N_{n+3}N_{n+2} - 2(3n+7)N_{n+3}N_{n+1} + 2(3n+10)N_{n+2}N_{n+1}) - 1)$.

In this work, we derive expressions for sums of second powers of generalized Tribonacci numbers. We present some works on sum formulas of powers of the numbers in the following Table 2.

Table 2. A few special study on sum formulas of second, third and arbitrary powers.

Name of sequence	sums of second powers	sums of third powers	sums of powers
Generalized Fibonacci	[3,4,10,11,12,24,33,30]	[9,25,27,28,31,32,39]	[5,8,15]
Generalized Tribonacci	[17,26,29]		
Generalized Tetranacci	[16,18,34]		

Let

$$\Delta = (-t^2x^3 + sx + rtx^2 + 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1).$$

THEOREM 1.1. *If* $\Delta \neq 0$ *then*

(a):

$$\sum_{k=0}^{n} x^k W_k^2 = \frac{\Delta_1}{\Delta}$$

(b):

$$\sum_{k=0}^{n} x^k W_{k+1} W_k = \frac{\Delta_2}{\Delta}$$

(c):

$$\sum_{k=0}^{n} x^k W_{k+2} W_k = \frac{\Delta_3}{\Delta},$$

where

$\Delta_1 = -x^{n+3}(t^2x^3+sx+rtx^2-1)W_{n+3}^2-x^{n+2}(r^2x+t^2x^3+sx+r^2t^2x^4+rtx^2+r^2sx^2+r^3tx^3+2rstx^3-1)W_{n+2}^2-x^{n+1}(r^2x+s^2x^2-s^3x^3+t^2x^3+sx+r^2t^2x^4+s^2t^2x^5+rtx^2+r^2sx^2+r^3tx^3+4rstx^3-rs^2tx^4-1)W_{n+1}^2+x^2(t^2x^3+sx+rtx^2-1)W_2^2+x(r^2x+t^2x^3+sx+r^2t^2x^4+rtx^2+r^2sx^2+r^3tx^3+2rstx^3-1)W_1^2+(r^2x+s^2x^2-s^3x^3+t^2x^3+sx+r^2t^2x^4+s^2t^2x^5+rtx^2+r^2sx^2+r^3tx^3+4rstx^3-rs^2tx^4-1)W_0^2+2x^{n+4}(r+tx)(s+rtx)W_{n+3}W_{n+2}+2x^{n+4}t(r+stx^2)W_{n+3}W_{n+1}-2x^{n+4}t(sx-1)(s+rtx)W_{n+2}W_{n+1}-2x^3(r+tx)(s+rtx)W_2W_1-2tx^3(r+stx^2)W_2W_0+2x^3t(sx-1)(s+rtx)W_1W_0$

and

$\Delta_2 = x^{n+3}(r+stx^2)W_{n+3}^2+x^{n+4}(t+rs)(s+rtx)W_{n+2}^2+x^{n+4}t^2(r+stx^2)W_{n+1}^2-x^{n+2}(r^2x+s^2x^2+t^2x^3+2rstx^3-1)W_{n+3}W_{n+2}+x^{n+3}t(r^2x-s^2x^2-t^2x^3+1)W_{n+3}W_{n+1}-x^{n+1}(r^2x+s^2x^2-s^3x^3+t^2x^3+sx+rtx^2+r^2sx^2+r^3tx^3-rt^3x^5-st^2x^4+2rstx^3-rs^2tx^4-1)W_{n+2}W_{n+1}-x^2(r+stx^2)W_2^2-x^3(t+rs)(s+rtx)W_1^2-x^3t^2(r+stx^2)W_0^2+x(r^2x+s^2x^2+t^2x^3+2rstx^3-1)W_2W_1-x^2t(r^2x-s^2x^2-t^2x^3+1)W_2W_0+(r^2x+s^2x^2-s^3x^3+t^2x^3+sx+rtx^2+r^2sx^2+r^3tx^3-rt^3x^5-st^2x^4+2rstx^3-rs^2tx^4-1)W_1W_0$

and

$\Delta_3 = x^{n+3}(s-s^2x+r^2+rtx)W_{n+3}^2+x^{n+2}(s-s^2x+r^2t^2x^3-r^2sx+rt^3x^4-rs^2tx^3)W_{n+2}^2+x^{n+4}t^2(s-s^2x+r^2+rtx)W_{n+1}^2-x^{n+2}(r+tx)(r^2x-s^2x^2+t^2x^3-1)W_{n+3}W_{n+2}-x^{n+1}(r^2x+s^2x^2-s^3x^3+t^2x^3+sx+r^2sx^2-st^2x^4+2rstx^3-1)W_{n+3}W_{n+1}+x^{n+2}t(sx-1)(r^2x-s^2x^2+t^2x^3-1)W_{n+2}W_{n+1}-x^2(s-s^2x+r^2+rtx)W_2^2+x(-s+s^2x-r^2t^2x^3+r^2sx-rt^3x^4+rs^2tx^3)W_1^2-x^3t^2(s-s^2x+r^2+rtx)W_0^2+x(r+tx)(r^2x-s^2x^2+t^2x^3-1)W_2W_1+(r^2x+s^2x^2-s^3x^3+t^2x^3+sx+r^2sx^2-st^2x^4+2rstx^3-1)W_2W_0-xt(sx-1)(r^2x-s^2x^2+t^2x^3-1)W_1W_0.$

Proof. The proof is given in [29, Theorem 3.1.].

2. Main Result

Let

$$\Omega = (-t^2x^3+sx+rtx^2+1)^2(r^2x-s^2x^2+t^2x^3+2sx+2rtx^2-1)^2$$

THEOREM 2.1. *Let x be a real or complex number. If $\Omega \neq 0$ then*

(a):

$$\sum_{k=0}^{n} kx^k W_k^2 = \frac{\Omega_1}{\Omega},$$

(b):

$$\sum_{k=0}^{n} kx^k W_{k+1} W_k = \frac{\Omega_2}{\Omega},$$

(c):

$$\sum_{k=0}^{n} kx^k W_{k+2}W_k = \frac{\Omega_3}{\Omega},$$

where

$$\Omega_1 = \sum_{k=1}^{12} \Gamma_k, \quad \Omega_2 = \sum_{k=1}^{12} \Phi_k, \quad \Omega_3 = \sum_{k=1}^{12} \Psi_k,$$

with

$\Gamma_1 = x^{n+3}(n(t^2x^3 - sx - rtx^2 - 1)(t^2x^3 + sx + rtx^2 - 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 2r^2x - 2s^2x^2 - 2s^3x^3 + 6t^2x^3 + s^4x^4 - 3t^4x^6 + 6sx - 2r^2s^2x^3 - 9r^2t^2x^4 - 2r^4t^2x^5 - 6s^2t^2x^5 - 4r^3t^3x^6 - 2r^2t^4x^7 + 4s^3t^2x^6 - s^2t^4x^8 + 6rtx^2 - 6rt^3x^5 - 6st^2x^4 - 12r^2st^2x^5 + 2rs^2t^3x^7 - 6rs^2tx^4 - 4r^3stx^4 + 2rs^3tx^5 - 12rst^3x^6 - 3 - 2r^2sx^2 - 6rstx^3 + r^2s^2t^2x^6 - 4r^3tx^3)W_{n+3}^2,$

$\Gamma_2 = x^{n+2}(n(r^2x + t^2x^3 + sx + r^2t^2x^4 + rtx^2 + r^2sx^2 + r^3tx^3 + 2rstx^3 - 1)(t^2x^3 - sx - rtx^2 - 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 4r^2x - 2r^4x^2 - 2s^2x^2 + 3t^2x^3 - t^6x^9 + 4sx - 5r^2s^2x^3 - 2r^2s^3x^4 - 2r^2t^2x^4 - 2r^4s^2x^4 + r^2s^4x^5 - 8r^4t^2x^5 - 7s^2t^2x^5 - 12r^3t^3x^6 - 11r^2t^4x^7 + 4s^3t^2x^6 - 2r^6t^2x^6 - 4r^5t^3x^7 - 2r^4t^4x^8 - 4r^4sx^3 - 2rt^3x^5 - 2st^2x^4 - 4r^5tx^4 - 2rt^5x^8 - 2st^4x^7 - 20r^2st^2x^5 - 14r^3s^2tx^5 + 4rs^2t^3x^7 + 2r^3s^3tx^6 - 16r^4st^2x^6 - 14r^3st^3x^7 + 2rstx^3 - 18r^2s^2t^2x^6 + 6r^2s^3t^2x^7 + r^4s^2t^2x^7 + 2r^3s^2t^3x^8 - r^2s^2t^4x^9 - 8rs^2tx^4 - 16r^3stx^4 - 6rs^3tx^5 - 18rst^3x^6 + 4rs^4tx^6 - 4r^5stx^5 - 2rst^5x^9 - 2 + 4rtx^2)W_{n+2}^2,$

$\Gamma_3 = x^{n+1}(n(t^2x^3 - sx - rtx^2 - 1)(r^2x + s^2x^2 - s^3x^3 + t^2x^3 + sx + r^2t^2x^4 + s^2t^2x^5 + rtx^2 + r^2sx^2 + r^3tx^3 + 4rstx^3 - rs^2tx^4 - 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 2r^2x - r^4x^2 + s^2x^2 - 4s^3x^3 + s^4x^4 + 2s^5x^5 - s^6x^6 + 3t^4x^6 - 2t^6x^9 + 2sx - 4r^2s^2x^3 - r^4s^2x^4 + 2r^2s^4x^5 - 4r^4t^2x^5 - 2s^2t^2x^5 - 8r^3t^3x^6 - 10r^2t^4x^7 - r^6t^2x^6 - 2r^5t^3x^7 - 2s^4t^2x^7 - 2r^4t^4x^8 - 8s^2t^4x^8 - 2r^3t^5x^9 + 2s^5t^2x^8 - r^2t^6x^{10} + 4s^3t^4x^9 - s^4t^4x^{10} + 2rt^3x^5 + 2st^2x^4 - 2r^5tx^4 - 4rt^5x^8 - 4st^4x^7 - 14r^2st^2x^5 - 8r^3s^2tx^5 + 4r^3s^3tx^6 - 10r^4st^2x^6 - 10r^3st^3x^7 - 8rs^3t^3x^8 - 6r^2st^4x^8 + 2rs^4t^3x^9 + 2rs^2t^5x^{10} + 6rstx^3 - 19r^2s^2t^2x^6 + 8r^2s^3t^2x^7 + 2r^4s^2t^2x^7 - r^2s^4t^2x^8 - 2r^2s^2t^4x^9 - 12rs^2tx^4 - 12r^3stx^4 - 4rs^3tx^5 - 16rst^3x^6 + 10rs^4tx^6 - 2r^5stx^5 - 2rs^5tx^7 - 8rst^5x^9 - 1 + 2rtx^2 - 2r^4sx^3)W_{n+1}^2,$

$\Gamma_4 = 2x^{n+4}(n(r + tx)(-t^2x^3 + sx + rtx^2 + 1)(s + rtx)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 2r^3s^2x^2 - 4rs + 8r^3t^2x^3 + 8r^2t^3x^4 + 2r^5t^2x^4 + 4r^4t^3x^5 - s^2t^3x^5 + 2r^3t^4x^6 + 3rs^2x + 3r^3sx - 5r^2tx + 2rs^3x^2 - 6rt^2x^2 - rs^4x^3 + 4r^4tx^2 + 4s^2tx^2 + 3s^3tx^3 + 6rt^4x^5 + 4st^3x^4 - 2s^4tx^4 + st^5x^7 - 5stx + 10r^2s^2tx^3 + 12rs^2t^2x^4 - 2r^2s^3tx^4 + 14r^3st^2x^4 - 5rs^3t^2x^5 + 13r^2st^3x^5 + rs^2t^4x^7 - r^3s^2t^2x^5 - 2r^2s^2t^3x^6 + 10r^2stx^2 + 10rst^2x^3 + 4r^4stx^3)W_{n+3}W_{n+2},$

$\Gamma_5 = 2tx^{n+4}(n(-t^2x^3 + sx + rtx^2 + 1)(r + stx^2)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 3r^3x - 4r + r^2t^3x^5 - 2s^2t^3x^6 + s^3t^3x^7 - 6stx^2 + 2rs^2x^2 + 2r^3sx^2 - rs^3x^3 + 2r^2tx^2 + 2rt^2x^3 + r^4tx^3 + 5s^2tx^3 + 4s^3tx^4 + 2rt^4x^6 + 6st^3x^5 - 3s^4tx^5 + 3rsx + 11rs^2t^2x^5 + 3r^3st^2x^5 - 2rs^3t^2x^6 + 2r^2st^3x^6 + 9r^2stx^3 + 4rst^2x^4 - rst^4x^7 + 4r^2s^2tx^4)W_{n+3}W_{n+1},$

$\Gamma_6 = 2tx^{n+4}(n(sx - 1)(s + rtx)(t^2x^3 - sx - rtx^2 - 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 8s^2x - 4s - 2s^3x^2 - 4s^4x^3 + 2s^5x^4 - 2r^2s^2x^2 - 3r^2s^3x^3 + 3r^2t^2x^3 + 2r^4t^2x^4 - 4s^2t^2x^4 + r^3t^3x^5 - s^2t^4x^7 + 4r^3tx^2 + 4rt^3x^4 + 2st^2x^3 + rt^5x^7 + 2st^4x^6 - 5rtx + 4r^2st^2x^4 - 6r^3s^2tx^4 + 2rs^2t^3x^6 - 3r^4st^2x^5 - 2r^3st^3x^6 - rs^3t^3x^7 + r^2st^4x^7 + 12rstx^2 - 14r^2s^2t^2x^5 + 2r^2s^3t^2x^6 - rs^2tx^3 - r^3stx^3 - 14rs^3tx^4 - 6rst^3x^5 + 4rs^4tx^5 + 3r^2sx)W_{n+2}W_{n+1},$

$\Gamma_7 = x^2(-r^2x + 2s^2x^2 - 3t^2x^3 + t^6x^9 - 4sx + r^2s^2x^3 + 8r^2t^2x^4 + r^4t^2x^5 + 7s^2t^2x^5 + 2r^3t^3x^6 + 2r^2t^4x^7 - 4s^3t^2x^6 - 4rtx^2 + 2r^2sx^2 + 4r^3tx^3 + 2rt^3x^5 + 2st^2x^4 + 2rt^5x^8 + 2st^4x^7 + 6r^2st^2x^5 - 2rs^2t^3x^7 + 8rstx^3 + 2r^3stx^4 + 10rst^3x^6 + 2)W_2^2,$

$\Gamma_8 = x(-2r^2x + r^4x^2 + 2s^2x^2 - 2s^3x^3 + s^4x^4 - 3t^4x^6 + 2t^6x^9 - 2sx + 2r^2s^2x^3 + 2r^2s^3x^4 + r^4s^2x^4 + 4r^4t^2x^5 + 8s^2t^2x^5 + 8r^3t^3x^6 + 10r^2t^4x^7 - 4s^3t^2x^6 + r^6t^2x^6 + 2r^5t^3x^7 + 2r^4t^4x^8 - s^2t^4x^8 + 2r^3t^5x^9 + r^2t^6x^{10} - 2rtx^2 + 2r^4sx^3 - 2rt^3x^5 - 2st^2x^4 + 2r^5tx^4 + 4rt^5x^8 + 4st^4x^7 + 10r^2st^2x^5 + 8r^3s^2tx^5 - 2rs^2t^3x^7 + 8r^4st^2x^6 + 10r^3st^3x^7 - 2rs^3t^3x^8 + 4r^2st^4x^8 + 2rstx^3 + 10r^2s^2t^2x^6 - 4r^2s^3t^2x^7 - 2r^3s^2t^3x^8 + 6r^3stx^4 + 6rs^3tx^5 + 12rst^3x^6 - 2rs^4tx^6 + 2r^5stx^5 + 4rst^5x^9 + 1)W_1^2,$

$\Gamma_9 = t^2x^3(-2r^2x + 2s^2x^2 + 2s^3x^3 - 6t^2x^3 - s^4x^4 + 3t^4x^6 - 6sx + 2r^2s^2x^3 + 9r^2t^2x^4 + 2r^4t^2x^5 + 6s^2t^2x^5 + 4r^3t^3x^6 + 2r^2t^4x^7 - 4s^3t^2x^6 + s^2t^4x^8 - 6rtx^2 + 2r^2sx^2 + 4r^3tx^3 + 6rt^3x^5 + 6st^2x^4 + 12r^2st^2x^5 - 2rs^2t^3x^7 + 6rstx^3 - r^2s^2t^2x^6 + 6rs^2tx^4 + 4r^3stx^4 - 2rs^3tx^5 + 12rst^3x^6 + 3)W_0^2,$

$\Gamma_{10} = -2x^3(-3rs + r^3s^2x^2 + 6r^3t^2x^3 + 5r^2t^3x^4 + r^5t^2x^4 + 2r^4t^3x^5 + 2r^3t^4x^6 + 2r^2t^5x^7 - s^3t^3x^6 + 2rs^2x + 2r^3sx - 4r^2tx + rs^3x^2 - 5rt^2x^2 + 3r^4tx^2 + 3s^2tx^2 + 2s^3tx^3 + 4rt^4x^5 + 2st^3x^4 - s^4tx^4 + rt^6x^8 + 2st^5x^7 - 4stx + 4r^2s^2tx^3 + 8rs^2t^2x^4 + 7r^3st^2x^4 - 4rs^3t^2x^5 + 10r^2st^3x^5 - 2r^2s^2t^3x^6 + 7r^2stx^2 + 6rst^2x^3 + 2r^4stx^3 + 3rst^4x^6)$
$W_2W_1,$

$\Gamma_{11} = -2tx^3(-3r + 2r^3x - r^3t^2x^4 + 2r^2t^3x^5 - s^2t^3x^6 - 5stx^2 + rs^2x^2 + r^3sx^2 + r^2tx^2 + 4s^2tx^3 + 3s^3tx^4 + 3rt^4x^6 + 4st^3x^5 - 2s^4tx^5 + st^5x^8 + 2rsx + 4r^2s^2tx^4 + 6rs^2t^2x^5 + 2r^3st^2x^5 - rs^3t^2x^6 + r^2st^3x^6 + 4r^2stx^3 + 4rst^2x^4)$
$W_2W_0,$

$\Gamma_{12} = 2tx^3(3s - 6s^2x + 2s^3x^2 + 2s^4x^3 - s^5x^4 + 2r^2s^2x^2 + 2r^2s^3x^3 - 2r^2t^2x^3 - r^4t^2x^4 + s^2t^2x^4 - r^2t^4x^6 + 2s^3t^2x^5 - s^4t^2x^6 + 2s^2t^4x^7 - 2r^2sx - 3r^3tx^2 - 2rt^3x^4 - 2rt^5x^7 - 3st^4x^6 + 4rtx + 4r^3s^2tx^4 + rs^2t^3x^6 + 2r^4st^2x^5 + r^3st^3x^6 - 9rstx^2 + 8r^2s^2t^2x^5 - r^2s^3t^2x^6 + 4rs^2tx^3 + 2r^3stx^3 + 7rs^3tx^4 + 2rst^3x^5 - 2rs^4tx^5 + rst^5x^8)W_1W_0$

and

$\Phi_1 = x^{n+3}(n(r + stx^2)(-t^2x^3 + sx + rtx^2 + 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 2r^3x - 3r - r^3t^2x^4 + 2r^2t^3x^5 - s^2t^3x^6 - 5stx^2 + rs^2x^2 + r^3sx^2 + r^2tx^2 + 4s^2tx^3 + 3s^3tx^4 + 3rt^4x^6 + 4st^3x^5 - 2s^4tx^5 + st^5x^8 + 2rsx + 4r^2s^2tx^4 + 6rs^2t^2x^5 + 2r^3st^2x^5 - rs^3t^2x^6 + r^2st^3x^6 + 4r^2stx^3 + 4rst^2x^4)W_{n+3}^2,$

$\Phi_2 = x^{n+4}(t + rs)(n(s + rtx)(-t^2x^3 + sx + rtx^2 + 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 3s^2x - 4s + 2s^3x^2 - s^4x^3 + 2r^2s^2x^2 + 3r^2t^2x^3 + 2r^4t^2x^4 + r^3t^3x^5 - s^3t^2x^5 + 3r^2sx + 4r^3tx^2 + 4rt^3x^4 + 2st^2x^3 + rt^5x^7 + 2st^4x^6 - 5rtx + 8r^2st^2x^4 + 6rstx^2 - r^2s^2t^2x^5 + 7rs^2tx^3 + 4r^3stx^3 - 2rs^3tx^4)W_{n+2}^2,$

$\Phi_3 = t^2x^{n+4}(n(r + stx^2)(-t^2x^3 + sx + rtx^2 + 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 3r^3x - 4r + r^2t^3x^5 - 2s^2t^3x^6 + s^3t^3x^7 - 6stx^2 + 2rs^2x^2 + 2r^3sx^2 - rs^3x^3 + 2r^2tx^2 + 2rt^2x^3 + r^4tx^3 + 5s^2tx^3 + 4s^3tx^4 + 2rt^4x^6 + 6st^3x^5 - 3s^4tx^5 + 3rsx + 4r^2s^2tx^4 + 11rs^2t^2x^5 + 3r^3st^2x^5 - 2rs^3t^2x^6 + 2r^2st^3x^6 + 9r^2stx^3 + 4rst^2x^4 - rst^4x^7)W_{n+1}^2,$

$\Phi_4 = x^{n+2}(n(t^2x^3 - sx - rtx^2 - 1)(r^2x + s^2x^2 + t^2x^3 + 2rstx^3 - 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 4r^2x - 2r^4x^2 + 4s^2x^2 - 2s^3x^3 + 3t^2x^3 - 2s^4x^4 + s^5x^5 - t^6x^9 + sx - 4r^2s^2x^3 - 2r^2s^3x^4 - 6r^2t^2x^4 + r^4t^2x^5 - 8s^2t^2x^5 - 4r^3t^3x^6 - 4r^2t^4x^7 + 2s^3t^2x^6 + s^4t^2x^7 - 2s^2t^4x^8 - 2r^2sx^2 - r^4sx^3 - 2r^3tx^3 - 2st^2x^4 + st^4x^7 - 10r^2st^2x^5 - 8r^3s^2tx^5 + 2rs^2t^3x^7 - 4r^4st^2x^6 - 2r^3st^3x^7 + 6rstx^3 - 14r^2s^2t^2x^6 + 2r^2s^3t^2x^7 - 8rs^2tx^4 - 8r^3stx^4 - 10rs^3tx^5 - 16rst^3x^6 + 4rs^4tx^6 - 2rst^5x^9 - 2)W_{n+3}W_{n+2},$

$\Phi_5 = tx^{n+3}(n(-t^2x^3 + sx + rtx^2 + 1)(r^2x - s^2x^2 - t^2x^3 + 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 3r^4x^2 - 2r^2x + 6s^2x^2 - 4s^3x^3 + 6t^2x^3 - 3s^4x^4 + 2s^5x^5 - 3t^4x^6 + 2sx - 2r^2s^2x^3 - 4r^2s^3x^4 - 4r^2t^2x^4 - 10s^2t^2x^5 - 2r^3t^3x^6 + 4s^3t^2x^6 - 2s^2t^4x^8 + rtx^2 - 2rt^3x^5 - 4st^2x^4 + r^5tx^4 + rt^5x^8 + 2st^4x^7 - 4r^2st^2x^5 - 2r^3s^2tx^5 + 2rs^2t^3x^7 - 2r^2s^2t^2x^6 - 2rs^2tx^4 + 4r^3stx^4 - 8rs^3tx^5 - 12rst^3x^6 + rs^4tx^6 - 3 + 4r^2sx^2 + 2r^4sx^3 + 2r^3tx^3)W_{n+3}W_{n+1},$

$\Phi_6 = x^{n+1}(n(t^2x^3 - sx - rtx^2 - 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1)(r^2x + s^2x^2 - s^3x^3 + t^2x^3 + sx + rtx^2 + r^2sx^2 + r^3tx^3 - rt^3x^5 - st^2x^4 + 2rstx^3 - rs^2tx^4 - 1) + 2r^2x - r^4x^2 + s^2x^2 - 4s^3x^3 + s^4x^4 + 2s^5x^5 - s^6x^6 + 3t^4x^6 - 2t^6x^9 + 2sx - 4r^2s^2x^3 - 5r^2t^2x^4 - r^4s^2x^4 + 2r^2s^4x^5 - 4s^2t^2x^5 - 2r^2t^4x^7 + 8s^3t^2x^6 - r^6t^2x^6 + 2r^4t^4x^8 - 3s^2t^4x^8 - s^5t^2x^8 - r^2t^6x^{10} + 2s^3t^4x^9 + 2rtx^2 - 3st^2x^4 - 2r^5tx^4 + 2rt^5x^8 + st^6x^{10} - 2r^4sx^3 - 4rt^3x^5 - 2r^2st^2x^5 - 4r^3s^2tx^5 + 16rs^2t^3x^7 + 4r^3s^3tx^6 - 5r^4st^2x^6 + 4r^3st^3x^7 - 2rs^3t^3x^8 + 8r^2st^4x^8 + 2rs^2t^5x^{10} - 2rstx^3 + 10r^2s^3t^2x^7 + 2r^4s^2t^2x^7 - r^2s^4t^2x^8 + 2r^3s^2t^3x^8 - 2r^2s^2t^4x^9 - 6rs^2tx^4 - 6r^3stx^4 + 8rs^4tx^6 - 2r^5stx^5 - 2rs^5tx^7 - 6rst^5x^9 - 1 - 4rst^3x^6)W_{n+2}W_{n+1},$

$\Phi_7 = x^2(2r - r^3x + 2r^3t^2x^4 - 3r^2t^3x^5 + s^3t^3x^7 + 4stx^2 - rs^3x^3 + 2rt^2x^3 + r^4tx^3 - 3s^2tx^3 - 2s^3tx^4 - 4rt^4x^6 - 2st^3x^5 + s^4tx^5 - 2st^5x^8 - rsx - 4r^2s^2tx^4 - rs^2t^2x^5 - r^3st^2x^5 + r^2stx^3 - 4rst^2x^4 - rst^4x^7)W_2^2,$

$\Phi_8 = x^3(t + rs)(3s - 2s^2x - s^3x^2 - r^2s^2x^2 - 2r^2t^2x^3 - r^4t^2x^4 - s^2t^2x^4 - r^2t^4x^6 + 2s^3t^2x^5 - 2r^2sx - 3r^3tx^2 - 2rt^3x^4 - 2rt^5x^7 - 3st^4x^6 + 4rtx - 3r^2st^2x^4 + rs^2t^3x^6 - 4rstx^2 - 2rs^2tx^3 - 2r^3stx^3 - 2rst^3x^5)W_1^2,$

$\Phi_9 = t^2x^3(3r - 2r^3x + r^3t^2x^4 - 2r^2t^3x^5 + s^2t^3x^6 + 5stx^2 - rs^2x^2 - r^3sx^2 - r^2tx^2 - 4s^2tx^3 - 3s^3tx^4 - 3rt^4x^6 - 4st^3x^5 + 2s^4tx^5 - st^5x^8 - 2rsx - 4r^2s^2tx^4 - 6rs^2t^2x^5 - 2r^3st^2x^5 + rs^3t^2x^6 - r^2st^3x^6 - 4r^2stx^3 - 4rst^2x^4)W_0^2,$

$\Phi_{10} = -x(2r^2x - r^4x^2 + 2s^2x^2 - s^4x^4 + 3t^4x^6 - 2t^6x^9 - 2r^2s^2x^3 - 2r^2s^3x^4 - 4r^2t^2x^4 + 2r^4t^2x^5 - 6s^2t^2x^5 - 4r^3t^3x^6 - 4r^2t^4x^7 + 2s^4t^2x^7 - 2s^2t^4x^8 - rtx^2 - 2r^2sx^2 - 2r^3tx^3 + 2rt^3x^5 + r^5tx^4 - rt^5x^8 - 8r^2st^2x^5 - 6r^3s^2tx^5 - 2rs^2t^3x^7 - 2r^4st^2x^6 + 2rs^3t^3x^8 - 2r^2st^4x^8 - 4r^2s^2t^2x^6 - 4rs^2tx^4 - 2r^3stx^4 - 4rs^3tx^5 - 8rst^3x^6 + rs^4tx^6 - 4rst^5x^9 - 1)W_2W_1,$

$\Phi_{11} = tx^2(2r^2x - 2r^4x^2 - 4s^2x^2 + 2s^3x^3 - 3t^2x^3 + 2s^4x^4 - s^5x^5 + t^6x^9 - sx + 2r^2s^2x^3 + 2r^2s^3x^4 + 6r^2t^2x^4 + r^4t^2x^5 + 8s^2t^2x^5 - 2r^2t^4x^7 - 2s^3t^2x^6 - s^4t^2x^7 + 2s^2t^4x^8 - 2r^2sx^2 - r^4sx^3 + 2st^2x^4 - st^4x^7 + 2r^2st^2x^5 + 4rstx^3 + 2r^2s^2t^2x^6 + 4rs^3tx^5 + 8rst^3x^6 + 2)W_2W_0,$

$\Phi_{12} = tx^3(3t - 6t^3x^3 + 3t^5x^6 + 6rs - 2r^3s^2x^2 - 2r^3t^2x^3 + 4r^2t^3x^4 - r^5t^2x^4 + 2s^2t^3x^5 + 2r^3t^4x^6 - 2s^3t^3x^6 - 4rs^2x - 4r^3sx + 2r^2tx - 2rs^3x^2 - rt^2x^2 - 3r^4tx^2 + 4s^2tx^2 - 4s^3tx^3 + 2rt^4x^5 + 4st^3x^4 - 3s^4tx^4 + 2s^5tx^5 - rt^6x^8 - 2st^5x^7 - 2stx - 6r^2s^2tx^3 - 6rs^2t^2x^4 - 4r^2s^3tx^4 - 2r^3st^2x^4 - 4rs^3t^2x^5 + rs^4t^2x^6 - 2rs^2t^4x^7 - 2r^3s^2t^2x^5 - 6r^2stx^2 - 2r^4stx^3 + 6rst^4x^6)W_1W_0$

and

$\Psi_1 = (n(-t^2x^3 + sx + rtx^2 + 1)(s - s^2x + r^2 + rtx)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1) + 2r^4x - 3s + 6s^2x - 2s^3x^2 - 2s^4x^3 + s^5x^4 - 3r^2 - r^2s^2x^2 - 2r^2s^3x^3 + 2r^2t^2x^3 - s^2t^2x^4 + 2r^3t^3x^5 + 4r^2t^4x^6 - 2s^3t^2x^5 + s^4t^2x^6 - 2s^2t^4x^7 + 4r^2sx + r^4sx^2 + 4r^3tx^2 + 2rt^3x^4 + 2rt^5x^7 + 3st^4x^6 - 4rtx + 4r^2st^2x^4 - 2rs^2t^3x^6 + 4rstx^2 - 2r^2s^2t^2x^5 + 2r^3stx^3 - 4rs^3tx^4 + 2rst^3x^5)x^{n+3}W_{n+3}^2,$

$\Psi_2 = (n(t^2x^3 - sx - rtx^2 - 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1)(-s + s^2x - r^2t^2x^3 + r^2sx - rt^3x^4 + rs^2tx^3) + 4s^2x - 2s - 2s^3x^2 - 4r^2s^2x^2 - 2r^2s^3x^3 - 5r^2t^2x^3 - r^4s^2x^3 + 4r^4t^2x^4 + 2s^2t^2x^4 + 8r^3t^3x^5 + 8r^2t^4x^6 - 4s^3t^2x^5 + 2r^5t^3x^6 + 2s^4t^2x^6 + 4r^4t^4x^7 - 3s^2t^4x^7 + 2r^3t^5x^8 - 2r^4sx^2 - 6rt^3x^4 - 2st^2x^3 + 6rt^5x^7 + 4st^4x^6 + 2r^2st^2x^4 - 4r^3s^2tx^4 - 2rs^2t^3x^6 - 4r^3s^3tx^5 + 4r^4st^2x^5 + 10r^3st^3x^6 - 2rs^3t^3x^7 + 8r^2st^4x^7 - 8r^2s^3t^2x^6 - 2r^4s^2t^2x^6 + r^2s^4t^2x^7 - 2r^3s^2t^3x^7 - 2r^2s^2t^4x^8 - 2r^3stx^3 - 2rs^3tx^4 + 8rst^3x^5 - 4rs^4tx^5 + 2rs^5tx^6 - 2rst^5x^8 + 4r^2sx)x^{n+2}W_{n+2}^2,$

$\Psi_3 = (n(-t^2x^3 + sx + rtx^2 + 1)(r^2x - s^2x^2 + t^2x^3 + 2sx + 2rtx^2 - 1)(s - s^2x + r^2 + rtx) + 3r^4x - 4s + 8s^2x - 2s^3x^2 - 4s^4x^3 + 2s^5x^4 - 4r^2 - 4r^2s^3x^3 + 5r^2t^2x^3 + 2r^4t^2x^4 - 4s^2t^2x^4 + 2r^3t^3x^5 + 2r^2t^4x^6 - s^2$

$t^4x^7+6r^2sx+2r^4sx^2+6r^3tx^2+4rt^3x^4+2st^2x^3+r^5tx^3+rt^5x^7+2st^4x^6-5rtx+8r^2st^2x^4-2r^3s^2tx^4+6rstx^2-3r^2s^2t^2x^5+4rs^2tx^3+8r^3stx^3-10rs^3tx^4+rs^4tx^5)t^2x^{n+4}W_{n+1}^2,$

$\Psi_4=(n(r+tx)(t^2x^3-sx-rtx^2-1)(r^2x-s^2x^2+t^2x^3-1)(r^2x-s^2x^2+t^2x^3+2sx+2rtx^2-1)+4r^3x-2r-2r^5x^2+6t^3x^4-3t^5x^7-3tx+2r^3s^2x^3+2r^3s^3x^4-8r^3t^2x^4-8r^2t^3x^5-4r^4t^3x^6-2s^2t^3x^6-8r^3t^4x^7-4r^2t^5x^8+2s^3t^3x^7+2stx^2-4rs^2x^2-2r^3sx^2+4rs^3x^3+6r^2tx^2+4rt^2x^3+2rs^4x^4-r^5sx^3-5r^4tx^3-4s^2tx^3-rs^5x^5+4s^3tx^4-2rt^4x^6-4st^3x^5+3s^4tx^5-2s^5tx^6+2st^5x^8+rsx+6r^2s^2tx^4+8r^2s^3tx^5-8r^3st^2x^5+10rs^3t^2x^6-12r^2st^3x^6-2rs^4t^2x^7+4rs^2t^4x^8+4r^3s^2t^2x^6+4r^2s^2t^3x^7-6r^2stx^3-2rst^2x^4-2r^4stx^4-11rst^4x^7)x^{n+2}W_{n+3}W_{n+2},$

$\Psi_5=(n(t^2x^3-sx-rtx^2-1)(r^2x+s^2x^2-s^3x^3+t^2x^3+sx+r^2sx^2-st^2x^4+2rstx^3-1)(r^2x-s^2x^2+t^2x^3+2sx+2rtx^2-1)+2r^2x-r^4x^2+s^2x^2-4s^3x^3+s^4x^4+2s^5x^5-s^6x^6+3t^4x^6-2t^6x^9+2sx-4r^2s^2x^3-4r^2t^2x^4-r^4s^2x^4+2r^2s^4x^5+2r^4t^2x^5-4s^2t^2x^5-4r^3t^3x^6-4r^2t^4x^7+8s^3t^2x^6-3s^2t^4x^8-s^5t^2x^8+2s^3t^4x^9-rtx^2-2r^4sx^3-2r^3tx^3+2rt^3x^5-3st^2x^4+r^5tx^4-rt^5x^8+st^6x^{10}-2r^2st^2x^5-6r^3s^2tx^5+6rs^2t^3x^7-r^4st^2x^6+2rs^3t^3x^8-4r^2st^4x^8-2r^2s^2t^2x^6+2r^2s^3t^2x^7-2r^3stx^4-4rs^3tx^5-8rst^3x^6+5rs^4tx^6-4rst^5x^9-1)x^{n+1}W_{n+3}W_{n+1},$

$\Psi_6=(n(sx-1)(-t^2x^3+sx+rtx^2+1)(r^2x-s^2x^2+t^2x^3+2sx+2rtx^2-1)(r^2x-s^2x^2+t^2x^3-1)+4r^2x-2r^4x^2-6s^2x^2+8s^3x^3+3t^2x^3-2s^4x^4-4s^5x^5+2s^6x^6-t^6x^9+4sx+4r^2s^2x^3-6r^2t^2x^4+2r^4s^2x^4-4r^2s^4x^5+r^4t^2x^5-4r^3t^3x^6-4r^2t^4x^7+4s^3t^2x^6-3s^4t^2x^7-8r^2sx^2+2r^4sx^3-2r^3tx^3-8st^2x^4+4st^4x^7+4r^2st^2x^5+4r^3s^2tx^5+14rs^2t^3x^7-2r^3s^3tx^6+4r^3st^3x^7-2rs^3t^3x^8+4r^2st^4x^8-5rstx^3+6r^2s^2t^2x^6-2r^2s^3t^2x^7+4rs^2tx^4+2r^3stx^4-6rst^3x^6-8rs^4tx^6+r^5stx^5+rs^5tx^7-rst^5x^9-2)tx^{n+2}W_{n+2}W_{n+1},$

$\Psi_7=(2s-r^4x-4s^2x+2s^3x^2+2r^2+2r^2s^2x^2+r^2t^2x^3+2r^4t^2x^4-2s^2t^2x^4-2r^3t^3x^5-6r^2t^4x^6+4s^3t^2x^5-2s^4t^2x^6+3s^2t^4x^7-2r^2sx-2r^3tx^2+2st^2x^3+r^5tx^3-3rt^5x^7-4st^4x^6+3rtx-2r^3s^2tx^4+4rs^2t^3x^6-2rstx^2+r^2s^2t^2x^5+4rs^2tx^3+4r^3stx^3-2rs^3tx^4-4rst^3x^5+rs^4tx^5)x^2W_2^2$

$\Psi_8=x(s-2s^2x+2s^3x^2-2s^4x^3+s^5x^4+3r^2s^2x^2+4r^2t^2x^3+r^2s^4x^4-3r^4t^2x^4-5s^2t^2x^4-6r^3t^3x^5-5r^2t^4x^6+6s^3t^2x^5-r^5t^3x^6-3s^4t^2x^6-2r^4t^4x^7+4s^2t^4x^7-2r^3t^5x^8-2r^2t^6x^9-2r^2sx+r^4sx^2+5rt^3x^4+4st^2x^3-4rt^5x^7-5st^4x^6-rt^7x^{10}-2r^2st^2x^4-2r^3s^2tx^4+2rs^2t^3x^6+4r^3s^3tx^5-4r^4st^2x^5-4r^3st^3x^6+2rs^3t^3x^7-4r^2st^4x^7-rs^4t^3x^8+2rs^2t^5x^9+rstx^2+2r^2s^3t^2x^6+r^4s^2t^2x^6+3r^2s^2t^4x^8+4rs^2tx^3+2r^3stx^3-4rs^3tx^4-8rst^3x^5+4rs^4tx^5-r^5stx^4-rs^5tx^6+rst^5x^8)W_1^2,$

$\Psi_9=t^2x^3(3s-2r^4x-6s^2x+2s^3x^2+2s^4x^3-s^5x^4+3r^2+r^2s^2x^2+2r^2s^3x^3-2r^2t^2x^3+s^2t^2x^4-2r^3t^3x^5-4r^2t^4x^6+2s^3t^2x^5-s^4t^2x^6+2s^2t^4x^7-4r^2sx-r^4sx^2-4r^3tx^2-2rt^3x^4-2rt^5x^7-3st^4x^6+4rtx-4r^2st^2x^4+2rs^2t^3x^6-4rstx^2+2r^2s^2t^2x^5-2r^3stx^3+4rs^3tx^4-2rst^3x^5)W_0^2,$

$\Psi_{10}=x(-r^6tx^4-2r^5t^2x^5+r^5x^2+2r^4s^2tx^5-3r^4stx^4+3r^4t^3x^6+4r^4tx^3-2r^3s^2t^2x^6-2r^3s^2x^3+4r^3st^2x^5+2r^3sx^2+8r^3t^4x^7+6r^3t^2x^4-2r^3x-r^2s^4tx^6-2r^2s^3tx^5-4r^2s^2t^3x^7-6r^2s^2tx^4+8r^2st^3x^6+10r^2stx^3+5r^2t^5x^8+4r^2t^3x^5-3r^2tx^2+2rs^4t^2x^7-rs^4x^4-6rs^3t^2x^6-4rs^3x^3-6rs^2t^4x^8+2rs^2t^2x^5+4rs^2x^2+8rst^4x^7+4rst^2x^4+2rt^6x^9-3rt^4x^6+r+s^5tx^6+s^4t^3x^8-2s^4tx^5-2s^3t^3x^7-4s^3tx^4-2s^2t^5x^9+4s^2t^3x^6+4s^2tx^3-st^5x^8+2st^3x^5-stx^2+t^7x^{10}-3t^3x^4+2tx)W_2W_1,$

$\Psi_{11}=tx^2(2r+2r^3x-2r^5x^2-6t^3x^4+3t^5x^7+3tx+2r^3s^3x^4+4r^3t^2x^4+4r^2t^3x^5+2s^2t^3x^6-2s^3t^3x^7-2stx^2-8rs^2x^2-6r^3sx^2+2r^2tx^2-4rt^2x^3+2rs^4x^4-r^5sx^3-3r^4tx^3+4s^2tx^3-rs^5x^5-4s^3tx^4+2rt^4x^6+4st^3x^5-3s^4tx^5+2s^5tx^6-2st^5x^8+5rsx-6r^2s^2tx^4+2rs^2t^2x^5-6rs^3t^2x^6+8r^2st^3x^6-2r^2stx^3+2rst^2x^4-2r^4stx^4+5rst^4x^7)W_2W_0,$

$$\Psi_{12} = tx(-r^5tx^4 - r^4s^2x^4 + r^4st^2x^6 - 2r^4sx^3 - 2r^4t^2x^5 + r^4x^2 + 2r^3s^2tx^5 - 4r^3st^3x^7 - 6r^3stx^4 + 4r^3t^3x^6 + 2r^3tx^3 + 2r^2s^4x^5 + 2r^2s^3t^2x^7 + 2r^2s^3x^4 - 6r^2s^2t^2x^6 - 4r^2s^2x^3 - 4r^2st^4x^8 - 2r^2st^2x^5 + 6r^2sx^2 + 4r^2t^4x^7 + 4r^2t^2x^4 - 2r^2x + 3rs^4tx^6 + 2rs^3t^3x^8 + 4rs^3tx^5 - 10rs^2t^3x^7 - 8rs^2tx^4 + 4rst^3x^6 + 8rstx^3 + rt^5x^8 - 2rt^3x^5 + rtx^2 - s^6x^6 - s^5t^2x^8 + 2s^5x^5 + 4s^4t^2x^7 + 3s^4x^4 + 2s^3t^4x^9 - 6s^3t^2x^6 - 8s^3x^3 - 3s^2t^4x^8 + 4s^2t^2x^5 + 5s^2x^2 - st^6x^{10} + 3st^2x^4 - 2sx + 2t^6x^9 - 3t^4x^6 + 1)W_1W_0.$$

Proof. First, we obtain $\sum_{k=0}^{n} kx^kW_k^2$. Using the recurrence relation

$$W_{n+3} = rW_{n+2} + sW_{n+1} + tW_n$$

or

$$tW_n = W_{n+3} - rW_{n+2} - sW_{n+1}$$

i.e.

$$t^2W_n^2 = (W_{n+3} - rW_{n+2} - sW_{n+1})^2 = W_{n+3}^2 + r^2W_{n+2}^2 + s^2W_{n+1}^2 - 2rW_{n+3}W_{n+2} - 2sW_{n+3}W_{n+1} + 2rsW_{n+2}W_{n+1}$$

we obtain

$$\begin{aligned}
t^2 \times n \times x^nW_n^2 &= n \times x^nW_{n+3}^2 + r^2 \times n \times x^nW_{n+2}^2 + s^2 \times n \times x^nW_{n+1}^2 \\
&\quad -2r \times n \times x^nW_{n+3}W_{n+2} - 2s \times n \times x^nW_{n+3}W_{n+1} + 2rs \times n \times x^nW_{n+2}W_{n+1} \\
t^2 \times (n-1) \times x^{n-1}W_{n-1}^2 &= (n-1) \times x^{n-1}W_{n+2}^2 + r^2 \times (n-1) \times x^{n-1}W_{n+1}^2 + s^2 \times (n-1) \times x^{n-1}W_n^2 \\
&\quad -2r \times (n-1) \times x^{n-1}W_{n+2}W_{n+1} - 2s \times (n-1) \times x^{n-1}W_{n+2}W_n \\
&\quad +2rs \times (n-1) \times x^{n-1}W_{n+1}W_n \\
t^2 \times (n-2) \times x^{n-2}W_{n-2}^2 &= (n-2) \times x^{n-2}W_{n+1}^2 + r^2 \times (n-2)x^{n-2}W_n^2 + s^2 \times (n-2) \times x^{n-2}W_{n-1}^2 \\
&\quad -2r \times (n-2) \times x^{n-2}W_{n+1}W_n - 2s \times (n-2) \times x^{n-2}W_{n+1}W_{n-1} \\
&\quad +2rs \times (n-2)x^{n-2}W_nW_{n-1} \\
&\quad \vdots \\
t^2 \times 1 \times x^1W_1^2 &= 1 \times x^1W_4^2 + r^2 \times 1 \times x^1W_3^2 + s^2 \times 1 \times x^1W_2^2 \\
&\quad -2r \times 1 \times x^1W_4W_3 - 2s \times 1 \times x^1W_4W_2 + 2rs \times 1 \times x^1W_3W_2 \\
t^2 \times 0 \times x^0W_0^2 &= 0 \times x^0W_3^2 + r^2 \times 0 \times x^0W_2^2 + s^2 \times 0 \times x^0W_1^2 \\
&\quad -2r \times 0 \times x^0W_3W_2 - 2s \times 0 \times x^0W_3W_1 + 2rs \times 0 \times x^0W_2W_1
\end{aligned}$$

If we add the equations side by side, we get

$$\begin{aligned}
t^2\sum_{k=0}^{n}kx^kW_k^2 &= (nx^nW_{n+3}^2+(n-1)x^{n-1}W_{n+2}^2+(n-2)x^{n-2}W_{n+1}^2\\
&\quad +1\times x^{-1}W_2^2+2\times x^{-2}W_1^2+3\times x^{-3}W_0^2+\sum_{k=0}^{n}(k-3)x^{k-3}W_k^2)\\
&\quad +r^2(nx^nW_{n+2}^2+(n-1)x^{n-1}W_{n+1}^2+1\times x^{-1}W_1^2+2\times x^{-2}W_0^2+\sum_{k=0}^{n}(k-2)x^{k-2}W_k^2)\\
&\quad +s^2(nx^nW_{n+1}^2+1\times x^{-1}W_0^2+\sum_{k=0}^{n}(k-1)x^{k-1}W_k^2)-2r(nx^nW_{n+3}W_{n+2}\\
&\quad +(n-1)x^{n-1}W_{n+2}W_{n+1}+1\times x^{-1}W_2W_1+2\times x^{-2}W_1W_0+\sum_{k=0}^{n}(k-2)x^{k-2}W_{k+1}W_k)\\
&\quad -2s(nx^nW_{n+3}W_{n+1}+1\times x^{-1}W_2W_0+\sum_{k=0}^{n}(k-1)x^{k-1}W_{k+2}W_k)\\
&\quad +2rs(nx^nW_{n+2}W_{n+1}+1\times x^{-1}W_1W_0+\sum_{k=0}^{n}(k-1)x^{k-1}W_{k+1}W_k)
\end{aligned}$$

and so

$$\begin{aligned}
t^2\sum_{k=0}^{n}kx^kW_k^2 &= x^{-3}(r^2x+s^2x^2+1)\sum_{k=0}^{n}kx^kW_k^2-x^{-3}(2r^2x+s^2x^2+3)\sum_{k=0}^{n}x^kW_k^2 \qquad (2.1)\\
&\quad -2sx^{-1}\sum_{k=0}^{n}kx^kW_kW_{k+2}+2sx^{-1}\sum_{k=0}^{n}x^kW_kW_{k+2}+2r(sx-1)x^{-2}\sum_{k=0}^{n}kx^kW_{k+1}W_k\\
&\quad +2r(2-sx)x^{-2}\sum_{k=0}^{n}x^kW_{k+1}W_k+nx^nW_{n+3}^2+(n+nr^2x-1)x^{n-1}W_{n+2}^2\\
&\quad +(n-r^2x+nr^2x+ns^2x^2-2)x^{n-2}W_{n+1}^2-2nrx^nW_{n+2}W_{n+3}\\
&\quad -2nsx^nW_{n+1}W_{n+3}+2r(-n+nsx+1)x^{n-1}W_{n+1}W_{n+2}\\
&\quad +x^{-1}W_2^2+(r^2x+2)x^{-2}W_1^2+(2r^2x+s^2x^2+3)x^{-3}W_0^2\\
&\quad -2rx^{-1}W_1W_2-2sx^{-1}W_0W_2+2r(sx-2)x^{-2}W_1W_0
\end{aligned}$$

Next we obtain $\sum_{k=0}^{n}kx^kW_{k+1}W_k$. Multiplying the both side of the recurrence relation

$$tW_n=W_{n+3}-rW_{n+2}-sW_{n+1}$$

by W_{n+1} we get

$$tW_{n+1}W_n=W_{n+3}W_{n+1}-rW_{n+2}W_{n+1}-sW_{n+1}^2.$$

Then using last recurrence relation, we obtain

$$\begin{aligned}
t\times n\times x^nW_{n+1}W_n &= nx^nW_{n+3}W_{n+1}-r\times n\times x^nW_{n+2}W_{n+1}-s\times n\times x^nW_{n+1}^2\\
t\times (n-1)\times x^{n-1}W_nW_{n-1} &= (n-1)\times x^{n-1}W_{n+2}W_n-r\times (n-1)\times x^{n-1}W_{n+1}W_n\\
&\quad -s\times (n-1)\times x^{n-1}W_n^2\\
t\times (n-2)\times x^{n-2}W_{n-1}W_{n-2} &= (n-2)\times x^{n-2}W_{n+1}W_{n-1}-r\times (n-2)\times x^{n-2}W_nW_{n-1}\\
&\quad -s\times (n-2)\times x^{n-2}W_{n-1}^2\\
&\quad \vdots\\
t\times 2\times x^2W_3W_2 &= 2\times x^2W_5W_3-r\times 2\times W_4W_3-s\times 2\times x^2W_3^2\\
t\times 1\times xW_2W_1 &= 1\times xW_4W_2-r\times 1\times xW_3W_2-s\times 1\times xW_2^2\\
t\times 0\times x^0W_1W_0 &= 0\times x^0W_3W_1-r\times 0\times x^0W_2W_1-s\times 0\times x^0W_1^2
\end{aligned}$$

If we add the equations side by side, we get

$$\begin{aligned}
t\sum_{k=0}^{n}kx^kW_{k+1}W_k &= (nx^nW_{n+3}W_{n+1}+1\times x^{-1}W_2W_0+\sum_{k=0}^{n}(k-1)x^{k-1}W_{k+2}W_k)\\
&\quad -r(nx^nW_{n+2}W_{n+1}+1\times x^{-1}W_1W_0+\sum_{k=0}^{n}(k-1)x^{k-1}W_{k+1}W_k)\\
&\quad -s(nx^nW_{n+1}^2+1\times x^{-1}W_0^2+\sum_{k=0}^{n}(k-1)x^{k-1}W_k^2)
\end{aligned}$$

and so

$$\begin{aligned}
t\sum_{k=0}^{n}kx^kW_{k+1}W_k &= -sx^{-1}\sum_{k=0}^{n}kx^kW_k^2+sx^{-1}\sum_{k=0}^{n}x^kW_k^2+x^{-1}\sum_{k=0}^{n}kx^kW_kW_{k+2}\\
&\quad -x^{-1}\sum_{k=0}^{n}x^kW_kW_{k+2}-rx^{-1}\sum_{k=0}^{n}kx^kW_kW_{k+1}+rx^{-1}\sum_{k=0}^{n}x^kW_kW_{k+1}\\
&\quad -nsx^nW_{n+1}^2+nx^nW_{n+3}W_{n+1}-nrx^nW_{n+2}W_{n+1}-\frac{s}{x}W_0^2+\frac{1}{x}W_2W_0-\frac{r}{x}W_1W_0
\end{aligned}\tag{2.2}$$

Next we obtain $\sum_{k=0}^{n}kx^kW_{k+2}W_k$. Multiplying the both side of the recurrence relation

$$tW_n=W_{n+3}-rW_{n+2}-sW_{n+1}$$

by W_{n+2} we get

$$tW_{n+2}W_n=W_{n+3}W_{n+2}-rW_{n+2}^2-sW_{n+2}W_{n+1}.$$

Then using last recurrence relation, we obtain

$$\begin{aligned}
t\times n\times x^nW_{n+2}W_n &= nx^nW_{n+3}W_{n+2}-r\times n\times x^nW_{n+2}^2-s\times n\times x^nW_{n+2}W_{n+1}\\
t\times(n-1)\times x^{n-1}W_{n+1}W_{n-1} &= (n-1)\times x^{n-1}W_{n+2}W_{n+1}-r\times(n-1)\times x^{n-1}W_{n+1}^2\\
&\quad -s\times(n-1)\times x^{n-1}W_{n+1}W_n\\
t\times(n-2)x^{n-2}W_nW_{n-2} &= (n-2)\times x^{n-2}W_{n+1}W_n-r\times(n-2)\times x^{n-2}W_n^2-s\times(n-2)\times x^{n-2}W_nW_{n-1}\\
&\quad \vdots\\
t\times 2\times x^2W_4W_2 &= 2\times x^2W_5W_4-r\times 2\times x^2W_4^2-s\times 2\times x^2W_4W_3\\
t\times 1\times x^1W_3W_1 &= 1\times x^1W_4W_3-r\times 1\times x^1W_3^2-s\times 1\times x^1W_3W_2\\
t\times 0\times x^0W_2W_0 &= 0\times x^0W_3W_2-r\times 0\times x^0W_2^2-s\times 0\times x^0W_2W_1
\end{aligned}$$

If we add the equations side by side, we get

$$\begin{aligned}
t\sum_{k=0}^{n}kx^kW_{k+2}W_k &= (nx^nW_{n+3}W_{n+2}+(n-1)x^{n-1}W_{n+2}W_{n+1}+1\times x^{-1}W_2W_1\\
&\quad +2\times x^{-2}W_1W_0+\sum_{k=0}^{n}(k-2)x^{k-2}W_{k+1}W_k)-r(nx^nW_{n+2}^2+(n-1)x^{n-1}W_{n+1}^2\\
&\quad +1\times x^{-1}W_1^2+2\times x^{-2}W_0^2+\sum_{k=0}^{n}(k-2)x^{k-2}W_k^2)-s(nx^nW_{n+2}W_{n+1}\\
&\quad +1\times x^{-1}W_1W_0+\sum_{k=0}^{n}(k-1)x^{k-1}W_{k+1}W_k)
\end{aligned}$$

and so

$$\begin{aligned}
t\sum_{k=0}^{n}kx^kW_{k+2}W_k &= -rx^{-2}\sum_{k=0}^{n}kx^kW_k^2+2rx^{-2}\sum_{k=0}^{n}x^kW_k^2-(sx-1)x^{-2}\sum_{k=0}^{n}kx^kW_{k+1}W_k \qquad (2.3)\\
&\quad +(sx-2)x^{-2}\sum_{k=0}^{n}x^kW_{k+1}W_k-nrx^nW_{n+2}^2-r(n-1)x^{n-1}W_{n+1}^2\\
&\quad +nx^nW_{n+3}W_{n+2}+(n-nsx-1)x^{n-1}W_{n+2}W_{n+1}-\frac{r}{x}W_1^2\\
&\quad -2\frac{r}{x^2}W_0^2+\frac{1}{x}W_2W_1-(sx-2)x^{-2}W_1W_0
\end{aligned}$$

Using Theorem 1.1 and solving the system (2.1)-(2.2)-(2.3), the results in (a), (b) and (c) follow.

3. Specific Cases

In this section, we present the closed form solutions (identities) of the sums $\sum_{k=0}^{n}kx^kW_k^2$, $\sum_{k=0}^{n}kx^kW_{k+2}W_k$ and $\sum_{k=0}^{n}kx^kW_{k+1}W_k$ for the specific case of sequence $\{W_n\}$.

3.1. The Case $x=1$. The case $x=1$ of Theorem 2.1 is given in [30]. In this subsection, we only consider the case $x=1$, $r=0,s=2,t=1$ and $x=1$, $r=1,s=1,t=2$ (these two special cases were not given in [30] because we can not use Theorem 2.1 directly). Observe that setting $x=1$, $r=0,s=2,t=1$ and $x=1$, $r=1,s=1,t=2$ (i.e. for the generalized Pell-Padovan case and for the generalized third order Jacobsthal case) in Theorem 2.1 (a), (b) and (c) makes the right hand side of the sum formulas to be an indeterminate form. Application of L'Hospital rule (using twice) however provides the evaluation of the sum formulas. If $x=1$, $r=0,s=2,t=1$ then we have the

following theorem (in fact taking $x = 1$, $r = 0, s = 2, t = 1$ in Theorem 2.1 and then using L'Hospital rule twice for $x = 1$ we obtain the following theorem).

THEOREM 3.1. *If $r = 0, s = 2, t = 1$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} kW_k^2 = \frac{1}{4}((2n^2+18n+69)W_{n+3}^2 + (2n^2+14n+53)W_{n+2}^2 + (2n^2+18n+85)W_{n+1}^2 - 4(n^2+8n+31)W_{n+3}W_{n+2} - 4(n^2+10n+40)W_{n+3}W_{n+1} + 4(n^2+10n+38)W_{n+2}W_{n+1} - 53W_2^2 - 41W_1^2 - 69W_0^2 - 116W_1W_0 + 124W_2W_0 + 96W_2W_1)$.

(b): $\sum_{k=0}^{n} kW_{k+1}W_k = \frac{1}{4}(-2(n^2+8n+31)W_{n+3}^2 - 2(n^2+6n+24)W_{n+2}^2 - 2(n^2+10n+40)W_{n+1}^2 + (4n^2+30n+111)W_{n+3}W_{n+2} + (4n^2+38n+145)W_{n+3}W_{n+1} - (4n^2+38n+137)W_{n+2}W_{n+1} + 48W_2^2 + 38W_1^2 + 62W_0^2 - 85W_2W_1 - 111W_2W_0 + 103W_1W_0)$.

(c): $\sum_{k=0}^{n} kW_{k+2}W_k = \frac{1}{4}(2(n^2+8n+29)W_{n+3}^2 + 2(n^2+6n+22)W_{n+2}^2 + 2(n^2+10n+38)W_{n+1}^2 - (4n^2+26n+103)W_{n+3}W_{n+2} - (4n^2+38n+137)W_{n+3}W_{n+1} + (4n^2+34n+125)W_{n+2}W_{n+1} - 44W_2^2 - 34W_1^2 - 58W_0^2 + 81W_2W_1 + 103W_2W_0 - 95W_1W_0)$.

From Theorem 3.1, we have the following corollary which gives sum formulas of Pell-Padovan numbers (take $W_n = R_n$ with $Q_0 = 1, R_1 = 1, R_2 = 1$).

COROLLARY 3.2. *For $n \geq 0$, Pell-Padovan numbers have the following properties:*

(a): $\sum_{k=0}^{n} kR_k^2 = \frac{1}{4}((2n^2+18n+69)R_{n+3}^2 + (2n^2+14n+53)R_{n+2}^2 + (2n^2+18n+85)R_{n+1}^2 - 4(n^2+8n+31)R_{n+3}R_{n+2} - 4(n^2+10n+40)R_{n+3}R_{n+1} + 4(n^2+10n+38)R_{n+2}R_{n+1} - 59)$.

(b): $\sum_{k=0}^{n} kR_{k+1}R_k = \frac{1}{4}(-2(n^2+8n+31)R_{n+3}^2 - 2(n^2+6n+24)R_{n+2}^2 - 2(n^2+10n+40)R_{n+1}^2 + (4n^2+30n+111)R_{n+3}R_{n+2} + (4n^2+38n+145)R_{n+3}R_{n+1} - (4n^2+38n+137)R_{n+2}R_{n+1} + 55)$.

(c): $\sum_{k=0}^{n} kR_{k+2}R_k = \frac{1}{4}(2(n^2+8n+29)R_{n+3}^2 + 2(n^2+6n+22)R_{n+2}^2 + 2(n^2+10n+38)R_{n+1}^2 - (4n^2+26n+103)R_{n+3}R_{n+2} - (4n^2+38n+137)R_{n+3}R_{n+1} + (4n^2+34n+125)R_{n+2}R_{n+1} - 47)$.

Taking $R_n = C_n$ with $C_0 = 3, C_1 = 0, C_2 = 2$ in Theorem 3.1, we have the following corollary which presents sum formulas of Pell-Perrin numbers.

COROLLARY 3.3. *For $n \geq 0$, Pell-Perrin numbers have the following properties:*

(a): $\sum_{k=0}^{n} kC_k^2 = \frac{1}{4}((2n^2+18n+69)C_{n+3}^2 + (2n^2+14n+53)C_{n+2}^2 + (2n^2+18n+85)C_{n+1}^2 - 4(n^2+8n+31)C_{n+3}C_{n+2} - 4(n^2+10n+40)C_{n+3}C_{n+1} + 4(n^2+10n+38)C_{n+2}C_{n+1} - 89)$.

(b): $\sum_{k=0}^{n} kC_{k+1}C_k = \frac{1}{4}(-2(n^2+8n+31)C_{n+3}^2 - 2(n^2+6n+24)C_{n+2}^2 - 2(n^2+10n+40)C_{n+1}^2 + (4n^2+30n+111)C_{n+3}C_{n+2} + (4n^2+38n+145)C_{n+3}C_{n+1} - (4n^2+38n+137)C_{n+2}C_{n+1} + 84)$.

(c): $\sum_{k=0}^{n} kC_{k+2}C_k = \frac{1}{4}(2(n^2+8n+29)C_{n+3}^2 + 2(n^2+6n+22)C_{n+2}^2 + 2(n^2+10n+38)C_{n+1}^2 - (4n^2+26n+103)C_{n+3}C_{n+2} - (4n^2+38n+137)C_{n+3}C_{n+1} + (4n^2+34n+125)C_{n+2}C_{n+1} - 80)$.

If $x = 1$, $r = 1, s = 1, t = 2$ then we have the following theorem (in fact taking $r = 1, s = 1, t = 2$ in Theorem 2.1 and then using L'Hospital rule twice for $x = 1$ we obtain the following theorem).

THEOREM 3.4. *If $r = 1, s = 1, t = 2$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} kW_k^2 = \frac{1}{1323}((63n^2+198n-4076)W_{n+3}^2 + 9(21n^2+31n-1381)W_{n+2}^2 + (252n^2-27n-16583)W_{n+1}^2 - 9(21n^2+45n-1366)W_{n+3}W_{n+2} - 2(63n^2+135n-4070)W_{n+3}W_{n+1} + 12(21n+19)W_{n+2}W_{n+1} + 4211W_2^2 + 12519W_1^2 + 16304W_0^2 - 12510W_1W_2 - 8284W_2W_0 + 24W_1W_0)$.

(b): $\sum_{k=0}^{n} kW_{k+1}W_k = \frac{1}{2646}(-(63n^2+9n-4142)W_{n+3}^2 - 3(63n^2+51n-4174)W_{n+2}^2 - 4(63n^2+135n-4070)W_{n+1}^2 + 3(63n^2+93n-4192)W_{n+3}W_{n+2} + 2(63n^2+387n-3968)W_{n+3}W_{n+1} - 6(189n+73)W_{n+2}W_{n+1} - 4088W_2^2 - 12486W_1^2 - 16568W_0^2 + 12666W_2W_1 + 8584W_0W_2 - 696W_0W_1)$.

(c): $\sum_{k=0}^{n} kW_{k+2}W_k = \frac{1}{2646}(-(63n^2-117n-4130)W_{n+3}^2 - 9(21n^2+73n-1336)W_{n+2}^2 - 4(63n^2+9n-4184)W_{n+1}^2 + 27(7n^2+29n-452)W_{n+3}W_{n+2} + 2(63n^2-180n-4187)W_{n+3}W_{n+1} - 12(21n+40)W_{n+2}W_{n+1} - 3950W_2^2 - 12492W_1^2 - 16520W_0^2 + 12\,798W_2W_1 + 7888W_2W_0 + 228W_1W_0)$.

From Theorem 3.4, we have the following corollary which gives sum formulas of third order Jacobsthal numbers (take $W_n = J_n$ with $J_0 = 0, J_1 = 1, J_2 = 1$).

COROLLARY 3.5. *For $n \geq 0$, third order Jacobsthal numbers have the following properties:*

(a): $\sum_{k=0}^{n} kJ_k^2 = \frac{1}{1323}((63n^2+198n-4076)J_{n+3}^2 + 9(21n^2+31n-1381)J_{n+2}^2 + (252n^2-27n-16583)J_{n+1}^2 - 9(21n^2+45n-1366)J_{n+3}J_{n+2} - 2(63n^2+135n-4070)J_{n+3}J_{n+1} + 12(21n+19)J_{n+2}J_{n+1} + 4220)$.

(b): $\sum_{k=0}^{n} kJ_{k+1}J_k = \frac{1}{2646}(-(63n^2+9n-4142)J_{n+3}^2 - 3(63n^2+51n-4174)J_{n+2}^2 - 4(63n^2+135n-4070)J_{n+1}^2 + 3(63n^2+93n-4192)J_{n+3}J_{n+2} + 2(63n^2+387n-3968)J_{n+3}J_{n+1} - 6(189n+73)J_{n+2}J_{n+1} - 3908)$.

(c): $\sum_{k=0}^{n} kJ_{k+2}J_k = \frac{1}{2646}(-(63n^2-117n-4130)J_{n+3}^2 - 9(21n^2+73n-1336)J_{n+2}^2 - 4(63n^2+9n-4184)J_{n+1}^2 + 27(7n^2+29n-452)J_{n+3}J_{n+2} + 2(63n^2-180n-4187)J_{n+3}J_{n+1} - 12(21n+40)J_{n+2}J_{n+1} - 3644)$.

Taking $W_n = j_n$ with $j_0 = 2, j_1 = 1, j_2 = 5$ in Theorem 3.4, we have the following corollary which presents sum formulas of third order Jacobsthal-Lucas numbers.

COROLLARY 3.6. *For $n \geq 0$, third order Jacobsthal-Lucas numbers have the following properties:*

(a): $\sum_{k=0}^{n} kj_k^2 = \frac{1}{1323}((63n^2+198n-4076)j_{n+3}^2 + 9(21n^2+31n-1381)j_{n+2}^2 + (252n^2-27n-16583)j_{n+1}^2 - 9(21n^2+45n-1366)j_{n+3}j_{n+2} - 2(63n^2+135n-4070)j_{n+3}j_{n+1} + 12(21n+19)j_{n+2}j_{n+1} + 37668)$.

(b): $\sum_{k=0}^{n} kj_{k+1}j_k = \frac{1}{2646}(-(63n^2+9n-4142)j_{n+3}^2 - 3(63n^2+51n-4174)j_{n+2}^2 - 4(63n^2+135n-4070)j_{n+1}^2 + 3(63n^2+93n-4192)j_{n+3}j_{n+2} + 2(63n^2+387n-3968)j_{n+3}j_{n+1} - 6(189n+73)j_{n+2}j_{n+1} - 33180)$.

(c): $\sum_{k=0}^{n} kj_{k+2}j_k = \frac{1}{2646}(-(63n^2-117n-4130)j_{n+3}^2 - 9(21n^2+73n-1336)j_{n+2}^2 - 4(63n^2+9n-4184)j_{n+1}^2 + 27(7n^2+29n-452)j_{n+3}j_{n+2} + 2(63n^2-180n-4187)j_{n+3}j_{n+1} - 12(21n+40)j_{n+2}j_{n+1} - 33996)$.

3.2. The Case $x = -1$. We now consider the case $x = -1$ in Theorem 2.1.

Taking $x = -1, r = s = t = 1$ in Theorem 2.1, we obtain the following Proposition.

PROPOSITION 3.7. *If $x = -1, r = s = t = 1$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} k(-1)^k W_k^2 = \frac{1}{4}((-1)^n((n+1)W_{n+3}^2 - (2n+1)W_{n+2}^2 + (3n+2)W_{n+1}^2 - 2(n+2)W_{n+1}W_{n+3} + 2W_{n+2}W_{n+1}) + W_1^2 - W_0^2 - 2W_2W_0 + 2W_1W_0)$.

(b): $\sum_{k=0}^{n} k(-1)^k W_{k+1}W_k = \frac{1}{4}((-1)^n((n+1)W_{n+3}^2 + W_{n+2}^2 - (n+2)W_{n+1}^2 - (2n+2)W_{n+3}W_{n+2} + 2nW_{n+2}W_{n+1}) + W_1^2 - W_0^2 - 2W_1W_0)$.

(c): $\sum_{k=0}^{n} k(-1)^k W_{k+2}W_k = \frac{1}{4}((-1)^n(nW_{n+3}^2 - (2n+1)W_{n+2}^2 - (n+1)W_{n+1}^2 + 2W_{n+3}W_{n+2} + 2nW_{n+3}W_{n+1} - 4(n+1)W_{n+2}W_{n+1}) - W_2^2 + W_1^2 + 2W_2W_1 - 2W_2W_0)$.

From Proposition 3.7, we have the following Corollary which gives sum formulas of Tribonacci numbers (take $W_n = T_n$ with $T_0 = 0, T_1 = 1, T_2 = 1$).

COROLLARY 3.8. *For $n \geq 0$, Tribonacci numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k T_k^2 = \frac{1}{4}((-1)^n ((n+1)T_{n+3}^2 - (2n+1)T_{n+2}^2 + (3n+2)T_{n+1}^2 - 2(n+2)T_{n+1}T_{n+3} + 2T_{n+2}T_{n+1}) + 1)$.

(b): $\sum_{k=0}^{n} k(-1)^k T_{k+1}T_k = \frac{1}{4}((-1)^n ((n+1)T_{n+3}^2 + T_{n+2}^2 - (n+2)T_{n+1}^2 - (2n+2)T_{n+3}T_{n+2} + 2nT_{n+2}T_{n+1}) + 1)$.

(c): $\sum_{k=0}^{n} k(-1)^k T_{k+2}T_k = \frac{1}{4}((-1)^n (nT_{n+3}^2 - (2n+1)T_{n+2}^2 - (n+1)T_{n+1}^2 + 2T_{n+3}T_{n+2} + 2nT_{n+3}T_{n+1} - 4(n+1)T_{n+2}T_{n+1}) + 2)$.

Taking $T_n = K_n$ with $K_0 = 3, K_1 = 1, K_2 = 3$ in Proposition 3.7, we have the following Corollary which presents sum formulas of Tribonacci-Lucas numbers.

COROLLARY 3.9. *For $n \geq 0$, Tribonacci-Lucas numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k K_k^2 = \frac{1}{4}((-1)^n ((n+1)K_{n+3}^2 - (2n+1)K_{n+2}^2 + (3n+2)K_{n+1}^2 - 2(n+2)K_{n+1}K_{n+3} + 2K_{n+2}K_{n+1}) - 20)$.

(b): $\sum_{k=0}^{n} k(-1)^k K_{k+1}K_k = \frac{1}{4}((-1)^n ((n+1)K_{n+3}^2 + K_{n+2}^2 - (n+2)K_{n+1}^2 - (2n+2)K_{n+3}K_{n+2} + 2nK_{n+2}K_{n+1}) - 14)$.

(c): $\sum_{k=0}^{n} k(-1)^k K_{k+2}K_k = \frac{1}{4}((-1)^n (nK_{n+3}^2 - (2n+1)K_{n+2}^2 - (n+1)K_{n+1}^2 + 2K_{n+3}K_{n+2} + 2nK_{n+3}K_{n+1} - 4(n+1)K_{n+2}K_{n+1}) - 20)$.

Taking $x = -1, r = 2, s = 1, t = 1$ in Theorem 2.1, we obtain the following Proposition.

PROPOSITION 3.10. *If $r = 2, s = 1, t = 1$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} k(-1)^k W_k^2 = \frac{1}{75}((-1)^n ((5n+2)W_{n+3}^2 - (45n+53)W_{n+2}^2 + (70n+68)W_{n+1}^2 + 2(5n+12)W_{n+3}W_{n+2} - 2(15n+31)W_{n+3}W_{n+1} + 2(10n+39)W_{n+2}W_{n+1}) - 3W_2^2 - 8W_1^2 - 2W_0^2 + 14W_2W_1 - 32W_0W_2 + 58W_1W_0)$.

(b): $\sum_{k=0}^{n} k(-1)^k W_{k+1}W_k = \frac{1}{75}((-1)^n ((15n+16)W_{n+3}^2 + 3(5n+17)W_{n+2}^2 - (15n+31)W_{n+1}^2 - (45n+58)W_{n+3}W_{n+2} - (15n+21)W_{n+3}W_{n+1} + (60n+49)W_{n+2}W_{n+1}) + W_2^2 + 36W_1^2 - 16W_0^2 - 13W_1W_2 - 6W_2W_0 - 11W_1W_0)$.

(c): $\sum_{k=0}^{n} k(-1)^k W_{k+2}W_k = \frac{1}{75}((-1)^n (20n+3)W_{n+3}^2 - (-1)^n (30n+17)W_{n+2}^2 - (-1)^n (20n+23)W_{n+1}^2 - (-1)^n (35n-11)W_{n+3}W_{n+2} + (-1)^n (30n+7)W_{n+3}W_{n+1} - (-1)^n (70n+83)W_{n+2}W_{n+1} - 17W_2^2 + 13W_1^2 - 3W_0^2 + 46W_1W_2 - 23W_2W_0 - 13W_1W_0)$.

From Proposition 3.10, we have the following Corollary which gives sum formulas of Third-order Pell numbers (take $W_n = P_n$ with $P_0 = 0, P_1 = 1, P_2 = 1$).

COROLLARY 3.11. *For $n \geq 0$, third-order Pell numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k P_k^2 = \frac{1}{75}((-1)^n ((5n+2)P_{n+3}^2 - (45n+53)P_{n+2}^2 + (70n+68)P_{n+1}^2 + 2(5n+12)P_{n+3}P_{n+2} - 2(15n+31)P_{n+3}P_{n+1} + 2(10n+39)P_{n+2}P_{n+1}) + 8)$.

(b): $\sum_{k=0}^{n} k(-1)^k P_{k+1}P_k = \frac{1}{75}((-1)^n ((15n+16)P_{n+3}^2 + 3(5n+17)P_{n+2}^2 - (15n+31)P_{n+1}^2 - (45n+58)P_{n+3}P_{n+2} - (15n+21)P_{n+3}P_{n+1} + (60n+49)P_{n+2}P_{n+1}) + 14)$.

(c): $\sum_{k=0}^{n} k(-1)^k P_{k+2}P_k = \frac{1}{75}((-1)^n (20n+3)P_{n+3}^2 - (-1)^n (30n+17)P_{n+2}^2 - (-1)^n (20n+23)P_{n+1}^2 - (-1)^n (35n-11)P_{n+3}P_{n+2} + (-1)^n (30n+7)P_{n+3}P_{n+1} - (-1)^n (70n+83)P_{n+2}P_{n+1} + 37)$.

Taking $W_n = Q_n$ with $Q_0 = 3, Q_1 = 2, Q_2 = 6$ in Proposition 3.10, we have the following Corollary which presents sum formulas of third-order Pell-Lucas numbers.

COROLLARY 3.12. *For $n \geq 0$, third-order Pell-Lucas numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k Q_k^2 = \frac{1}{75}((-1)^n((5n+2)Q_{n+3}^2-(45n+53)Q_{n+2}^2+(70n+68)Q_{n+1}^2+2(5n+12)Q_{n+3}Q_{n+2}-2(15n+31)Q_{n+3}Q_{n+1}+2(10n+39)Q_{n+2}Q_{n+1})-218)$.

(b): $\sum_{k=0}^{n} k(-1)^k Q_{k+1}Q_k = \frac{1}{75}((-1)^n((15n+16)Q_{n+3}^2+3(5n+17)Q_{n+2}^2-(15n+31)Q_{n+1}^2-(45n+58)Q_{n+3}Q_{n+2}-(15n+21)Q_{n+3}Q_{n+1}+(60n+49)Q_{n+2}Q_{n+1})-294)$.

(c): $\sum_{k=0}^{n} k(-1)^k Q_{k+2}Q_k = \frac{1}{75}((-1)^n(20n+3)Q_{n+3}^2-(-1)^n(30n+17)Q_{n+2}^2-(-1)^n(20n+23)Q_{n+1}^2-(-1)^n(35n-11)Q_{n+3}Q_{n+2}+(-1)^n(30n+7)Q_{n+3}Q_{n+1}-(-1)^n(70n+83)Q_{n+2}Q_{n+1}-527)$.

From Proposition 3.10, we have the following Corollary which gives sum formulas of third-order modified Pell numbers (take $W_n = E_n$ with $E_0 = 0, E_1 = 1, E_2 = 1$).

COROLLARY 3.13. *For $n \geq 0$, third-order modified Pell numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k E_k^2 = \frac{1}{75}((-1)^n((5n+2)E_{n+3}^2-(45n+53)E_{n+2}^2+(70n+68)E_{n+1}^2+2(5n+12)E_{n+3}E_{n+2}-2(15n+31)E_{n+3}E_{n+1}+2(10n+39)E_{n+2}E_{n+1})+3)$.

(b): $\sum_{k=0}^{n} k(-1)^k E_{k+1}E_k = \frac{1}{75}((-1)^n((15n+16)E_{n+3}^2+3(5n+17)E_{n+2}^2-(15n+31)E_{n+1}^2-(45n+58)E_{n+3}E_{n+2}-(15n+21)E_{n+3}E_{n+1}+(60n+49)E_{n+2}E_{n+1})+24)$.

(c): $\sum_{k=0}^{n} k(-1)^k E_{k+2}E_k = \frac{1}{75}((-1)^n(20n+3)E_{n+3}^2-(-1)^n(30n+17)E_{n+2}^2-(-1)^n(20n+23)E_{n+1}^2-(-1)^n(35n-11)E_{n+3}E_{n+2}+(-1)^n(30n+7)E_{n+3}E_{n+1}-(-1)^n(70n+83)E_{n+2}E_{n+1}+42)$.

Taking $x = -1, r = 0, s = 1, t = 1$ in Theorem 2.1, we obtain the following Proposition.

PROPOSITION 3.14. *If $r = 0, s = 1, t = 1$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} k(-1)^k W_k^2 = \frac{1}{25}((-1)^n((15n+14)W_{n+3}^2-(15n-1)W_{n+2}^2+(10n-4)W_{n+1}^2+2(5n+8)W_{n+3}W_{n+2}-2(10n+11)W_{n+2}W_{n+1}-2(5n+13)W_{n+3}W_{n+1})-W_2^2+16W_1^2-14W_0^2+6W_1W_2-16W_2W_0-2W_1W_0)$.

(b): $\sum_{k=0}^{n} k(-1)^k W_{k+1}W_k = \frac{1}{25}((-1)^n((5n+8)W_{n+3}^2-(5n+3)W_{n+2}^2-(5n+13)W_{n+1}^2-(5n-2)W_{n+3}W_{n+2}+(10n-9)W_{n+2}W_{n+1}+(5n+3)W_{n+3}W_{n+1})+3W_2^2+2W_1^2-8W_0^2+7W_2W_1-2W_2W_0-19W_1W_0)$.

(c): $\sum_{k=0}^{n} k(-1)^k W_{k+2}W_k = \frac{1}{25}((-1)^n((10n+1)W_{n+3}^2-(10n-9)W_{n+2}^2-(10n+11)W_{n+1}^2+(15n+19)W_{n+3}W_{n+2}+(10n-9)W_{n+3}W_{n+1}-(30n+23)W_{n+2}W_{n+1})-9W_2^2+19W_1^2-W_0^2+4W_2W_1-19W_2W_0+7W_1W_0)$.

From Proposition 3.14, we have the following Corollary which gives sum formulas of Padovan numbers (take $W_n = P_n$ with $P_0 = 1, P_1 = 1, P_2 = 1$).

COROLLARY 3.15. *For $n \geq 0$, Padovan numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k P_k^2 = \frac{1}{25}((-1)^n((15n+14)P_{n+3}^2-(15n-1)P_{n+2}^2+(10n-4)P_{n+1}^2+2(5n+8)P_{n+3}P_{n+2}-2(10n+11)P_{n+2}P_{n+1}-2(5n+13)P_{n+3}P_{n+1})-11)$.

(b): $\sum_{k=0}^{n} k(-1)^k P_{k+1}P_k = \frac{1}{25}((-1)^n((5n+8)P_{n+3}^2-(5n+3)P_{n+2}^2-(5n+13)P_{n+1}^2-(5n-2)P_{n+3}P_{n+2}+(10n-9)P_{n+2}P_{n+1}+(5n+3)P_{n+3}P_{n+1})-17)$.

(c): $\sum_{k=0}^{n} k(-1)^k P_{k+2}P_k = \frac{1}{25}((-1)^n((10n+1)P_{n+3}^2-(10n-9)P_{n+2}^2-(10n+11)P_{n+1}^2+(15n+19)P_{n+3}P_{n+2}+(10n-9)P_{n+3}P_{n+1}-(30n+23)P_{n+2}P_{n+1})+1)$.

Taking $W_n = E_n$ with $E_0 = 3, E_1 = 0, E_2 = 2$ in Proposition 3.14, we have the following Corollary which presents sum formulas of Perrin numbers.

COROLLARY 3.16. *For $n \geq 0$, Perrin numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k E_k^2 = \frac{1}{25}((-1)^n((15n+14)E_{n+3}^2 - (15n-1)E_{n+2}^2 + (10n-4)E_{n+1}^2 + 2(5n+8)E_{n+3}E_{n+2} - 2(10n+11)E_{n+2}E_{n+1} - 2(5n+13)E_{n+3}E_{n+1}) - 226)$.

(b): $\sum_{k=0}^{n} k(-1)^k E_{k+1}E_k = \frac{1}{25}((-1)^n((5n+8)E_{n+3}^2 - (5n+3)E_{n+2}^2 - (5n+13)E_{n+1}^2 - (5n-2)E_{n+3}E_{n+2} + (10n-9)E_{n+2}E_{n+1} + (5n+3)E_{n+3}E_{n+1}) - 72)$.

(c): $\sum_{k=0}^{n} k(-1)^k E_{k+2}E_k = \frac{1}{25}((-1)^n((10n+1)E_{n+3}^2 - (10n-9)E_{n+2}^2 - (10n+11)E_{n+1}^2 + (15n+19)E_{n+3}E_{n+2} + (10n-9)E_{n+3}E_{n+1} - (30n+23)E_{n+2}E_{n+1}) - 159)$.

From Proposition 3.14, we have the following Corollary which gives sum formulas of Padovan-Perrin numbers (take $W_n = S_n$ with $S_0 = 0, S_1 = 0, S_2 = 1$).

COROLLARY 3.17. *For $n \geq 0$, Padovan-Perrin numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k S_k^2 = \frac{1}{25}((-1)^n((15n+14)S_{n+3}^2 - (15n-1)S_{n+2}^2 + (10n-4)S_{n+1}^2 + 2(5n+8)S_{n+3}S_{n+2} - 2(10n+11)S_{n+2}S_{n+1} - 2(5n+13)S_{n+3}S_{n+1}) - 1)$.

(b): $\sum_{k=0}^{n} k(-1)^k S_{k+1}S_k = \frac{1}{25}((-1)^n((5n+8)S_{n+3}^2 - (5n+3)S_{n+2}^2 - (5n+13)S_{n+1}^2 - (5n-2)S_{n+3}S_{n+2} + (10n-9)S_{n+2}S_{n+1} + (5n+3)S_{n+3}S_{n+1}) + 3)$.

(c): $\sum_{k=0}^{n} k(-1)^k S_{k+2}S_k = \frac{1}{25}((-1)^n((10n+1)S_{n+3}^2 - (10n-9)S_{n+2}^2 - (10n+11)S_{n+1}^2 + (15n+19)S_{n+3}S_{n+2} + (10n-9)S_{n+3}S_{n+1} - (30n+23)S_{n+2}S_{n+1}) - 9)$.

Observe that setting $x = -1$, $r = 0, s = 2, t = 1$ (i.e. for the generalized Pell-Padovan case) in Theorem 2.1 (a), (b) and (c) makes the right hand side of the sum formulas to be an indeterminate form. Application of L'Hospital rule (using twice) however provides the evaluation of the sum formulas. If $x = -1$, $r = 0, s = 2, t = 1$ then we have the following theorem (in fact taking $x = -1$, $r = 0, s = 2, t = 1$ in Theorem 2.1 and then using L'Hospital rule twice for $x = -1$ we obtain the following theorem).

THEOREM 3.18. *If $r = 0, s = 2, t = 1$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} k(-1)^k W_k^2 = \frac{1}{100}((-1)^n((20n^2 - 30n - 1569)W_{n+3}^2 - (20n^2 - 70n - 1519)W_{n+2}^2 - (20n^2 - 90n - 1679)W_{n+1}^2 + 4(5n^2 - 401)W_{n+3}W_{n+2} - 4(5n^2 + 10n - 396)W_{n+3}W_{n+1} - 4(15n^2 - 10n - 1198)W_{n+2}W_{n+1}) - 1519W_2^2 + 1429W_1^2 + 1569W_0^2 - 1584W_2W_1 + 1604W_2W_0 + 4692W_1W_0)$.

(b): $\sum_{k=0}^{n} k(-1)^k W_{k+1}W_k = \frac{1}{100}((-1)^n(2(5n^2 - 401)W_{n+3}^2 - 2(5n^2 - 10n - 396)W_{n+2}^2 - 2(5n^2 + 10n - 396)W_{n+1}^2 + (10n^2 - 10n - 827)W_{n+3}W_{n+2} - (10n^2 + 10n - 827)W_{n+3}W_{n+1} - (30n^2 - 50n - 2461)W_{n+2}W_{n+1}) - 792W_2^2 + 762W_1^2 + 802W_0^2 - 807W_2W_1 + 827W_2W_0 + 2381W_1W_0)$.

(c): $\sum_{k=0}^{n} k(-1)^k W_{k+2}W_k = \frac{1}{100}((-1)^n(2(15n^2 - 40n - 1173)W_{n+3}^2 - 2(15n^2 - 70n - 1118)W_{n+2}^2 - 2(15n^2 - 10n - 1198)W_{n+1}^2 + (30n^2 - 10n - 2381)W_{n+3}W_{n+2} - (30n^2 - 50n - 2461)W_{n+3}W_{n+1} - (90n^2 - 90n - 7103)W_{n+2}W_{n+1}) - 2236W_2^2 + 2066W_1^2 + 2346W_0^2 - 2341W_2W_1 + 2381W_2W_0 + 6923W_1W_0)$.

From Theorem 3.18, we have the following corollary which gives sum formulas of Pell-Padovan numbers (take $W_n = R_n$ with $Q_0 = 1, R_1 = 1, R_2 = 1$).

COROLLARY 3.19. *For $n \geq 0$, Pell-Padovan numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k R_k^2 = \frac{1}{100}((-1)^n((20n^2 - 30n - 1569)R_{n+3}^2 - (20n^2 - 70n - 1519)R_{n+2}^2 - (20n^2 - 90n - 1679)R_{n+1}^2 + 4(5n^2 - 401)R_{n+3}R_{n+2} - 4(5n^2 + 10n - 396)R_{n+3}R_{n+1} - 4(15n^2 - 10n - 1198)R_{n+2}R_{n+1}) + 6191)$.

(b): $\sum_{k=0}^{n} k(-1)^k R_{k+1}R_k = \frac{1}{100}((-1)^n\,(2(5n^2-401)R_{n+3}^2-2(5n^2-10n-396)R_{n+2}^2-2(5n^2+10n-396)R_{n+1}^2+(10n^2-10n-827)R_{n+3}R_{n+2}-(10n^2+10n-827)R_{n+3}R_{n+1}-(30n^2-50n-2461)R_{n+2}R_{n+1})+3173)$.

(c): $\sum_{k=0}^{n} k(-1)^k R_{k+2}R_k = \frac{1}{100}((-1)^n\,(2(15n^2-40n-1173)R_{n+3}^2-2(15n^2-70n-1118)R_{n+2}^2-2(15n^2-10n-1198)R_{n+1}^2+(30n^2-10n-2381)R_{n+3}R_{n+2}-(30n^2-50n-2461)R_{n+3}R_{n+1}-(90n^2-90n-7103)R_{n+2}R_{n+1})+9139)$.

Taking $W_n = C_n$ with $C_0 = 3, C_1 = 0, C_2 = 2$ in Theorem 3.18, we have the following corollary which presents sum formulas of Pell-Perrin numbers.

COROLLARY 3.20. *For $n \geq 0$, Pell-Perrin numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k C_k^2 = \frac{1}{100}((-1)^n\,((20n^2-30n-1569)C_{n+3}^2-(20n^2-70n-1519)C_{n+2}^2-(20n^2-90n-1679)C_{n+1}^2+4(5n^2-401)C_{n+3}C_{n+2}-4(5n^2+10n-396)C_{n+3}C_{n+1}-4(15n^2-10n-1198)C_{n+2}C_{n+1})+17669)$.

(b): $\sum_{k=0}^{n} k(-1)^k C_{k+1}C_k = \frac{1}{100}((-1)^n\,(2(5n^2-401)C_{n+3}^2-2(5n^2-10n-396)C_{n+2}^2-2(5n^2+10n-396)C_{n+1}^2+(10n^2-10n-827)C_{n+3}C_{n+2}-(10n^2+10n-827)C_{n+3}C_{n+1}-(30n^2-50n-2461)C_{n+2}C_{n+1})+9012)$.

(c): $\sum_{k=0}^{n} k(-1)^k C_{k+2}C_k = \frac{1}{100}((-1)^n\,(2(15n^2-40n-1173)C_{n+3}^2-2(15n^2-70n-1118)C_{n+2}^2-2(15n^2-10n-1198)C_{n+1}^2+(30n^2-10n-2381)C_{n+3}C_{n+2}-(30n^2-50n-2461)C_{n+3}C_{n+1}-(90n^2-90n-7103)C_{n+2}C_{n+1})+26456)$.

Taking $x = -1, r = 0, s = 1, t = 2$ in Theorem 2.1, we obtain the following proposition.

PROPOSITION 3.21. *If $r = 0, s = 1, t = 2$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} k(-1)^k W_k^2 = \frac{1}{64}((-1)^n\,((12n+5)\,W_{n+3}^2-(12n-7)W_{n+2}^2+(16n-4)W_{n+1}^2+2(4n+1)W_{n+3}W_{n+2}-4(4n+5)W_{n+3}W_{n+1}-4(4n-1)W_{n+2}W_{n+1})-7W_2^2+19W_1^2-20W_0^2-6W_2W_1-4W_2W_0+20W_1W_0)$.

(b): $\sum_{k=0}^{n} k(-1)^k W_{k+1}W_k = \frac{1}{64}((-1)^n\,((4n+1)\,W_{n+3}^2-(4n-3)W_{n+2}^2-4(4n+5)W_{n+1}^2-(8n-2)W_{n+3}W_{n+2}+2(8n+6)W_{n+3}W_{n+1}+(16n-12)W_{n+2}W_{n+1})-3W_2^2+7W_1^2-4W_0^2+10W_2W_1-4W_2W_0-28W_1W_0)$.

(c): $\sum_{k=0}^{n} k(-1)^k W_{k+2}W_k = \frac{1}{64}((-1)^n\,((4n-5)\,W_{n+3}^2-(4n-9)W_{n+2}^2-4(4n-1)W_{n+1}^2+(24n+14)W_{n+3}W_{n+2}+(16n-12)W_{n+3}W_{n+1}-2(24n+2)W_{n+2}W_{n+1})-9W_2^2+13W_1^2+20W_0^2-10W_2W_1-28W_2W_0+44W_1W_0)$.

From Proposition 3.21, we have the following Corollary which gives sum formulas of Jacobsthal-Padovan numbers (take $W_n = Q_n$ with $Q_0 = 1, Q_1 = 1, Q_2 = 1$).

COROLLARY 3.22. *For $n \geq 0$, Jacobsthal-Padovan numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k Q_k^2 = \frac{1}{64}((-1)^n\,((12n+5)\,Q_{n+3}^2-(12n-7)Q_{n+2}^2+(16n-4)Q_{n+1}^2+2(4n+1)Q_{n+3}Q_{n+2}-4(4n+5)Q_{n+3}Q_{n+1}-4(4n-1)Q_{n+2}Q_{n+1})+2)$.

(b): $\sum_{k=0}^{n} k(-1)^k Q_{k+1}Q_k = \frac{1}{64}((-1)^n\,((4n+1)\,Q_{n+3}^2-(4n-3)Q_{n+2}^2-4(4n+5)Q_{n+1}^2-(8n-2)Q_{n+3}Q_{n+2}+2(8n+6)Q_{n+3}Q_{n+1}+(16n-12)Q_{n+2}Q_{n+1})-22)$.

(c): $\sum_{k=0}^{n} k(-1)^k Q_{k+2}Q_k = \frac{1}{64}((-1)^n\,((4n-5)\,Q_{n+3}^2-(4n-9)Q_{n+2}^2-4(4n-1)Q_{n+1}^2+(24n+14)Q_{n+3}Q_{n+2}+(16n-12)Q_{n+3}Q_{n+1}-2(24n+2)Q_{n+2}Q_{n+1})+30)$.

Taking $W_n = L_n$ with $L_0 = 3, L_1 = 0, L_2 = 2$ in Proposition 3.21, we have the following Corollary which presents sum formulas of Jacobsthal-Perrin numbers.

COROLLARY 3.23. *For $n \geq 0$, Jacobsthal-Perrin numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k L_k^2 = \frac{1}{64}((-1)^n((12n+5)L_{n+3}^2 - (12n-7)L_{n+2}^2 + (16n-4)L_{n+1}^2 + 2(4n+1)L_{n+3}L_{n+2} - 4(4n+5)L_{n+3}L_{n+1} - 4(4n-1)L_{n+2}L_{n+1}) - 232)$.

(b): $\sum_{k=0}^{n} k(-1)^k L_{k+1}L_k = \frac{1}{64}((-1)^n((4n+1)L_{n+3}^2 - (4n-3)L_{n+2}^2 - 4(4n+5)L_{n+1}^2 - (8n-2)L_{n+3}L_{n+2} + 2(8n+6)L_{n+3}L_{n+1} + (16n-12)L_{n+2}L_{n+1}) - 72)$.

(c): $\sum_{k=0}^{n} k(-1)^k L_{k+2}L_k = \frac{1}{64}((-1)^n((4n-5)L_{n+3}^2 - (4n-9)L_{n+2}^2 - 4(4n-1)L_{n+1}^2 + (24n+14)L_{n+3}L_{n+2} + (16n-12)L_{n+3}L_{n+1} - 2(24n+2)L_{n+2}L_{n+1}) - 24)$.

Taking $x=-1, r=1, s=0, t=1$ in Theorem 2.1, we obtain the following Proposition.

PROPOSITION 3.24. *If $r=1, s=0, t=1$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} k(-1)^k W_k^2 = \frac{1}{9}((-1)^n((3n+7)W_{n+3}^2 - (6n+5)W_{n+2}^2 + (6n-1)W_{n+1}^2 - 6W_{n+3}W_{n+2} - 2(3n+7)W_{n+3}W_{n+1} + 2(3n+10)W_{n+2}W_{n+1}) + 4W_2^2 + W_1^2 - 7W_0^2 - 6W_2W_1 - 8W_2W_0 + 14W_1W_0)$.

(b): $\sum_{k=0}^{n} k(-1)^k W_{k+1}W_k = \frac{1}{9}((-1)^n((3n+4)W_{n+3}^2 + (3n+10)W_{n+2}^2 - (3n+7)W_{n+1}^2 - (9n+15)W_{n+3}W_{n+2} + (3n+10)W_{n+3}W_{n+1} + (6n-4)W_{n+2}W_{n+1}) + W_2^2 + 7W_1^2 - 4W_0^2 - 6W_2W_1 + 7W_2W_0 - 10W_1W_0)$.

(c): $\sum_{k=0}^{n} k(-1)^k W_{k+2}W_k = \frac{1}{9}((-1)^n(-3W_{n+3}^2 - 3W_{n+2}^2 + 3W_{n+1}^2 + 9W_{n+3}W_{n+2} + (9n+6)W_{n+3}W_{n+1} - (9n+15)W_{n+2}W_{n+1}) - 3W_2^2 - 3W_1^2 + 3W_0^2 + 9W_2W_1 - 3W_2W_0 - 6W_1W_0)$.

From Proposition 3.24, we have the following corollary which gives sum formulas of Narayana numbers (take $W_n = N_n$ with $N_0=0, N_1=1, N_2=1$).

COROLLARY 3.25. *For $n \geq 0$, Narayana numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k N_k^2 = \frac{1}{9}((-1)^n((3n+7)N_{n+3}^2 - (6n+5)N_{n+2}^2 + (6n-1)N_{n+1}^2 - 6N_{n+3}N_{n+2} - 2(3n+7)N_{n+3}N_{n+1} + 2(3n+10)N_{n+2}N_{n+1}) - 1)$.

(b): $\sum_{k=0}^{n} k(-1)^k N_{k+1}N_k = \frac{1}{9}((-1)^n((3n+4)N_{n+3}^2 + (3n+10)N_{n+2}^2 - (3n+7)N_{n+1}^2 - (9n+15)N_{n+3}N_{n+2} + (3n+10)N_{n+3}N_{n+1} + (6n-4)N_{n+2}N_{n+1}) + 2)$.

(c): $\sum_{k=0}^{n} k(-1)^k N_{k+2}N_k = \frac{1}{9}((-1)^n(-3N_{n+3}^2 - 3N_{n+2}^2 + 3N_{n+1}^2 + 9N_{n+3}N_{n+2} + (9n+6)N_{n+3}N_{n+1} - (9n+15)N_{n+2}N_{n+1}) + 3)$.

Taking $W_n = U_n$ with $U_0=3, U_1=1, U_2=1$ in Proposition 3.24, we have the following corollary which presents sum formulas of Narayana-Lucas numbers.

COROLLARY 3.26. *For $n \geq 0$, Narayana-Lucas numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k U_k^2 = \frac{1}{9}((-1)^n((3n+7)U_{n+3}^2 - (6n+5)U_{n+2}^2 + (6n-1)U_{n+1}^2 - 6U_{n+3}U_{n+2} - 2(3n+7)U_{n+3}U_{n+1} + 2(3n+10)U_{n+2}U_{n+1}) - 46)$.

(b): $\sum_{k=0}^{n} k(-1)^k U_{k+1}U_k = \frac{1}{9}((-1)^n((3n+4)U_{n+3}^2 + (3n+10)U_{n+2}^2 - (3n+7)U_{n+1}^2 - (9n+15)U_{n+3}U_{n+2} + (3n+10)U_{n+3}U_{n+1} + (6n-4)U_{n+2}U_{n+1}) - 43)$.

(c): $\sum_{k=0}^{n} k(-1)^k U_{k+2}U_k = \frac{1}{9}((-1)^n(-3U_{n+3}^2 - 3U_{n+2}^2 + 3U_{n+1}^2 + 9U_{n+3}U_{n+2} + (9n+6)U_{n+3}U_{n+1} - (9n+15)U_{n+2}U_{n+1}) + 3)$.

From Proposition 3.24, we have the following corollary which gives sum formulas of Narayana-Perrin numbers (take $W_n = H_n$ with $H_0=3, H_1=0, H_2=2$).

COROLLARY 3.27. *For $n \geq 0$, Narayana-Perrin numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k H_k^2 = \frac{1}{9}((-1)^n((3n+7)H_{n+3}^2 - (6n+5)H_{n+2}^2 + (6n-1)H_{n+1}^2 - 6H_{n+3}H_{n+2} - 2(3n+7)H_{n+3}H_{n+1} + 2(3n+10)H_{n+2}H_{n+1}) - 95)$.

(b): $\sum_{k=0}^{n} k(-1)^k H_{k+1}H_k = \frac{1}{9}((-1)^n((3n+4)H_{n+3}^2 + (3n+10)H_{n+2}^2 - (3n+7)H_{n+1}^2 - (9n+15)H_{n+3}H_{n+2} + (3n+10)H_{n+3}H_{n+1} + (6n-4)H_{n+2}H_{n+1}) + 10)$.

(c): $\sum_{k=0}^{n} k(-1)^k H_{k+2}H_k = \frac{1}{9}((-1)^n(-3H_{n+3}^2 - 3H_{n+2}^2 + 3H_{n+1}^2 + 9H_{n+3}H_{n+2} + (9n+6)H_{n+3}H_{n+1} - (9n+15)H_{n+2}H_{n+1}) - 3)$.

Taking $x=-1, r=1, s=1, t=2$ in Theorem 2.1, we obtain the following proposition.

PROPOSITION 3.28. *If $r=1, s=1, t=2$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} k(-1)^k W_k^2 = \frac{1}{150}((-1)^n((20n+19)W_{n+3}^2 - (30n-19)W_{n+2}^2 + (70n-6)W_{n+1}^2 - (10n+37)W_{n+3}W_{n+2} - 2(30n+31)W_{n+3}W_{n+1} + 4(10n+22)W_{n+2}W_{n+1}) - W_2^2 + 49W_1^2 - 76W_0^2 - 27W_2W_1 - 2W_2W_0 + 48W_1W_0)$.

(b): $\sum_{k=0}^{n} k(-1)^k W_{k+1}W_k = \frac{1}{300}((-1)^n((30n+1)W_{n+3}^2 + (30n+51)W_{n+2}^2 - 4(30n+31)W_{n+1}^2 - (90n+23)W_{n+3}W_{n+2} + 2(30n+51)W_{n+3}W_{n+1} + (60n-148)W_{n+2}W_{n+1}) - 29W_2^2 + 21W_1^2 - 4W_0^2 + 67W_2W_1 + 42W_2W_0 - 208W_1W_0)$.

(c): $\sum_{k=0}^{n} k(-1)^k W_{k+2}W_k = \frac{1}{900}((-1)^n((30n-69)W_{n+3}^2 - (270n+219)W_{n+2}^2 - 4(30n-39)W_{n+1}^2 + (210n+387)W_{n+3}W_{n+2} + (360n-138)W_{n+3}W_{n+1} - 2(420n+144)W_{n+2}W_{n+1}) - 99W_2^2 + 51W_1^2 + 276W_0^2 + 177W_2W_1 - 498W_2W_0 + 552W_1W_0)$.

From Proposition 3.28, we have the following corollary which gives sum formulas of third order Jacobsthal numbers (take $W_n = J_n$ with $J_0=0, J_1=1, J_2=1$).

COROLLARY 3.29. *For $n \geq 0$, third order Jacobsthal numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k J_k^2 = \frac{1}{150}((-1)^n((20n+19)J_{n+3}^2 - (30n-19)J_{n+2}^2 + (70n-6)J_{n+1}^2 - (10n+37)J_{n+3}J_{n+2} - 2(30n+31)J_{n+3}J_{n+1} + 4(10n+22)J_{n+2}J_{n+1}) + 21)$.

(b): $\sum_{k=0}^{n} k(-1)^k J_{k+1}J_k = \frac{1}{300}((-1)^n((30n+1)J_{n+3}^2 + (30n+51)J_{n+2}^2 - 4(30n+31)J_{n+1}^2 - (90n+23)J_{n+3}J_{n+2} + 2(30n+51)J_{n+3}J_{n+1} + (60n-148)J_{n+2}J_{n+1}) + 59)$.

(c): $\sum_{k=0}^{n} k(-1)^k J_{k+2}J_k = \frac{1}{900}((-1)^n((30n-69)J_{n+3}^2 - (270n+219)J_{n+2}^2 - 4(30n-39)J_{n+1}^2 + (210n+387)J_{n+3}J_{n+2} + (360n-138)J_{n+3}J_{n+1} - 2(420n+144)J_{n+2}J_{n+1}) + 129)$.

Taking $W_n = j_n$ with $j_0=2, j_1=1, j_2=5$ in Proposition 3.28, we have the following corollary which presents sum formulas of third order Jacobsthal-Lucas numbers.

COROLLARY 3.30. *For $n \geq 0$, third order Jacobsthal-Lucas numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k j_k^2 = \frac{1}{150}((-1)^n((20n+19)j_{n+3}^2 - (30n-19)j_{n+2}^2 + (70n-6)j_{n+1}^2 - (10n+37)j_{n+3}j_{n+2} - 2(30n+31)j_{n+3}j_{n+1} + 4(10n+22)j_{n+2}j_{n+1}) - 339)$.

(b): $\sum_{k=0}^{n} k(-1)^k j_{k+1}j_k = \frac{1}{300}((-1)^n((30n+1)j_{n+3}^2 + (30n+51)j_{n+2}^2 - 4(30n+31)j_{n+1}^2 - (90n+23)j_{n+3}j_{n+2} + 2(30n+51)j_{n+3}j_{n+1} + (60n-148)j_{n+2}j_{n+1}) - 381)$.

(c): $\sum_{k=0}^{n} k(-1)^k j_{k+2}j_k = \frac{1}{900}((-1)^n((30n-69)j_{n+3}^2 - (270n+219)j_{n+2}^2 - 4(30n-39)j_{n+1}^2 + (210n+387)j_{n+3}j_{n+2} + (360n-138)j_{n+3}j_{n+1} - 2(420n+144)j_{n+2}j_{n+1}) - 4311)$.

Taking $x=-1, r=2, s=3, t=5$ in Theorem 2.1, we obtain the following Proposition.

PROPOSITION 3.31. *If $r = 2, s = 3, t = 5$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} k(-1)^k W_k^2 = \frac{1}{680625}((-1)^n ((15675n + 11674) W_{n+3}^2 - (9075n - 81169)W_{n+2}^2 + (288750n - 3100)W_{n+1}^2 - 2(17325n + 30966)W_{n+3}W_{n+2} - 10 (14025n + 6407) W_{n+3}W_{n+1} + 10 (23100n + 20113) W_{n+2}W_{n+1}) - 4001W_2^2 + 90244W_1^2 - 291850W_0^2 - 27282W_2W_1 + 76180W_2W_0 - 29870W_1W_0)$.

(b): $\sum_{k=0}^{n} k(-1)^k W_{k+1}W_k = \frac{1}{680625}((-1)^n ((14025n - 7618) W_{n+3}^2 + 11(5775n + 697)W_{n+2}^2 - 25(14025n + 6407)W_{n+1}^2 - (66825n - 22224)W_{n+3}W_{n+2} + 5(10725n + 18973)W_{n+3}W_{n+1} - (8250n + 254035)W_{n+2}W_{n+1}) - 21643W_2^2 - 55858W_1^2 + 190450W_0^2 + 89049W_2W_1 + 41240W_2W_0 - 245785W_1W_0)$.

(c): $\sum_{k=0}^{n} k(-1)^k W_{k+2}W_k = \frac{1}{75625}((-1)^n (550n - 1361)W_{n+3}^2 - 11(-1)^n(2200n + 981)W_{n+2}^2 - 25(-1)^n(550n - 811)W_{n+1}^2 - 5(-1)^n(14\,300n - 1011)W_{n+2}W_{n+1} + 5(-1)^n(2200n - 1429)W_{n+3}W_{n+1} + (-1)^n(10\,725n + 9073)W_{n+3}W_{n+2} - 1911W_2^2 + 13\,409W_1^2 + 34\,025W_0^2 - 1652W_2W_1 - 18\,145W_2W_0 + 76\,555W_1W_0)$.

From Proposition 3.31, we have the following corollary which gives sum formulas of 3-primes numbers (take $W_n = G_n$ with $G_0 = 0, G_1 = 1, G_2 = 2$).

COROLLARY 3.32. *For $n \geq 0$, 3-primes numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k G_k^2 = \frac{1}{680625}((-1)^n ((15675n + 11674) G_{n+3}^2 - (9075n - 81169)G_{n+2}^2 + (288750n - 3100)G_{n+1}^2 - 2(17325n + 30966)G_{n+3}G_{n+2} - 10(14025n + 6407)G_{n+3}G_{n+1} + 10(23100n + 20113)G_{n+2}G_{n+1}) + 19676)$.

(b): $\sum_{k=0}^{n} k(-1)^k G_{k+1}G_k = \frac{1}{680625}((-1)^n ((14025n - 7618) G_{n+3}^2 + 11(5775n + 697)G_{n+2}^2 - 25(14025n + 6407)G_{n+1}^2 - (66825n - 22224)G_{n+3}G_{n+2} + 5(10725n + 18973)G_{n+3}G_{n+1} - (8250n + 254035)G_{n+2}G_{n+1}) + 35668)$.

(c): $\sum_{k=0}^{n} k(-1)^k G_{k+2}G_k = \frac{1}{75625}((-1)^n (550n - 1361) G_{n+3}^2 - 11 (-1)^n (2200n + 981)G_{n+2}^2 - 25(-1)^n(550n - 811)G_{n+1}^2 - 5 (-1)^n (14\,300n - 1011)G_{n+2}G_{n+1} + 5 (-1)^n (2200n - 1429)G_{n+3}G_{n+1} + (-1)^n (10\,725n + 9073)G_{n+3}G_{n+2} + 2461)$.

Taking $W_n = H_n$ with $H_0 = 3, H_1 = 2, H_2 = 10$ in Proposition 3.31, we have the following corollary which presents sum formulas of Lucas 3-primes numbers.

COROLLARY 3.33. *For $n \geq 0$, Lucas 3-primes numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k H_k^2 = \frac{1}{680625}((-1)^n ((15675n + 11674) H_{n+3}^2 - (9075n - 81169)H_{n+2}^2 + (288750n - 3100)H_{n+1}^2 - 2(17325n + 30966)H_{n+3}H_{n+2} - 10(14025n + 6407)H_{n+3}H_{n+1} + 10(23100n + 20113)H_{n+2}H_{n+1}) - 1105234)$.

(b): $\sum_{k=0}^{n} k(-1)^k H_{k+1}H_k = \frac{1}{680625}((-1)^n ((14025n - 7618) H_{n+3}^2 + 11(5775n + 697)H_{n+2}^2 - 25(14025n + 6407)H_{n+1}^2 - (66825n - 22224)H_{n+3}H_{n+2} + 5(10725n + 18973)H_{n+3}H_{n+1} - (8250n + 254035)H_{n+2}H_{n+1}) + 869788)$.

(c): $\sum_{k=0}^{n} k(-1)^k H_{k+2}H_k = \frac{1}{75625}((-1)^n (550n - 1361) H_{n+3}^2 - 11 (-1)^n (2200n + 981)H_{n+2}^2 - 25(-1)^n(550n - 811)H_{n+1}^2 - 5 (-1)^n (14\,300n - 1011)H_{n+2}H_{n+1} + 5 (-1)^n (2200n - 1429)H_{n+3}H_{n+1} + (-1)^n (10\,725n + 9073)H_{n+3}H_{n+2} + 50701)$.

From Proposition 3.31, we have the following corollary which gives sum formulas of modified 3-primes numbers (take $W_n = E_n$ with $E_0 = 0, E_1 = 1, E_2 = 1$).

COROLLARY 3.34. *For $n \geq 0$, modified 3-primes numbers have the following properties:*

(a): $\sum_{k=0}^{n} k(-1)^k E_k^2 = \frac{1}{680625}((-1)^n\left((15675n+11674\right)E_{n+3}^2 - (9075n-81169)E_{n+2}^2 + (288750n-3100)E_{n+1}^2 - 2(17325n+30966)E_{n+3}E_{n+2} - 10(14025n+6407)E_{n+3}E_{n+1} + 10(23100n+20113)E_{n+2}E_{n+1}) + 58961)$.

(b): $\sum_{k=0}^{n} k(-1)^k E_{k+1}E_k = \frac{1}{680625}((-1)^n\left((14025n-7618\right)E_{n+3}^2 + 11(5775n+697)E_{n+2}^2 - 25(14025n+6407)E_{n+1}^2 - (66825n-22224)E_{n+3}E_{n+2} + 5(10725n+18973)E_{n+3}E_{n+1} - (8250n+254035)E_{n+2}E_{n+1}) + 11548)$.

(c): $\sum_{k=0}^{n} k(-1)^k E_{k+2}E_k = \frac{1}{75625}((-1)^n\left(550n-1361\right)E_{n+3}^2 - 11\left(-1\right)^n\left(2200n+981\right)E_{n+2}^2 - 25(-1)^n(550n-811)E_{n+1}^2 - 5\left(-1\right)^n\left(14\,300n-1011\right)E_{n+2}E_{n+1} + 5\left(-1\right)^n\left(2200n-1429\right)E_{n+3}E_{n+1} + \left(-1\right)^n\left(10\,725n+9073\right)E_{n+3}E_{n+2} + 9846)$.

3.3. The Case $x = i$. We now consider the complex case $x = i$ in Theorem 2.1. The following proposition presents some summing formulas of generalized Fibonacci numbers with positive subscripts.

Taking $x = i, r = s = t = 1$ in Theorem 2.1, we obtain the following Proposition.

PROPOSITION 3.35. *If $r = s = t = 1$ then for $n \geq 0$ we have the following formulas:*

(a): $\sum_{k=0}^{n} ki^k W_k^2 = \frac{-i}{4}(i^n(i\left(\left(1+i\right)n+2+i\right)W_{n+3}^2 + (2in-2+4i)W_{n+2}^2 - i((1+3i)n+2+i)W_{n+1}^2 + 2\left((1-i)n+2-3i\right)W_{n+3}W_{n+2} + (2+2i)W_{n+3}W_{n+1} - 2((1+i)n+4+i)W_{n+2}W_{n+1}) - W_2^2 - (2+2i)W_1^2 + (1-2i)W_0^2 + (4+2i)W_2W_1 - 6iW_1W_0 - (2-2i)W_2W_0)$.

(b): $\sum_{k=0}^{n} ki^k W_{k+1}W_k = \frac{-i}{4}(i^n((1-i)W_{n+3}^2 - 2(in+1+2i)W_{n+2}^2 + (1+i)W_{n+1}^2 + (2in+4i)W_{n+3}W_{n+2} + i(2in+2+4i)W_{n+3}W_{n+1} - i((2+4i)n+4+4i)W_{n+2}W_{n+1}) + (1+i)W_2^2 + (2-2i)W_1^2 - (1-i)W_0^2 - 2W_2W_1 - (2+2i)W_2W_0 + 2W_1W_0)$.

(c): $\sum_{k=0}^{n} ki^k W_{k+2}W_k = \frac{-i}{4}(i^n(i\left(\left(1+i\right)n+2-i\right)W_{n+3}^2 + (2n+2-2i)W_{n+2}^2 - ((1+i)n+3)W_{n+1}^2 - (2+2i)W_{n+3}W_{n+2} - i((2+2i)n+2)W_{n+3}W_{n+1} - (2-2i)W_{n+2}W_{n+1}) - (1-2i)W_2^2 + 2W_1^2 - (1+2i)W_0^2 + (2-2i)W_2W_1 - 2iW_2W_0 - (2+2i)W_1W_0)$.

From Proposition 3.35, we have the following Corollary which gives sum formulas of Tribonacci numbers (take $W_n = T_n$ with $T_0 = 0, T_1 = 1, T_2 = 1$).

COROLLARY 3.36. *For $n \geq 0$, Tribonacci numbers have the following properties:*

(a): $\sum_{k=0}^{n} ki^k T_k^2 = \frac{-i}{4}(i^n(i\left(\left(1+i\right)n+2+i\right)T_{n+3}^2 + (2in-2+4i)T_{n+2}^2 - i((1+3i)n+2+i)T_{n+1}^2 + 2((1-i)n+2-3i)T_{n+3}T_{n+2} + (2+2i)T_{n+3}T_{n+1} - 2((1+i)n+4+i)T_{n+2}T_{n+1}) + 1)$.

(b): $\sum_{k=0}^{n} ki^k T_{k+1}T_k = \frac{-i}{4}(i^n((1-i)T_{n+3}^2 - 2(in+1+2i)T_{n+2}^2 + (1+i)T_{n+1}^2 + (2in+4i)T_{n+3}T_{n+2} + i(2in+2+4i)T_{n+3}T_{n+1} - i((2+4i)n+4+4i)T_{n+2}T_{n+1}) + 1 - i)$.

(c): $\sum_{k=0}^{n} ki^k T_{k+2}T_k = \frac{-i}{4}(i^n(i\left(\left(1+i\right)n+2-i\right)T_{n+3}^2 + (2n+2-2i)T_{n+2}^2 - ((1+i)n+3)T_{n+1}^2 - (2+2i)T_{n+3}T_{n+2} - i((2+2i)n+2)T_{n+3}T_{n+1} - (2-2i)T_{n+2}T_{n+1}) + 3)$.

Taking $W_n = K_n$ with $K_0 = 3, K_1 = 1, K_2 = 3$ in Proposition 3.35, we have the following Corollary which presents sum formulas of Tribonacci-Lucas numbers.

COROLLARY 3.37. *For $n \geq 0$, Tribonacci-Lucas numbers have the following properties:*

(a): $\sum_{k=0}^{n} ki^k K_k^2 = \frac{-i}{4}(i^n(i\left(\left(1+i\right)n+2+i\right)K_{n+3}^2 + (2in-2+4i)K_{n+2}^2 - i((1+3i)n+2+i)K_{n+1}^2 + 2\left((1-i)n+2-3i\right)K_{n+3}K_{n+2} + (2+2i)K_{n+3}K_{n+1} - 2((1+i)n+4+i)K_{n+2}K_{n+1}) - 8 - 14i)$.

(b): $\sum_{k=0}^{n} ki^k K_{k+1}K_k = \frac{-i}{4}(i^n((1-i)K_{n+3}^2 - 2(in+1+2i)K_{n+2}^2 + (1+i)K_{n+1}^2 + (2in+4i)K_{n+3}K_{n+2} + i(2in+2+4i)K_{n+3}K_{n+1} - i((2+4i)n+4+4i)K_{n+2}K_{n+1}) - 16 - 2i)$.

(c): $\sum_{k=0}^{n} ki^k K_{k+2}K_k = \frac{-i}{4}(i^n(i\left((1+i)\,n+2-i\right)K_{n+3}^2+(2n+2-2i)K_{n+2}^2-((1+i)n+3)K_{n+1}^2-(2+2i)$ $K_{n+3}K_{n+2}-i((2+2i)n+2)K_{n+3}K_{n+1}-(2-2i)K_{n+2}K_{n+1})-16-30i)$.

Corresponding sums of the other third order linear sequences can be calculated similarly when $x=i$.

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Research Article

BIOMETRIC SCREENINGS: THE ROUTE TO OCCUPATIONAL SAFETY AND HEALTH

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ABSTRACT

Biometric characteristics such as fingerprints, palmprints, iris, or face recognition have been used at organizations to identify an individual, grant access to physical or digital facilities, and to control employees' time and attendance. Nowadays, employers recognize the importance of workers' health. For this reason, wellness programs are gaining popularity among enterprises. As part of these programs, other biometric traits such as height, weight, blood samples are acquired at biometric screening events. Their main objective is to promote healthy habits within the workforce via early prevention and timely interventions of diseases. The present work goes beyond the biometric screenings' health benefits that are broadly reported and analyzes the implication of the screenings over occupational safety and health

For this purpose, a literature review was carried out. Literature research and analysis conveyed relevant information regarding the application of biometrics in the workplace via biometric screenings. The revision of pertinent scientific documents showed that biometric characteristics acquired in a biometric screening event and the subsequent results' analysis can aid in the identification of unconventional hazards that can affect occupational safety and health. Furthermore, the present study describes different examples on how a biometric screening results can be associated with occupational hazards and consequently affect occupational safety and health.

Keywords: *Biometric Screenings, Occupational, Safety, Health*

1. INTRODUCTION

Fingerprints, iris, palm print, and face recognition are biometric characteristics commonly used in the workplace for granting physical or virtual access to the facilities and controlling employees' time and attendance. The acquisition of biometric identifiers that characterize employees' health conditions is becoming prevalent in the workplace. Nowadays, companies collect different biometric traits to enhance safety and health at work via biometric screening events, which are part of workplace wellness programs.

Corporate wellness programs are gaining popularity within organizations. In the United States, 54% of employees working full time have access to a workplace health program while in Europe is 23% The tendency is to upscale the existent programs and the introduction of new ones in multinational companies(Yeung & Johnston, 2016)

Biometric screenings fall under the scope of workplace wellness programs. These screenings are defined as the process of measuring biometric characteristics such as height, weight, blood pressure, cholesterol, blood glucose, physical activity tests and more acquired at the workplace to assess the health condition of the workforce and monitor the changes throughout time(Centers for Disease Control and Prevention, 2018)

Research about wellness programs and biometric screenings is predominantly health-based(Goetzel & Ozminkowski, 2008). Regarding the employees, research focuses on health benefits such as early detection of chronic diseases(Giese, 2018)(Vanichkachorn et al., 2017), motivation into healthy behaviors(Smith, 2017), promotion of healthy lifestyles, and education (Breaux-shropshire et al., 2012)(Ryan et al., 2014). As for the company, research topics include identification of the organization's benefits such as the return of investment, cut in corporate health plans(Vanichkachorn et al., 2017)(Rameswarapu et al., 2014)(Maeng et al., 2017), ways on how to deploy effective and successful screenings via participation rate(McLellan et al., 2009)(Sherman & Addy, 2018)(Breaux-shropshire et al., 2012) and incentives (Cuellar et al., 2017)(Heathfield, 2019)(Fronstin & Roebuck, 2015).

Research about the impact of biometric screenings on safety is not well documented. Some studies focus on one or two biometric characteristics such as height and weight to identify occupational risk and linkage to work-related diseases (Poston et al., 2011; Rosen, 2014; Schulte et al., 2007; Tucker & Friedman, 1998). In other studies, self-assessment tools are used to identify relationships with occupational incidents and health risks(White et al., 2015)(Chau et al., 2009).

This study presents an extensive revision of scientific literature regarding how biometric screenings impact workplace safety and health.

2. OCCUPATIONAL SAFETY AND HEALTH

Occupational Safety and Health (OSH) comprises different scientific disciplines that focus on the analysis, recognition, and monitor of occupational hazards to protect and guarantee the workers' wellbeing. OSH examines all the parameters related to health and safety to prevent risks and hazards at work. Moreover, OSH encompasses laws and guidelines for safeguarding employees in the workplace. These laws have basic outlines but differ in severity depending on the country and region. Occupational accidents and fatalities can be prevented by the application of safety procedures and methods which contribute to considerable benefits to the society, businesses, and enterprises (Alli, 2008).

2.1 Safety versus Health

Occupational Safety and Health is treated as a unified concept regarding policies, initiatives, management, and more. Nonetheless, safety and health in the workplace share

some similarities; they differ in important aspects. Safety addresses situations that can cause immediate harm or injuries. It also involves hazards that can affect workers due to unforeseen or harsh conditions. Health mainly deals with circumstances that can cause diseases and unfavorable reactions to long term hazards that are dangerous but not that severe as an accident(Goetsch, 2011).

Although safety and health have marked differences, under the occupational scope, these two concepts cannot be analyzed and understood separately. OSH professionals should be knowledgeable in these two topics to prevent and give timely responses to challenging work-related hazards such as stress or workplace violence.

Traditionally, OSH management systems were concentrated on preventing workplace accidents by utilizing different procedures such as training staff, implementing laws and regulations, designing ergonomic machinery(Vargas Cruz et al., 2018), and others. Nowadays, this approach is becoming broader by taking into consideration the employees' wellbeing as the base of the whole safety and health efforts.

Wellness programs, including biometric screenings, are part of OSH initiatives towards the enhancement of a safe and healthy workplace environment via the improvement of workforce welfare. As a result, OSH procedures are a legal duty for the company, but most important, they are becoming a moral obligation for the employers(Ruiz Salvador & Thinh, 2016).

3. BIOMETRIC SCREENINGS

Wellness programs have become very popular among medium and large enterprises. They consist of a series of activities developed at the workplace, aiming to improve the wellbeing of the employees. Biometric screenings are an essential component of wellness and health-promoting initiatives. These tests provide an insight into the worker's health condition to arrange and put in practice prevention efforts(Cuellar et al., 2017).

Fingerprints, face, hand geometry, or palm recognition are biometric characteristics commonly used in the workplace. These characteristics are restricted to serve as identification tools. Nowadays, as part of a workplace wellness program, other biometric traits such as height, weight, blood pressure, cholesterol, and more are acquired to improve employees' welfare. This process is denominated: "Biometric Screening."

Biometric screenings provide a quantitative value of the health condition of the employees. Moreover, employers' main objectives towards the deployment of these screenings at the organization are the reduction of medical care costs and to achieve a high return of investment (ROI). For instance, biometric screenings can identify high-risk individuals who have above average or abnormal values in the screening results(Vanichkachorn et al., 2017). Appropriate healthcare solutions tailored to these individuals will cut down costs in health plans by preventing or managing the development of chronic diseases(Breaux-shropshire et al., 2012). Furthermore, the RAND corporation report on workplace wellness stated that a positive ROI value could be obtained by the introduction of wellness programs, including biometric screenings(Soeren, Mattke, Hangsheng, Liu, John P.Caloyeras, Christina Y.Huang, Kristin R. Van Busum, Dmitry, Khodyakog, Victoria, 2013).

These screenings can also disclose valuable information regarding occupational injuries, diseases, unknown or chronic health conditions that can be asymptomatic. Moreover, a screening event is probably the only opportunity a worker has to detect a chronic illness; that without intervention can result in a catastrophic event. For example, presymptomatic type 1 diabetes can be recognized by detecting abnormal values of hemoglobin A 1c and fasting glucose thanks to the analysis of a blood sample collected in a biometric screening

event(Giese, 2018). Additionally, high levels of cholesterol, blood pressure, and obesity reveal a risk of a cardiovascular disease that can lead to a heart attack or stroke. Due to timely biometric screening, these risks can be mitigated and treated(Breaux-shropshire et al., 2012).

3.1 Biometric Screenings and Safety

OSH initiatives such as wellness programs shifted the accident causation and prevention efforts to focus on the workers' wellbeing. Biometric screenings are part of corporate wellness programs. They provide a complete characterization of the worker's health by the acquisition of various biometric measures, including blood, height, weight, and more(Fronstin & Roebuck, 2015). Furthermore, biometric screenings focus on how the work is affecting the employees' health, and according to the results taking timely and corrective actions.

Occupational Safety and Health's overarching goal is to identify, prevent, and reduce workplace hazards(Alli, 2008). However, hazards are prevalent in every work environment. An occupational hazard is defined as any object or event that has the potential to harm an employee. Hazards can be divided into two groups:

1. Safety hazards, which have the potential to harm workers physically
2. Health hazards, that have the potential for developing a disease (Government of Ontario, Ministry of Labour, 2016).

Biometric screenings take a step further in this classification by detecting nonconventional hazards such as health indicators and diseases, which not only can affect the worker's health but the ability to perform their work duties and can hinder safety behavior.

Biometric tests performed during the screenings classify each employee according to his/her health status. The early detection of health conditions such as high blood pressure, high triglycerides, and high cholesterol can prevent serious health problems such as physical and mental problems. These issues can lead to safety incidents and, in the long term, can become a burden to the company and society(PDHI, 2018).

Abnormal biometric values, such as high blood pressure levels, can indicate high levels of stress(Daniellou et al., 2012). Stressed individuals can be easily distracted from work, which can contribute to mistakes, unsafe behaviors, accidents, and workplace violence. Moreover, it can be the cause of chronic diseases such as cardiovascular events. Stressed workers are more likely to make unhealthier choices, such as alcohol and tobacco consumption(Musick, 2016) (Institute of Medicine (US) Committee on Health and & Behavior: Research, Practice, 2001)(Sauter et al., 2009).

Cotinine tests performed to a blood sample during a biometric screening event can easily detect smoking prevalence among the workforce(Quest Diagnostics, 2019). Besides the severe health issues related to tobacco usage, smoking can also be considered as an occupational hazard. Smokers take more breaks during working hours than a non-smoking employee, disrupting the working procedures. Additionally, loss of productivity due to these breaks, presenteeism, absenteeism, and health insurance costs are higher for a smoker (Berman et al., 2014)(Baker et al., 2017)(Halpern et al., 2001).

These screenings can easily detect if a person is overweight or obese by the calculation of the Body Mass Index (BMI). Obesity can also be linked to chronic diseases such as heart diseases, diabetes, sleep apnea, of cancer, and even workplace injuries(Rosen, 2014). Overweight individuals present deterioration in cognitive performance and more prolonged time reactions compared with a normal weight person (Steenbergen & Colzato, 2017). Additionally, obese people tend to unintentionally injure themselves more often and present

impairment in work activities(Rodbard et al., 2009). Overexertion and falls are the most frequent types of work-related injuries and accidents. Higher BMI values are closely connected to missed workdays and absenteeism(Tucker & Friedman, 1998). Obesity screening can be advantageous to prevent from occupational risks that can be associated with occupational asthma, the immune response to chemical exposures and diseases caused by occupational neurotoxins(Schulte et al., 2007).

Prediabetes and diabetes can be quickly spotted at a biometric screening event by reading the results on blood glucose levels(Adams et al., 2015). Prediabetes can be often reverted if it is timely diagnosed, and health-based corrective actions are taken in place(Centers for Disease Control and Prevention, 2014). Diabetes is an indicator of serious complications such as blindness, kidney failure, heart disease, stroke, and loss of toes, feet, or legs(Diabetes UK, 2019). Vision loss, dizziness, and loose of consciousness due to a glucose imbalance can be potential hazards for occupational incidents. Diabetes significantly impacts the ability to work; it can increase absenteeism and production loss. Diabetic individuals are more susceptible to fatigue, overweight, early retirement, and disability(Tunceli et al., 2005)(Fit for Work, 2017)(Trotto, 2015).

Biometric screenings offer a snapshot of the physical capabilities of the employees. This aspect is relevant in companies where manual labor is required, and fitness can be a breaking point in work safety, such as the case of firefighters(Poston et al., 2011).

Furthermore, as computers are becoming an essential tool for executing any job, sitting, sedentary work, and low activity workplaces are rapidly expanding(Parry & Straker, 2013). Biometric screenings can detect sedentary behavior among employees by analyzing biometric characteristics such as waist circumference, body mass index, triglyceride levels, and others. Since workers spend at least eight hours per day sitting in an office without counting the commuting time to the workplace, sedentarism can be considered as an occupational hazard(Strakeer et al., 2016)(Coenen et al., 2017)(Carr et al., 2016). Sedentary behavior can lead to non-communicable chronic diseases such as diabetes, cardiovascular diseases, cancer, and premature mortality. Moreover, musculoskeletal disorders, overweight, obesity, poor cognitive function can also be associated with a sedentary work environment(Owen et al., 2010)(González et al., 2017)(Middelbeek & Breda, 2013)(Panahi & Tremblay, 2018).

Biometric screenings serve as an assessment tool to check if the workplace conditions are safe. Screenings report common health trends among the employees that can be caused by the work environment. These commonalities can pinpoint unsafe conditions such as ergonomic problems or lack of safety procedures. Additionally, as these screenings are performed every year, it is possible to detect an increasing trend in work-related illnesses due to the workplace environment and the possibility to prevent and treat them via specific safety and health interventions in machinery or workspace design(Carr et al., 2016)(NIOSH, 2018).

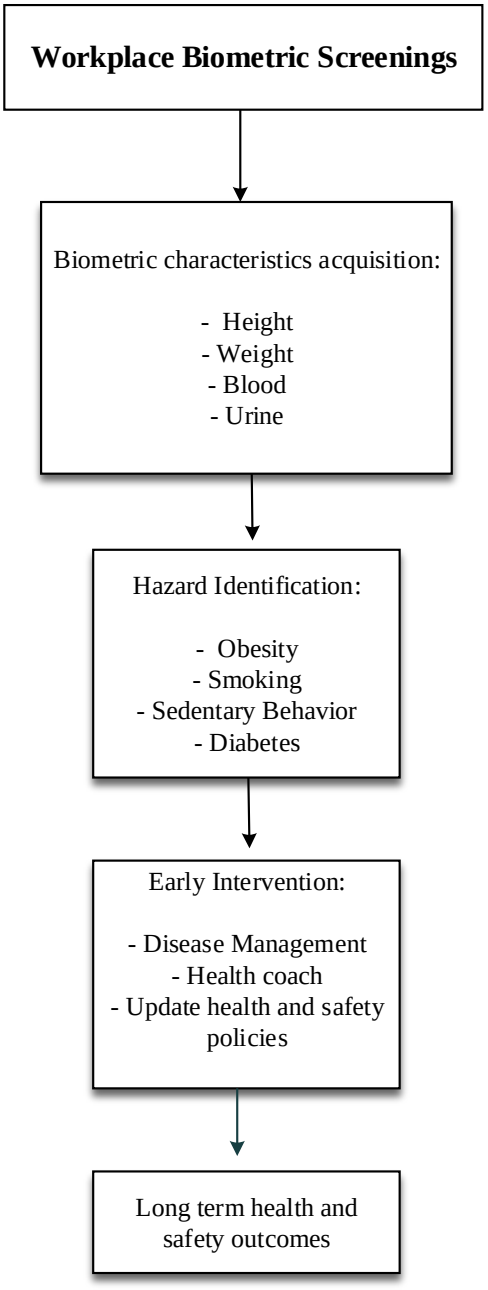
4. HEALTH AND SAFETY AT WORK: OVERLAPPING CONCEPTS

Occupational safety and health are two elements that are interconnected. Workforce's health is closely linked to performance and safety in an organization(Daniellou et al., 2012). Employees that enjoy good health are more productive, resilient, and less prone to safety incidents. Screenings boost morale among the workers. They understand and appreciate the organization's efforts towards their health, which is translated into employee retention, a feeling of ownership to the business, and motivation towards safety behaviors(Goetzel et al., 2007). Furthermore, according to the survey on the future of wellness at work, employees that recognize the company is paying attention to their health report improvement in factors such as stress level and job satisfaction(Yeung & Johnston, 2016).

McLellan et al. study (McLellan et al., 2009) found that participation rates in biometric screenings are positively correlated with the perception of safety at work. A combination of a positive perception of safety and an adequate health plan in the organization anticipates higher participation rates in biometric screenings. High participation rates are decisive for measuring the effectiveness and success of a program. It also contributes to a fair promotion and instauration of safety and health policies that benefit the majority of the employees. If a predominant number of employees participate in a biometric screening event, the results will sufficiently portray the necessities of the workforce and direct towards customized initiatives. Thus, biometric screenings and workplace safety operate together in a cycle where the perception of safety is enhanced as biometric screenings are implemented in the workplace.

Figure 1 shows a summary of the main aspects of how biometric screenings contribute to occupational safety and health outcomes.

Figure 1: Biometric Screenings Towards Workplace Health and Safety Outcom



5. DISCUSSION

Research on biometric screenings is mainly oriented to the health benefits given to employees and the organization. The purpose of this study was to explain in detail which biometric screenings are, but also go beyond the health approach and analyze the benefits of these tests in occupational safety and health. As described in the previous sections, health and safety at the workplace are directly connected since healthy employees are motivated to safety behaviors, which decrease work-related accidents.

Screening results provide critical health information to prevent and execute timely interventions regarding diseases and unhealthy habits within the workforce. Additionally, the processes that come after the tests such as education, health provider's appointments, follow-ups, and medical resources are essential for the success of the screenings.

The literature review indicates that biometric screening results can identify intrinsic hazards in the employees, which not only affect their health but can contribute to unsafe behaviors.

Furthermore, biometric screenings can be a primary tool for choosing or keeping employees in safety-sensitive positions. Work roles such as operating heavy machinery, jobs where firearms are employed or driving emergency vehicles in which not only the individual, coworker, and public safety are at risk.

6. CONCLUSIONS

Biometrics' purpose is to serve as a means of identification. The present work wanted to extend this concept into not just identifying the person itself but the hazards within the employee and how these hazards affect occupational safety and health.

The workforce is a crucial element in organizations and for the economy. Employees spend at least a third of the day in a company without counting the commuting or extra hours. For this reason, health-promoting programs are becoming a must at any organization. Employees' health is highly connected to productivity, satisfaction, safety practices, and savings in healthcare costs. Nevertheless, these benefits, the employer has the moral duty to provide a safe and healthy environment in a workplace.

Biometric screenings constitute a vital part of the wellness programs since, at one event, it is possible to collect several biometric characteristics such as height, weight, blood pressure, heart rate, and others. The screening results provide a holistic vision of the workforce's health status. Moreover, it represents an opportunity to detect chronic diseases or health risk factors to prevent and treat them. The impact of this specific program keeps present after the screenings. It goes beyond health benefits because it also aids in the identification of hazards, which are critical elements in risk analysis and consequently enhance safety behaviors at the workplace.

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Research Article

EFFECT OF SILANE COUPLING AGENT CONTENT ON MECHANICAL PROPERTIES OF HYDROXYAPATITE/POLY(METHYL METHACRYLATE) DENTURE BASE COMPOSITE

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ABSTRACT

In removable prosthodontics, poly(methyl methacrylate) (PMMA) is the most suitable for the construction of denture bases. Intra-orally, the subjected stress intensity during the function accelerate the fracture of acrylic resin denture bases. Extra-orally, fracture occurs when dentures are accidentally dropped on a hard surface. The aim of the current study was to investigate the effect of coupling agent concentration on the mechanical properties of Hydroxyapatite/Poly(methyl methacrylate) (HA/PMMA) denture base composite. The Hydroxyapatite (HA) treated with four different ratios (i.e. 0, 5, 7 and 10 wt%) of 3-(trimethoxysily) propyl methacrylate (γMPS) silane coupling agent was added into the PMMA matrix. The mechanical performance of the composite was evaluated by conducting fracture toughness, flexural and tensile tests. An improvement of 13.83% and 9.62% in the tensile and flexural strength respectively, was achieved. The tensile and flexural modulus of the composite increased by 19.04% and 12.5% respectively. A significant improvement of 29.26% in the fracture toughness was observed at 10 wt% of γ-MPS. 10 wt% of γ-MPS is the optimum amount of coupling agent for obtaining balanced mechanical properties.

Keywords: *Dental Composites; Denture Base materials; Poly (methyl methacrylate); Mechanical Properties; Hydroxyapatite; Silane Coupling Agent.*

1. INTRODUCTION

Coupling agents are able to create a strong interface bond between inorganic and organic materials. In all cases, the coupling agents form a bridge at the interface of both components(Elshereksi et al., 2017a). Therefore, silane coupling agents are more often being used because they are more efficient, easy to spread in the composite and having very good chemical resistance. They are classified as organo-silane compounds which have as a minimum two different types of reactive groups bonded to the silicone in a molecule(Goyal et al., 2006, Šinkovec and Mušič, 2020). The main purpose of using coupling agents is to improve the mechanical properties of nanocomposites by introducing interactions between filler particles and the matrix(Fu et al., 2019).

Using silane coupling agents is a common practice in the construction of dental composite materials (Antonucci et al., 2005). they have been used widely to enhance the bonding strength of the composites' two phases (Elshereksi et al., 2017b). It has been established that isocyanates have the ability to chemically bond to the surface of HA through the reaction of isocyanate with the surface hydroxyl groups of nano apatite (Wang et al., 2011). Therefore, isocyanate and diisocyanate can be used to introduce chemical bonding between HA particles and various polymer materials among them, poly (methyl methacrylate) PMMA(Mei et al., 2019). Similarly, with the hexamethylene diisocyanate, HA filler particles and polymer matrix can be bonded with covalent bond. As a result, the mechanical properties of the composite can be significantly enhanced (Turon et al., 2018).

It was reported that methacryloxy propyl trimethoxysilane (γ-MPS) as a coupling agent considerably increased the tensile, flexural strength, and the hardness of composites for dental applications. Silane coupling agent is more often being used because it is more efficient, easy to spread in the matrix and having very good chemical resistance(Khaje and Jamshidi, 2015, Alrahlah, 2018, Della Bona et al., 2008). The aim of the present research is to study the effect of coupling agent ratio on the mechanical properties of HA/PMMA composite.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Powder Components

The powder components consisted of poly(methyl methacrylate) PMMA (Sigma-Aldrich, USA), Hydroxyapatite (HA) (Particle size 5±1 μm, Fluidinova, Portugal) and Benzoyl Peroxide (BPO) (Particle size ≈ 106 μm, MCC, Germany).

2.1.2 Liquid Components

The liquid components consisted of methyl methacrylate (MMA) **(**Aldrich USA**),** 0.0025% of hydroquinone as stabilizer and 10% ethylene glycol dimethacrylate (EGDMA) as cross linking agent **(**Aldrich USA**)**.

2.1.3 Coupling Agent

The silane coupling agent [3-(methacryloxy) propyl trimethoxysilane] also known as 3-(trimethoxysily) propyl methacrylate (γ-MPS), (Boiling Point ≈ 190°C and Flash Point ≈ 92.22°C, Sigma-Aldrich. UAS).

2.2. Methods

2.2.1. HA Filler Treatment

HA was treated with different ratios (0% wt, 5% wt, 7% wt, and 10% wt) of silane coupling agent (γ-MPS) using a 70/30 acetone-water mixture. The HA powder was added to

the liquid mixture (acetone-water-silane) and mixed with stirrer for 4 h, subsequently the mixture was dried in oven at 110°C for 24 h. The procedures were previously described by Kundie et al. (2018a).

2.2.2. Sample Preparation

The treated HA with. 0, 5, 7 and 10 wt% of [3-(methacryloxy) propyl trimethoxysilane] (Aldrich USA) was used as reinforcement filler for the acrylic denture base material. The planetary ball milling technique (PBM) was used to mix powder components the for 30 min. The running speed was set at (150 rpm) and the powder to ball weight ratio (PBR) was 1:10. The milling was stopped every 3 min and continued after 6 min during the run time to avoid the overheating and premature polymerization problems. The powder components were added to the liquid components and mixed in accordance to standard dental laboratory manual (SDL). After reaching the dough stage, the mixture was packed into a stainless steel mold and compression molded at room temperature. The heat treatment process was done at 78°C for 90 min using a water bath. The procedure followed in the current study was according to the conventional technique of the acrylic denture base fabrication.(McCabe and Walls, 2013).

2.3. Mechanical Properties

To evaluate the mechanical performance of PMMA/HA composites in the current study, a total of three tests were carried out. The testing procedures are outlined in the following sub-section.

2.3.1. Tensile Test

A tensile test was carried out according to ASTM D 638-2005 type IV using (INSTRON 5582 10 kN tensile testing machine). The crosshead speed was set at 5 mm/min and gauge length at 50 mm.

2.3.2. Flexural Test

According to the ASTM standard D790-2005, a three point flexural tests was carried out using (INSTRON 5582 10 kN tensile testing machine). The diameter of the loading nose and supports was 20 mm and 10 mm, respectively. The span length was set at 50 mm whilst the crosshead speed at 2 mm/min. The flexural modulus and the flexural strength were calculated using the following equation(Campo, 2008, Hamizah et al., 2012, Kundie et al., 2018a):

$$\text{flexural modulus} = \frac{L^3 m}{4bd^3} \quad (1)$$

$$\text{flexural strength} = \frac{3PL}{2bd^2} \quad (2)$$

Whereby, b = specimen width, m = tangent gradient of the initial straight line of load versus deflection curve, d = specimen thickness, P = maximum load, L = span length.

2.3.3. Fracture Toughness

According to ISO 13586:2000, fracture toughness was measured using (SEN-B). The overall length = 80 mm, span length S = 64 mm, thickness, t = 4 mm, notch length a = 4 mm, width w = 20 mm. The crosshead speed of 1.00 mm/min. the values for K_{IC} was calculated using the following equation(Kundie et al., 2018a, Hamizah et al., 2012):

$$K_{IC} = \frac{3PSa^{1/2}Y}{2tw^2} \quad (3)$$

Geometrical correction factor (Y)

$$Y = 1.93 - 3.07\left(\frac{a}{W}\right) + 14.53\left(\frac{a}{W}\right)^2 - 25.1\left(\frac{a}{W}\right)^3 + 25.8\left(\frac{a}{W}\right)^4 \quad (4)$$

whereby: S = span length (mm), w = specimen width (mm), a = notch length (mm), P = load at peak (N), t = specimen thickness (mm).

3. RESULT AND DISCUSSION

3.1 Tensile Properties

Table 1 shows the effect of γ-MPS silane coupling agent amount on the tensile properties of PMMA/HA composite. The tensile strength and modulus increased with increasing the amount of γ-MPS and reached a maximum value of 59.81 MPa and 2.5 GPa, respectively, for the samples treated with 10% of γ-MPS. It can be seen that the treated composites show slightly higher tensile modulus compared with the untreated composite. The presence of silane coupling agent did not only improve the PMMA-HA interaction but also facilitated the dispersion of the HA particles in the PMMA matrix. The improvement in HA dispersion increased the ability of distributing the stresses uniformly in the composite, which led to higher resistance towards deformation leading to higher tensile modulus(Liu and Webster, 2010, Amdjadi et al., 2017). silane coupling agent improves interfacing bond between the inorganic filler and polymer matrix with both strong chemical bond and strong mechanical interlocking that formed between hydroxyapatite and the matrix. Eventually as a result, the tensile strength is improved (Wang and Bonfield, 2001, Marghalani, 2014).

Table 1: The tensile properties of PMMA/ HA 5 wt% as a function of different γ-MPS silane coupling agent concentration.

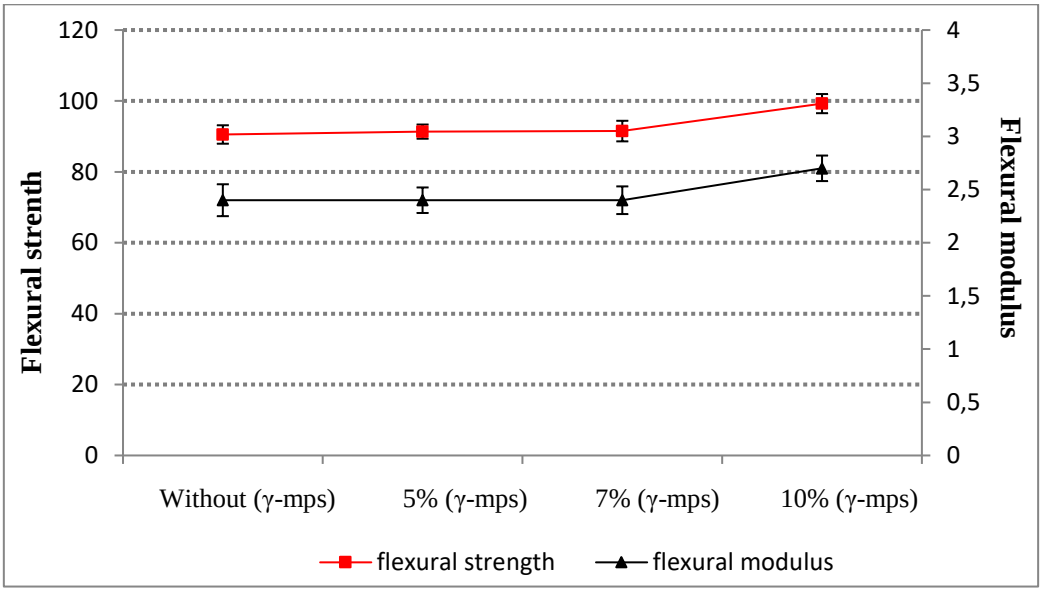
Content of γ-MPS	Tensile strength (MPa)	Tensile modulus (GPa)
Without (γ-mps)	52.54 ± 1.7	2.10 ± 0.10
5% (γ-mps)	54.88 ± 1.8	2.20 ± 0.09
7% (γ-mps)	55.50 ± 1.6	2.37± 0.06
10% (γ-mps)	59.81 ± 0.8	2.50 ± 0.05

3.2 Flexural properties

Figure 1 shows the effect of the amount of γ-MPS silane coupling agent on the flexural properties of HA/PMMA composites. The flexural strength and modulus rapidly increased with the increasing of γ-MPS amount and reached a maximum value of 99.25 MPa and 2.7 GPa, respectively, for the samples treated with 10% of γ-MPS. This observation is in agreement with the one made by (Tham et al., 2010, Kundie et al., 2018a, Chow et al., 2008). The flexural strength increased as a result the γ-MPS treatment. The silane coupling agent improved the filler-matrix compatibility which led eventually to a better distribution of the stress in the

composite thus yielding better composites properties. Synergistic effect was obtained as a result of the silanization of hydroxyapatite which improve the adhesion and interaction between the filler and the matrix. (Sideridou and Karabela, 2009, XAVIER et al., 2015). It can be seen that the flexural modulus is slightly higher for the composite treated with 10 wt% of γ-MPS compared to the other formulations. This can be attributed the strengthened filler-matrix interface which indirectly allows the treated composite to sustain external load and be able to distribute the stress fairly in composite(Marghalani, 2014, Pratap et al., 2019).

Fig. 1: The flexural properties of PMMA/ HA 5 wt% as a function of different γ-MPS silane coupling agent concentration.

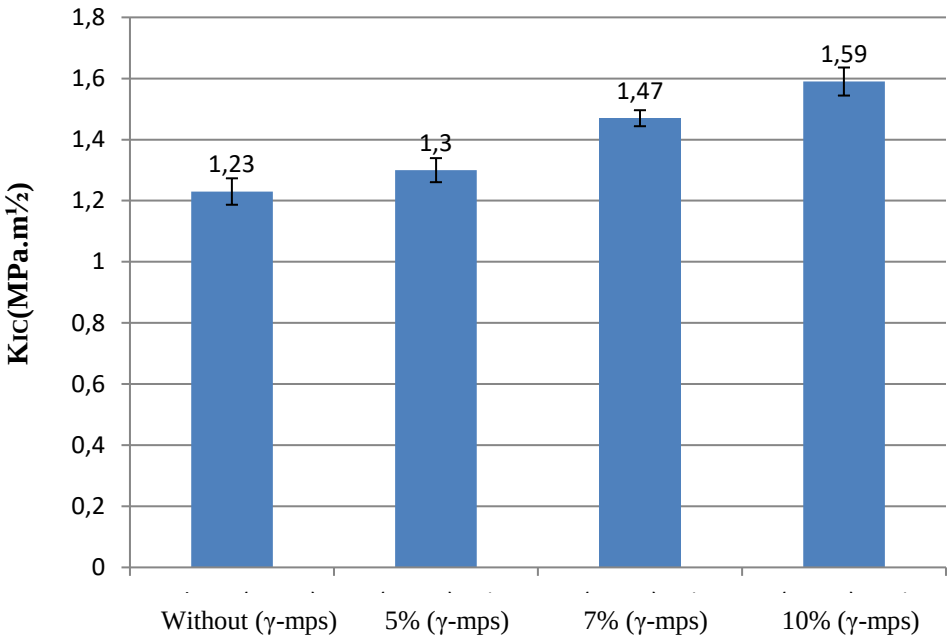


3.3 Fracture toughness

Figure 2 shows the effect of the amount of γ-MPS silane coupling agent on the fracture toughness of PMMA/HA composites. The fracture toughness reached the maximum value of 1.59 MPa.m^{½}, for the samples treated with 10% of γ-MPS. The toughening mechanisms are known to be associated with distribution of the filler particles. The homogenous distribution of the filler allows good plastic deformation which prevents crazing of the matrix resulting in high fracture toughness(Bartczak and Galeski, 2014, Lauke, 2008, Cotterell et al., 2007). The toughening efficiency indirectly depends on the amount of the coupling agent. Enough amount of coupling agent improves the distribution of filler particles (Kundie et al., 2018b, Fu et al., 2008). Homogeneously dispersed filler particles significantly effect the mechanical properties the polymer matrix, mainly in fracture toughness(Nilagiri Balasubramanian and Ramesh, 2018, Li et al., 2019).

If there is poor interfacing between the two phases of the composite result will be premature failure. Therefore, The filler-matrix interlocking is a very important factor (Šupová, 2009). The γ-MPS silane coupling agent can chemically link inorganic and organic materials in composites. The chemical structure of the silane, the silanization process and the silane content play a key role in determination of durability and strength of this interphase bond (Antonucci et al., 2005, Nakatani et al., 2011).

Fig. 2: The fracture toughness of PMMA/ HA 5 wt% as a function of different γ-MPS silane coupling agent concentration.



4. CONCLUSION

The use of silane coupling agent improved the chemical adhesion between the HA filler particles and the PMMA matrix. which led to improved mechanical properties of the denture base material. The unique dual functionality of silane coupling agent have the ability to chemically bridge organic and inorganic materials by creating an adhesive interphase in composites. The strong interfacial bond doesn't only aids the mixing of the two phases but also benefits the overall properties of the composite. It can also resist fracture through improving the stress transferring and distribution between the strong and brittle HA filler particles and flexible acrylic resin matrix.

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Research Article

THE EFFECT OF TRANS-THEORETICAL MODEL BASED-MOTIVATIONAL INTERVIEWING ON PROMOTING EXERCISE BEHAVIOR IN HEALTHY OLDER ADULTS: STUDY PROTOCOL OF A RANDOMIZED-CONTROLLED TRIAL

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ABSTRACT

Individualized health promotion activities are needed to bring the disadvantaged groups into healthy lifestyle behaviors. The older adult is both the most inactive and the most affected by physical inactivity in the community. Primary care nurses can help 65+ adults to gain exercise behavior by using their educator and guidance roles. The Trans-Theoretical Model (TTM) is an individualized counselling model that evaluates the behavior change as a process. TTM with motivational interviews (MI) is used to promote various health behaviors. This randomized-controlled trial will be conducted to investigate the effect of TTM based-MI on promoting exercise behavior in healthy older adults. The population is constituted of voluntary seniors who meet the inclusion criteria (n=117) from 65-74 aged adults (N=1630) who registered to a family health center. A power analysis was performed to sample size estimation with .30 effect size and .80 power. The projected sample size was found 90. The participants are divided into stratums, blocking according to the age, gender and exercise behavior stages of change. The seniors from the created stratums is allocated into intervention and control groups randomly. The data will be gathered via a questionnaire, TTM scales, KATZ Activities of Daily Living Scale (KATZ-ADL) and Physical Activity Scale for Elderly (PASE). Waist circumferences will be measured and average weekly step counts will be calculated via a pedometer. The data will be collected via an independent researcher, blinded to the study groups. TTM-based MI for gaining exercise behavior program is planned to apply to the intervention group overall six times (face-to-face for four times biweekly and twice via telephone by four weeks). Intervention period is planned as six months. The results obtained are expected to guide the community health nurses in terms of gaining exercise behavior of the older adults. This study is registered to clinicaltrials.gov, NCT04128553.

Keywords: *Older Adult, Community Health Nursing, Exercise, Motivational Interviewing, Transtheoretical Model*

1. INTRODUCTION

Physical inactivity is known to be responsible for 6% of all deaths among the global causes of mortality. It is estimated that approximately 21-25% of breast and colon cancer burden, %27 of diabetes and approximately 30% of ischemic heart disease burden are mainly attributed to physical inactivity (WHO, 2010:10).

Physical activity is proven as important for all the individuals at any age. Since the seniors have been the most physically inactive and the most affected group by physical inactivity in the community, promoting physical activity is accepted even more significant in this population (WHO, 2018: 7; WHO, 2010: 10,30-33). Physical activity is one of the effective non-pharmacological method to maintain the health and the functional independence especially for older adults. Therefore, it is very vital to initiate and progress to exercising slowly with the wisdom of a physically active lifestyle is a lifelong goal (Paterson et al., 2007). Gaining physical activity behavior contributes greatly to healthy aging (WHO, 2018: 7; WHO, 2010: 10,30-33). Regular physical activity in seniors reduces the risks of non-communicable diseases, such as coronary heart disease, stroke, diabetes, hypertension, colon and breast cancers, and depression. There are also benefits of being physically active in old ages, such as reducing the fall risk, improving the cognitive functions and controlling body weight (WHO, 2010: 30-31, 10).

While the physical activity is known to have numerous health benefits, a considerable proportion of older adults do not exercise and many of them indicate musculoskeletal system disorders or other systemic diseases as reasons for not exercising (Nied & Franklin, 2002). Centers for Disease Control and Prevention (CDC) states that even those with co-morbidities such as arthritis, diabetes or heart diseases, should not be inactive and regular physical activity can improve the quality of life and reduce the any complication risks related to the present co-morbidities of the older adult (CDC, 2020).

Although the health promotion activities are needed in seniors, the lack of motivation is one of the barriers of promoting the elder's health (Kitiş, 2017: 119-120). Adams-Fryatt (2010) states that, changing the physical inactivity behavior of older adults can be challenging, but it is not impossible. In a study, approximately half of the older adults were found not to exercise due to the lack of motivation; and individual and comprehensive approaches are needed in the exercise programs which are provided to older adults, is stated (Cohen-Mansfield et al., 2003). Community health nurse, as a primary care server, can identify the needs and barriers related to exercising by interviewing and monitoring the seniors one-by-one. They can motivate by guiding the older adults on this issue (Nies & Mcewen, 2019:777). The Trans-Theoretical Model (TTM), one of the most widely used models in the health promotion, recognizes the importance of the motivation and emphasizes the importance of strengthening the factors or the processes which trigger the behavior change (Emmons & Rollnick, 2001; Velicer et al., 1998; Spencer et al., 2006). Nurses can play a role in the acquisition of healthy lifestyle habits for the seniors via improving their knowledge and skills about the TTM, which is one of the individualized behavior change models, and motivational interviewing (MI), which is an individual-based counselling and guiding approach to be ensured behavior changes via helping individuals to recognize and solve their ambivalences (Cangöl & Şahin, 2017; Emmons & Rollnick, 2001).

There are different studies which investigate the effectiveness of the TTM on various health behaviors conducted by nurses in the literature. In the study conducted by Kim and Kang (2013), it is stated that the TTM-based physical activity intervention has positive impacts on reducing the cardiovascular disease risk. In the study of Cornacchione and Smith (2012), related to smoking, it is found that the TTM-based intervention has positive effects on

smoking cessation. In the research of Fahrenwald et al. (2005), TTM-based intervention was found effective to be increased the physical activity levels of the participants. Studies in the literature showed that the MI also has effects on improving the physical activity levels of individuals (Tse et al., 2013; Lin et al., 2016).

In the systematic reviews and meta-analysis in the literature, it is stated that even 15 minutes-long MIs make positive impacts on acquiring physical activity habit and other health promoting behaviors (McKenzie et al., 2015; Rubak et al., 2005; Van Der Bij et al., 2002). Physical activity self-efficacy levels of the individuals can be improved via MIs (Lilienthal et al., 2014; Tse et al., 2013). Moreover, feel of confidence and solving individual's problems and conflicts would be provided by personal communication (Emmons & Rollnick, 2001; Rollnick et al., 1992).

To the best of our knowledge, there are currently no studies evaluating the effectiveness TTM-based MI in the seniors in our country. Therefore, it is needed to assess the efficacy of TTM-based MI for gaining the physical activity behavior in the older adults in this study.

This study will be conducted to investigate the effect of TTM based-MI on promoting exercise behavior in healthy older adults. The main hypothesis and the sub-hypotheses of the research are as follows:

H_1: The TTM-based MI makes progress on the exercise behavior stages of change of the older adults.

$H_{1\text{-}1}$:The TTM-based MI increases the average score of processes of change in exercise of the older adults.

$H_{1\text{-}2}$:The TTM-based MI increases the average score of exercise self-efficacy of the older adults.

$H_{1\text{-}3}$:The TTM-based MI increases the average score of balance of decision-making of the older adults.

$H_{1\text{-}4}$: The TTM-based MI increases the physical activity levels of the older adults.

2. METHODS

2.1. Type of Study

This is a randomized controlled study.

2.2. Study Population and Inclusion-Exclusion Criteria

The study population is constituted of volunteer individuals who meet the inclusion criteria (n=117) from 65-74 aged adults (N=1630) who registered to a family health center (FHC) in a city, located in the middle region of the country.

Inclusion criteria are as follows:

- 65-74 aged seniors, who will voluntarily participate in the study
- Seniors, at least literate or elementary school graduated
- Seniors, at the pre-contemplation or contemplation stages of exercise behavior change
- No cognitive impairment which affects the interpersonal communication
- No muscle-joint problems to be a barrier for the physical activity
- No neuropsychiatric disorder

Exclusion criteria are as follows:

- Individuals whose age are less than 65 and bigger than 75.

- Seniors whose Mini Mental State Test (MMT) scores under 24 points (Mild Cognitive Impairment)
- Seniors whose Geriatric Depression Scale (GDS) scores above 5 points
- Seniors diagnosed with Cancer, Insulin-dependent Type-2 Diabetes Mellitus, Uncontrolled-Hypertension, Heart Failure, Chronic Obstructive Pulmonary Disease (COPD) and Asthma

Termination criteria are changing in the mental status of the senior during the follow-ups, requesting for leaving the research at any stage and any situation that requires hospitalization needs of the senior.

2.3. Study Group and Power Analysis

A power analysis was performed for sample size estimation with .30 effect size and .80 power. The projected sample size was found (at least) 90 with the allocation ratio 1:1.

2.4. Randomization and Blinding

In randomization, the seniors, planned to participate in this research, will be divided into stratums according to the age (65-69, 70-74), gender (male-female) and exercise behavior stages of change (pre-contemplation and contemplation). The block randomization will be used for bringing balance between the stratums. The seniors will be divided into two groups according to their exercise stages of change (pre-contemplation and contemplation), determined according to the Stage of Change Level Short Question Form. The seniors from the created stratums will be allocated into intervention and control groups randomly. The simple-random assignment of the research groups will be performed via an independent statistician to prevent the selection bias.

Blinding will not be applied to the participants due to the nature of the study. The data will be gathered, recorded and analyzed via an independent co-researcher who is blinded to the study groups, to avoid the bias. This co-researcher will be informed about the data collection process by the main research team.

2.5. Data Collection Tools and Implementation Process

A questionnaire, The Trans-Theoretical Model (TTM) Scales, KATZ Activities of Daily Living Scale (KATZ-ADL) and Physical Activity Scale for Elderly (PASE) will be used for the data collection. A pedometer (JP-600) will be used for calculating the weekly step counts of each participant. Waist circumference of the seniors will be measured via a tape-measure. The data will be gathered via the co-researcher through face-to-face interviews in the selected FHC. The flowchart of the study procedure is depicted in Figure 1. Outcomes and follow-ups are depicted in Table 1.

Table 1. Outcomes and Measurement Intervals (n =117)

Variables*	**Intervention**									**Control**	
	Weeks										
	0	**2**	**4**	**6**	**8**	**12**	**16**	**20**	**24**	**0**	**24**
The questionnaire	x									x	
The TTM Scales	x					x			x	x	x
KATZ-ADL	x					x			x	x	x
PASE	x					x			x	x	x
Waist circumference	x					x			x	x	x
Weekly average step counts	x				x	x			x	x	x
MI		x	x	x	x		x	x			

*The TTM Scales: The Trans-Theoretical Model Scales; KATZ-ADL: KATZ Activities of Daily Living Scale; PASE: Physical Activity Scale for Elderly; MI: Motivational Interviewing

Implementation of the intervention

In the first meeting (Week 0., the first follow-up), the research team will meet the participants in the intervention group, objective of the study will be explained to the seniors and the pretest (the questionnaire, the TTM Scales, KATZ-ADL, PASE) will be applied along with the body weight, height and waist circumference measurements. A pedometer (JP-600) will be handed out every senior in the intervention group and asked to carry it for one week (end of week 0.) to calculate the average weekly step counts (end of the 1st week.). The face-to-face individualized MI will be performed by the research team member (ES) in the following 2nd (the second follow-up), 4th (the third follow-up), 6th (the fourth follow-up) and 8th (the fifth follow-up) weeks. Stage of Change Level Short Question Form will be applied before every MI to maintain the next MI according to the senior's current stage of change. Following the fifth follow-up, the intervention group will be asked to carry the given pedometer for one week (end of 8th week) and the average weekly step counts will be calculated (end of 9th week). An inter-test (the TTM Scales, KATZ-ADL, PASE) will be applied along with the body weight and waist circumference measurements in the 12th week (the sixth follow-up). The intervention group will be asked to carry the given pedometer for one week again (end of 12th week) and the average weekly step counts will be calculated (end of 13th week). In this phase, the MI will be performed by the same researcher via telephone calls in the 16th (the seventh follow-up) and 20th (the eighth follow-up) weeks. The posttest (the TTM Scales, KATZ-ADL, PASE) will be applied along with the weight and waist circumference measurements in the 24th week of the study (the ninth follow-up). The intervention group will be asked to carry the given pedometer for one week again (end of 24th week) and the average weekly step counts will be calculated (end of 25th week). The MI will be performed six times in total, four times face-to-face and two times via telephone calls, to the intervention group during the study period (6 months). The MI will be conducted either the elder's own home or in a reserved-private room of the FHC, according to the individuals' choice.

For the control group, the pretest will be applied along with the body weight, height and waist circumference measurements to the seniors in the first consultation (Week 0., the first follow-up). A same brand pedometer will be handed out every senior in the control group and asked to carry it for one week (end of week 0.) to calculate the average weekly step counts (end of 1st week.). The posttest will be applied along with the body weight and waist circumference measurements in the 24th week of the study (the second follow-up). The control group will be asked to carry the given pedometer for one week again (end of 24th week) and the average weekly step counts will be calculated (end of 25th week). The MI will not be performed to the control group.

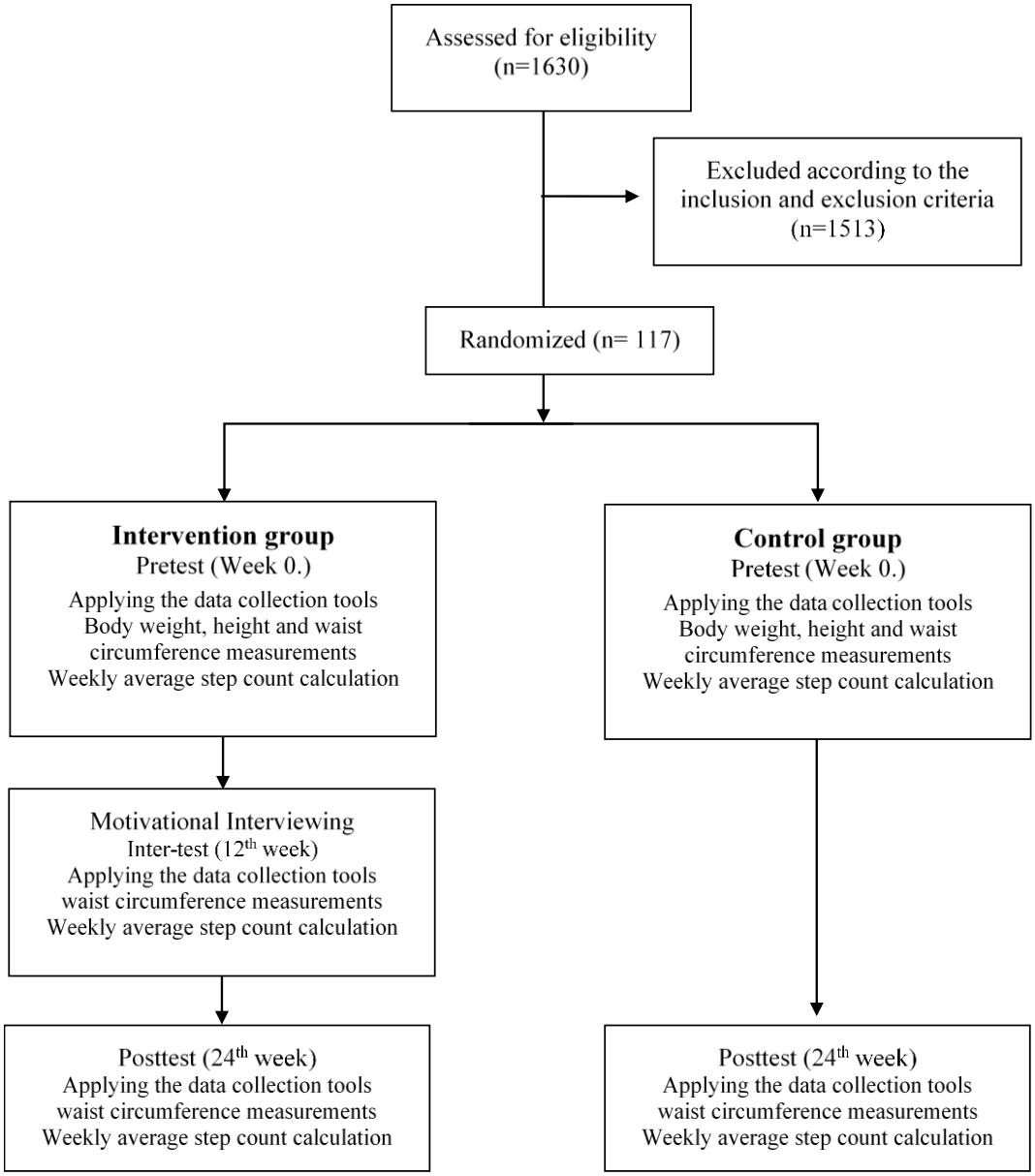
2.6. Data Analysis

The data will be analyzed via the Statistical Package for the Social Sciences (SPSS) version 18.0. Frequencies will be used to present the socio-demographic characteristics. The chi-square test will be used for comparing the categorical variables. Independent samples t-test or Mann Whitney-U test will be used according to the result of the normality test. Correlation analysis will be performed to explore the relation between follow-up scores of the scales.

2.7. Ethical Considerations

The ethical approval was gathered from the university's Clinical Research Ethics Committee (Approval number/date: 838/12.11.2018). The written permissions were obtained to the selected FHC. Both verbal and written consents of the seniors will be gathered before initiating the study.

Figure 1. Flowchart of the Study Procedure



3. RESULTS

The study is ongoing.

4. DISCUSSION AND CONCLUSION

The effect of TTM based-MI on promoting exercise behavior in healthy older adults will be investigated in this study. The results obtained in this study are expected to guide the community health nurses in terms of gaining the exercise behavior of the older adults who are under their care. The results also can present further evidences to the geriatric care researchers.

Acknowledgement

This is the protocol of a PhD. thesis with the same topic, which was registered to the clinicaltrials.gov, on 15.10.2019. The registration number is NCT04128553.

Conflict of interest

No conflict of interest has been declared by the authors.

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Research Article

THE EFFECTS OF HIGH SALT DIET AND EXERCISE ON THE WATER-SALT BALANCE AND BLOOD PRESSURE IN RATS

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ABSTRACT

In the study which was prepared based on the factors that can take place in essential hypertension pathology; We aimed to investigate the interactions of intensive exercise, high salt and partial NOS inhibition applications with each other, the effects on water-salt balance and blood pressure, changes in the intrarenal dopaminergic system, which is an important natriuretic system, and the participation of oxidative stress. The rats were given intensive exercise on a treadmill at a speed of 25 m / min at 5% inclination for 30 minutes a day, LNNA at a concentration of 50 mg / L and

a high salt diet of 4% for 7 days either separately or together. Blood pressures of the rats were measured on the first and last days of the experiment, and the rats were taken into metabolic cages; 24-hour water intake and urinevolume were measured. Dopamine levels were measured in 24-hour urine to detect intrarenal dopamine synthesis. In addition, oxidative stress parameters in the serums of rats; TAS, TOS and OSI levels were measured. Blood pressure was found to be high in the groups in which intensive exercise was applied together with LNNA and high salt diet. While there was no change in the water balance of this group, it was found that sodium excretion and dopamine levels increased in 24-hour urine. In addition, it was found that the total oxidant status increased in this group, and oxidative stress developed as a result of insufficient antioxidant system. It suggests that the reason of hypertension that develops with the application of intensive exercise together with LNNA and high salt diet may be due to the vascular resistance increasing effect of oxidative stress rather than water-salt retention and it points out the necessity of studies to fully detect vascular tissue oxidative stress markers and vascular oxidative damage.

Keywords: *Hypertension, Intensive Exercise, Intrarenal Dopamine, Oxidative Stress*

1. INTRODUCTION

Essential hypertension is a chronic progressive cardiovascular-kidney disease, which is manifested by constant high systemic arterial blood pressure, and whose etiopathogenesis is not fully known. Pathophysiological factors that play a role in the development of essential hypertension; excessive activity of sodium-retaining hormones and vasoconstrictor agents, insufficiency of vasodilator and natriuretic agents, increased sympathetic nervous system (SNS) activity, imbalances in renin production, high salt diet and oxidative stress (Mark et al., 1975, Carey, 2001, Touyz,2004, Chrysant, 2016).

Nitric oxide (NO) is an endogenous vasodilator agent which is released from the vascular endothelium and plays a role in the local regulation of vascular tone (Napoli and Ignarro, 2009). Also, NO, which is synthesized tonically in kidneys, plays an important role in controlling blood pressure by regulating renal hemodynamics and sodium excretion (Baylis et al., 1990, Granger and Alexander, 2000). Partial or total inhibition of NO synthesis with nitric oxide synthase (NOS) inhibitors such as L-nitro-N-arginine (LNNA) or decreased bioavailability of NO could lead to increased blood pressure (Manning et al., 1993, Vapaatalo et al., 2000, Aekthammarat et al., 2019).

High salt diet does not always lead to high blood pressure due to the balancing of natriuretic protective systems in the organism. (Titze and Luft, 2017). However, if there is renal damage due to high salt diet and an insufficiency in natriuretic systems such as intrarenal dopaminergic system, NO , autoregulatory mechanisms cause increased peripheral vascular resistance and vascular reactivity and lead to hypertension (Shultz and Tolins, 1993, Tolins and Shultz, 1994, Yuasa et al., 2000, Banday et al., 2008, Mente, et al. 2014). The effect of the salt on blood pressure includes mixed mechanisms and these mechanisms can not be fully explained. It is suggested that high salt diet causes oxidative stress (Kopkan and Majid, 2005, Feng et al., 2017).

It is thought that stimulation of the antioxidant system in the body with regular moderate exercise prevents the formation of oxidative stress, increases NO synthesis and bioactivity, decreases peripheral vascular resistance thus contributes to the regulation of blood pressure (Kitiyakara et al., 2003).However ,increased oxygen consumption and metabolic rate with intensive, exhaustive exercise effect the mitochondrial electron transport chain, increase the catecholamine and lactic acid levels,cause temporary hypoxia and reoxygenation in some

tissues and lead to hyperthermia. As a result, it could lead to increasing of the formation of free radicals, disruption of antioxidant-oxidant balance and oxidative stress (Alessio, 1993, Jil, 1999, Husain, 2003).

Studies have shown that development of oxidative stress, decreased nitric oxide bioavailability and hypertension occurs as a result of applying oxidant agents to test subjects. In addition to that, application of antioxidant vitamins to these test subjects with hypertension was found to increase NO bioavailability and lead blood pressure to normal levels (Banday et al., 2008, Schultz et al., 2017). Increased renal and vascular O_2 cause to decreased NO bioavailability, NO mediated vasodilation disruption, decreased renal sodium reabsorption and the development of salt sensitive hypertension (Touyz,2004, Vaziri, 2000).

When salt consumption increases, with the introduction of endogenous natriuretic systems, excess salt is discharged and blood pressure remains at normal levels. In this case, intrarenal dopamine exchanges Na^+/H^+ and inhibits Na^+ K^+ ATPase pump via D1-like receptor activation, located in the proximal tubular cells and increases urinary sodium excretion (Carey, 2001). However, in the presence of oxidative stress, D1-like receptor-G protein binding is impaired in renal proximal tubule cells. Oxidative stress causes D1 receptor dysfunction, Na^+ K^+ ATPase pump inhibition ability of produced dopamine is impaired, natriuresis can not occur and blood pressure increases (Majid and Kopkan, 2007, Banday et al., 2008). Oxidative stress causes increasing in blood pressure due to disruption in the activities of natriuretic and vasodilator systems.

In this study; It is aimed to investigate the effects of intensive exercise, LNNA and high salt diet individually and in combination on water-salt balance and blood pressure at a dose and time that will not increase blood pressure when administered alone.It was purposed to investigate the participation of these systems in the development of water-salt balance and hypertension by measuring parameters related to intrarenal dopamine synthesis and oxidative stress.

2. METHODS

Experiments were carried out on 48 male *Wistar Albino* rats which were 8 weeks old, weighing 190-205 gr. Rats were housed under standard conditions with a 12-hour light–dark cycle in standard cages in a room with a controlled humidity of 40% and a temperature of 22°C. On the last day of the experiment, rats were taken to individual metabolic cages.

They had ad libitum access to food and water. For 7 days, rats were fed one of the specially prepared high salt diet with 4% salt or one of the standard rat feeds at 0.8% salt. LNNA was administered to the subjects with drinking water. Considering the amount of water consumed per day, LNNA at an average concentration of 50mg / L was prepared daily and administered for 7 days. (The dose of LNNA applied in the experiment and the high salt diet rate were determined by pre-experiments.)

In the exercise program, "May TME 0804 Treadmill Exerciser" branded four-lane small experimental animal treadmill was used. The treadmill has an adjustable speed indicator in 0.1 km / hour steps and a mechanism that provides angulation between -10^o and $+20^o$. The subjects who do not want to run were warned by using the electrical shock switch continuously or when desired, between 1-6 levels.

Practice exercises were not applied to the subjects. The rats were applied intensive running exercise on the treadmill at a speed of 25 m / min and at a 5% incline for 30 minutes a day for 7 days. It has been reported that this exercise model is a intensive-consuming exercise protocol with a VO_{2max}> 75% (Hegde and Solomon, 2015). If the subjects could not turn from

the electric grid onto the band despite physical stimulation, they were considered exhausted and their exercises were terminated (Ji et al., 2004).

Rats were divided into eight groups with 6 rats in each group (n = 48). 1. Control(C)(%0,8 salt diet and drinking water), 2. high salt group (HS) (%4 high salt diet and drinking water), 3. Exercise group (E) (intensive exercise;25 m/min speed and %5 slope for 30 minutes in a day),4. LNNA group (LNNA 50 mg/L concentration with water), 5. HS+E (%4 high salt diet and intensive exercise), 6. E+LNNA (LNNA 50 mg/L concentration with water and intensive exercise), 7. LNNA+ HS (LNNA 50 mg/L concentration with water and high salt diet), 8. E+LNNA+ HS (intensive exercise, LNNA 50 mg/L concentration with water and high salt diet). Systolic blood pressure measurements of rats were made by indirect tail cuff method from the tail on days 0 and 7 of the experiment (MAY BPHR 9610-PC TAIL-CUFF Indirect Blood Pressure Recorder, Ankara, Turkey). The measurements of the conscious subjects were made in a quiet laboratory environment after approximately 20 minutes of rest period, when the regular signal sound was received. The systolic blood pressure values were recorded in the computer. Systolic blood pressure averages were calculated by taking 3 measurements from each rat.

Rats were transferred to metabolic cages on the last day of the experimental protocol and the amount of water they drank and the volume of urine they produced for 24 hours were determined. At the end of the experiment, intracardiac blood was collected from rats under mild ether anesthesia and the subjects were decapitated. Na^+ levels in serum and urine samples were measured using an autoanalyzer (ROCHE COBAS 6000).

The water balance of the rats (volume of water intaken- volume of urine = water balance) was calculated by measuring the 24 hour water intake and urine volumeof the rats taken into metabolic cages. Urine dopamine levels were measured by the Duzen Laboratories group using a chemical detector and high pressure liquid chromatography device (HPLC) from the collected 24 hour urine samples.

Serum Total Antioxidant (TAS), Serum Total Oxidant levels were measured by a fully automated method developed by Erel (Erel, 2004, Erel, 2005). The oxidative stress index was expressed as the percentage of the total oxidant status levels of the samples to the total antioxidant status of the samples. OSI was calculated as TOS (μmol H2O2 equivalent/L) / TAS (μmol Trolox equivalent/L)x100. In case the OSI value is greater than 1, it was evaluated as oxidative stress. Samples were studied in Rel Assay Laboratory, Gaziantep, with colorimetric method in a fully automatic biochemistry autoanalyzer of Vital Scientific brand in accordance with the Erel Method. RL 0017 coded Rel Assay kits were used for TAS and RL 0024 coded Rel Assay kits were used for TOS.

The data obtained were expressed as mean ± standard error (SD). Statistical differences were calculated using “independent student-t” tests in independent groups. Paired student-t test was used to evaluate the difference between the values of the same group on days 0 and 7. Student's t test was used in the interpretation of the obtained results and a p value of <0.05 was considered statistically significant.

3. RESULTS

There was not statistically significant difference between the first and last weights of the groups.

3.1. Blood Pressures:

The initial blood pressure of the subjects was determined similarly. At the end of the experiment, it was determined that the blood pressure values of the groups were not affected

by these applications when the final blood pressure values of the groups that were applied intensive exercise alone, high salt or LNNA were compared with the initial blood pressure values (Table 1).

It was found that the final blood pressure values of the groups which were applied Exercise + Salt, Exercise + LNNA and LNNA + High Salt increased significantly compared to the initial blood pressure values. In addition, it was found that the combination of intensive exercise, high salt and LNNA significantly increased blood pressure values (n = 6; 168.38 ± 4.25) compared to the other groups (P <0.05) (Table 1).

Table 1. Comparison of the blood pressure values of the groups measured at the beginning and at the end of the experiment.

	First measured (mmHg)	**Last measured (mmHg)**
Control (n=6)	127,33 ± 1,1	127,70 ± 1,1
High Salt (n=6)	128,88 ± 1,15	129,86 ± 1,4
Exercise (n=6)	128,42 ± 1,12	134,92 ± 2,93
LNNA (n=6)	125,48 ± 2,32	131,30 ± 1,44
Exercise + High Salt (n=6)	128,08 ± 0,95	140,85 ±1,96**
Exercise + LNNA (n=6)	128,40 ± 1,92	135,45 ± 1,79**
High Salt + LNNA (n=6)	128,53 ± 2,41	141,08 ± 1,84**
Exercise + High Salt + LNNA (n=6)	127,17 ± 2,01	168,38 ± 4,25** β

* Compared to control group, p < 0.05

** P <0.05 Compared to baseline blood pressure values within the group β P <0.05 according to the other applications groups

3.2. Effects on Water Intake, Urine Volume and Water Balances

On the last day of the experiment, 24 hour water intake and urinevolume of the subjects which were taken into metabolic cages were determined and their water balances were calculated.

Administration of high salt diet alone significantly increased 24 hour water intake (n = 6; 36.5 ± 0.89) compared to both the control and the high salt and exercise group (P <0.05) (Table 2). The 24 hour urine output of this group (n = 6; 7.67 ± 0.42) was found lower than the groups which were administered exercise and LNNA (Table 2). Only high salt application increased 24 hours water intake and decreased the volume of urine. Therefore, the water balance of high salt diet (n = 6; 28.8 ± 1.14); increased compared to control group and the groups in which high salt administered with exercise or LNNA. LNNA application alone did not change the 24 hour water intake, but significantly reduced the volume of urine (n = 6; 6.17 ± 0.49). Only the water balance of the LNNA group (n = 6; 28.3 ± 1.58) increased compared to the control and the groups in which LNNA was administered with salt and / or exercise, water-salt retention occurred in the body (Table 2).

Table2. Comparison of the 24 hour water intake, urine volume and water balance data of the groups.

	Water intake (ml/day)	**Urine output (ml/day)**	**Water balances (ml/day)**
Control (n=6)	31 ± 1,2	9,3 ± 1,2	21,6 ± 0,3
High Salt (n=6)	36,5 ± 0,89 *€	7,67 ± 0,42€**	28,8 ± 1,14 ***€
Exercise (n=6)	34 ± 2,35	8 ± 1,03	26 ± 1,9
LNNA (n=6)	34,5 ± 1,38	6,17 ± 0,49*#Ω**	28,3 ± 1,58 *#Ω**
Exercise + High Salt (n=6)	30 ± 2,24	8 ± 0,37	22 ± 2,03
Exercise + LNNA (n=6)	32,33 ± 2,14	8,01 ± 0,6	24,1 ± 1,84
High Salt + LNNA (n=6)	30,8 ± 2,51	11,5 ± 1,36	19,33 ± 2,17
Exercise + High Salt + LNNA (n=6)	33,67 ± 2,07	12,33 ± 1,84	22,8 ± 1,8

* Compared to control group, p < 0.05 € Compared to Exercise +high salt group, p < 0.05
Ω Compared to Exercise + LNNA group, p < 0.05 # Compared to high salt + LNNA group, p < 0.05
** Compared to Exercise, high salt + LNNA group, p < 0. 05

3.3. Serum Sodium and Urine Sodium Values

High salt, exercise and LNNA applications did not affect serum sodium values. While LNNA administration alone did not change 24 hour urinary sodium level, urinary sodium concentrations of the group in which LNNA was administered with high salt diet (n = 6; 1.81 ± 0.12) was found higher than control group, LNNA group or high salt diet group (P <0.05). Simultaneously LNNA administration increased urinary sodium excretion in subjects which were given high salt diet. The 24 hours urinary sodium values of the group in which exercise, high salt diet and LNNA were applied together (n = 6; 3.23 ± 0.45) were found to be statistically significantly higher than both the control and the other groups (P <0, 05). (Table 3).

Table 3: Comparison of serum sodium values of subjects and sodium concentration in milliliter measured from 24 hour urine samples collected from subjects.

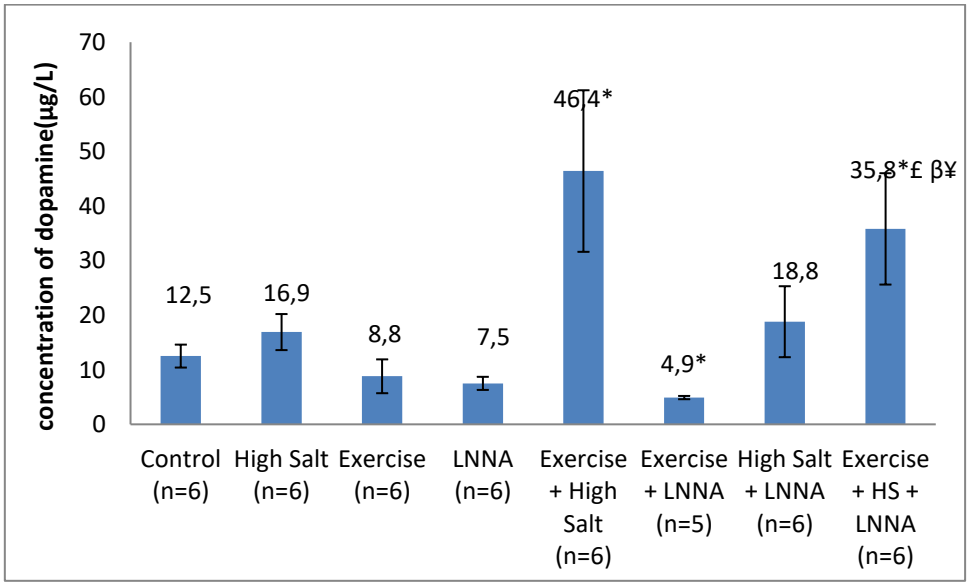
	Serum sodium concentrations *(mEq/l)*	**Urine sodium concentrations *(mEq/l)***
Control (n=6)	144,1 ± 0,6	0,77 ± 0,1
High Salt (n=6)	143,67 ± 0,92	0,76 ± 0,08
Exercise (n=6)	143,83 ± 0,17	0,86 ± 0,11
LNNA (n=6)	143,17 ± 0,7	0,72 ± 0,20
Exercise + High Salt (n=6)	143 ± 0,63	0,78 ± 0,08
Exercise + LNNA (n=5)	144,17 ± 0,4	1,69 ± 0,34
High Salt + LNNA (n=6)	144 ± 1	1,81 ± 0,12 *# £
Exercise + High Salt + LNNA (n=6)	143,33 ± 1,23	3,23 ± 0,45*£#β€∞

* Compared to control group, p < 0.05 # Compared to high salt group, p < 0.05
£ Compared to LNNA group, p < 0.05 β Compared to exercise group, p < 0.05
€ Compared to Exercise +high salt group, p < 0.05 ∞ Compared to high salt + LNNA group, p < 0.05

3.4. Urine Dopamine Values

The urine dopamine concentration of the high salt diet and exercise group was found to be higher than the control group. In addition, the urine dopamine concentration of this group is higher than the other groups (n = 6; 46.4 ± 14.8) however, it is not statistically significant since its standard error is high. The urine dopamine concentration (n = 6; 35.8 ± 10.2) of the group in which high salt diet, exercise and LNNA were applied together was found to be higher than the groups in which exercise and LNNA were administered separately and together and the control group (P <0, 05) (Figure 1).

Figure 1. Comparison of 24 Hour urine Dopamine concentrations



* Compared to control group, p < 0.05 £ Compared to LNNA group, p < 0.05
β Compared to Exercise group, p < 0.05 ¥ Compared to exercise + LNNA group, p < 0.05

3.5. Serum Total Oxidant Status (TOS), Total Antioxidant Status (TAS) and Oxidative Stress Index (OSI) values

Application of exercise and LNNA alone or together did not significantly affect serum total oxidant status values. TOS values of the group in which high salt diet was applied alone (n = 6; 24.11 ± 1.6) were found to be higher than TOS values of the control group and the groups in which high salt diet was applied with LNNA and/or exercise. In addition, serum total oxidant status values (n = 6; 18.05 ± 2.4) of the group in which high salt diet was applied with LNNA and exercise were found to be higher than the control group (P <0.05). Serum total antioxidant status values (n = 6; 1.91 ± 0.09) of the group in which the high salt diet (n = 6; 1.91 ± 0.09) was applied alone were found to be higher than the serum total antioxidant status values of the groups in which the high salt diet was applied with LNNA and / or exercise. Other applications did not significantly affect serum total antioxidant status.

Exercise or LNNA alone did not affect the oxidative stress index (OSI) values. However, the oxidative stress index (OSI) values of the group in which exercise and LNNA (n = 6; 1.06 ± 0.12) were applied together were found to be higher than the control group. The OSI values (n = 6; 1.27 ± 0.09) of the high salt diet alone were found to be significantly higher than the control group (Table 4).

Table 4: Comparison of serum TOS = Total Oxidant Status, TAS = total antioxidant Status, OSI = oxidative stress index values of the groups.

	TAS	**TOS**	**OSI**
Control (n=6)	1,71 ± 0,08	11,96 ± 1,8	0,64 ± 0,09
High Salt (n=6)	1,91 ± 0,09 €**∞	24,11 ± 1,6 *€∞	1,27 ± 0,09 *
Exercise (n=6)	1,41 ± 0,06	13,31 ± 2,1	0,93 ± 0,13
LNNA (n=6)	1,52 ± 0,06	14,38 ± 2,2	0,93 ±0,11
Exercise + High Salt (n=6)	1,58 ± 0,12	16,03 ± 2,1	0,99 ± 0,09 *
Exercise + LNNA (n=6)	1,47 ± 0,05	16,05 ± 3,1	1,06 ± 0,12*
High Salt + LNNA (n=6)	1,05 ± 0,06	16,06 ± 2,4	1,04 ± 0,12*
Exercise + High Salt + LNNA (n=6)	1,61 ± 0,07	18,05 ± 2,4*	1,12 ± 0,14 *

* Compared to control group, $p < 0.05$ ᵝ Compared to Exercise group, $p < 0.05$

€ Compared to Exercise +high salt group, p < 0.05 ∞ Compared to high salt + LNNA group, p < 0.05 ** Compared to Exercise, high salt + LNNA group, p < 0.05

In addition, the oxidative stress index (OSI) values of the groups in which the high salt diet was administered with exercise and / or LNNA (n = 6; 1.12 ± 0.14) were found to be significantly higher than the control group (P <0.05).

4. DISCUSSION

Various mechanisms that are claimed to be participating in essential hypertension; it was aimed to investigate the effects on blood pressure, water-salt balance, intrarenal dopamine synthesis and oxidative stress development due to applying intensive exercise and high salt diet separately and in combination with the NO synthesis inhibitor. In this study, it was shown for the first time that LNNA, high salt diet and intense exercise have no effect on blood pressure at the dose and time when they were applied alone. However it was found that they increased the blood pressure when they were applied together. In addition, when three of these factors were applied together, the increase in blood pressure was significantly more aggravated compared when two of these factors were applied together.

NOS inhibition and exercise studies have been conducted previously, however, these practices were generally aimed at investigating the effect of regular and moderate exercise on healing hypertension (Cornelissen and Smart, 2013, Hegde and Solomon, 2015). For example, Kuru et al. found that the NOS activity of the group that was regularly exercised increased and blood pressure decreased significantly when the hypertensive group that exercised moderately and regularly was compared with the sedentary hypertensive group in hypertension which was developed with L-NAME at a dose of 25mg / kg (Kuru et al., 2009). It is known that regular, moderate exercise reduces blood pressure in hypertension (Kitiyakara et al., 2003, Cornelissen and Smart, 2013). In contrast to regular and moderate exercise, intensive exercise causes oxidative stress. Oxidative stress is known to be a participant in the development of hypertension (Kopkan and Majid, 2005, Powers and Jackson, 2008, Schultz et al., 2017). However, the effect of intensive exercise on blood pressure is not fully known. Although intensive exercise alone is not expected to cause hypertension, these informations suggest that when it is applied together with other factors which participate to the pathogenesis of hypertension, intensive exercise may cause or aggravate hypertension. Therefore, in this study, it was predicted that oxidative stress caused by intensive exercise may contribute to the development of hypertension. Indeed, this study revealed the blood pressure increasing effect of intensive exercise, partial NOS inhibition or high salt diet. It is interesting that while LNNA or high salt diet did not increase blood pressure when they were applied alone, blood pressure was increased when intensive exercise application was added to them. When the reasons for the increase in blood pressure are questioned; parameters for water-salt balance, serum sodium concentration, urine dopamine levels and oxidative stress should be evaluated.

In this study, when the 24 hour water intake between the groups was compared, it was found that was increased in the high salt diet group and the other applications did not significantly affect water intake. In addition, when 24 hour urine volumes were compared; LNNA administration significantly reduced it. In the LNNA group, the decrease in urine volume disappeared with the addition of high salt diet. (Table 2). When the water balance table was examined, it was seen that there was a significant increase in LNNA or high salt applications alone. With LNNA application; while 24 hour water intake was not affected, a significant decrease in thevolume of urine suggests that the amount of water which was retained in the body has increased. It has been determined that high salt application increased 24 hour water intake and decreased the volume of urine, thus it increased the water balance. Increasing water balance suggests that there may be water retention in the body, but although these applications increased water balance, a significant increase in body weight did not occur.

While the water balance of both the high salt diet and LNNA group and the group to which exercise was added to these applications, did not change, the 24 hour urine volume increased. It suggests that the increased volume of urine in the groups which were applied

high salt diet or LNNA alone may be associated with increased blood pressure when it is considered that blood pressure of these groups increased. When blood pressure increases, sodium and water excretion from the kidneys increases, fluid volume decreases and blood pressure returns to normal levels. This phenomenon is defined as the relationship of pressure-natriuresis (Johnson and Freeman, 1992, Titze and Luft, 2017). As a matter of fact, urinary sodium and water excretion of these groups also increased. Subsequent autoregulatory mechanisms cause hypertension by increasing extrarenal peripheral vascular resistance and vascular reactivity. In high salt diet, the systems that regulate the salt balance of the organism come into play, the excess salt taken is removed and the blood pressure remains at normal levels. Intrarenal dopamine plays an important role in the maintenance of blood pressure and sodium-water homeostasis, with its vasodilator and natriuretic activity (Carey, 2001, Banday and Lokhandwala, 2020). Intrarenal dopamine achieves this effect generally through renal D1-like receptors (DA-1) -G protein coupling. DA-1 receptors inhibit Na + / H + exchange (apical) and Na^+ K^+ATPase pump (basolateral) especially in the proximal tubule and increase urinary sodium excretion. Inadequate intrarenal dopamine synthesis or activity is one of the factors that play a role in the pathogenesis of hypertension and indicator of intrarenal dopamine synthesis is dopamine levels in urine (Banday et al., 2008,a, Banday and Lokhandwala,b, 2020).

Wang et al. administered normal (0.28%) salt or high (4%) salt for 5 days to anesthetized rats to investigate the effectiveness of intrarenal dopamine in diuresis and natriuresis and they showed that the water-sodium excretions and urine dopamine concentrations of the high salt group increased (Wang, et al., 1997). In another study, increasing the amount of salt consumed for 8 days by 209-259 mEq in normal individuals who receive 9 mEq of salt per day increased the sodium and dopamine levels measured in 24 hour urine in these individuals (Wayne et al., 1974). In our study, administration of high salt (4%) alone did not significantly increase the urinary dopamine concentration being statistically compared to the normal (0.8%) salt applied group. Interestingly, the combination of high salt diet with LNNA and intensive exercise significantly increased dopamine levels in 24 hour urine (Figure 1). Increased urinary dopamine levels indicate increased intra renal dopamine synthesis and seem to occur with the increase in urinary sodium excretion. However, this situation could not prevent the increase in blood pressure. As intrarenal dopamine synthesis increases during high salt (1%) intake, it causes natriuresis with Na^+K^+ATPase pump inhibition via D1 receptors and maintains blood pressure at normal levels but with high salt diet and oxidative stress with agents such as BSO (butionine sulfoximine). It has been suggested that intrarenal dopamine synthesis is not affected, but as a result of dysfunction of dopaminergic receptors, intrarenal produced dopamine loses its Na^+K^+ ATPase pump inhibition ability and increases blood pressure by causing water-salt retention (Banday et al., 2008,a, Banday and Lokhandwala,b, 2020). In this study, sodium excretion, urinary dopamine levels and blood pressure increased with the application of intensive exercise together with LNNA and high salt diet. Although these results show that the natriuretic function of intrarenal dopamine is not impaired, the possibility of D1 receptor dysfunction that may occur with the formation of oxidative stress in this group cannot be excluded. In this study, despite the increase in dopamine synthesis, there is a possibility of D1 receptor dysfunction due to oxidative stress. However, the increase in urinary salt excretion is thought to be due to the increased blood pressure. Although the intrarenal dopaminergic system does not work, natriuresis occurs as a result of increased blood pressure. The degree of efficacy of intrarenal dopaminergic activity on natriuresis, in other words, the degree of increase and satiety of dopaminergic activity with the degree of salt loading have not been determined yet, and there is not enough information on the behavior pattern in high blood pressure.

People with mild hypertension were exercised with a $VO_{2\ max}$ of 40-60%; It has been found that sodium excretions and urine dopamine levels increase, blood pressure decreases (Arakawa et al., 1995). In another study, salt-sensitive Dahl rats were given a 4% high salt diet for 2 weeks, followed by 8m / min 60 minutes of $VO_{2\ max}$ 50% running exercise 5 days a week for 4 weeks. It was found that salt excretions and blood pressures of rats that were exercised were similar to rats that were not exercised, but rats that were applied moderate exercise had high urinary dopamine levels and that moderate exercise increased renal dopamine production (Maeda et al., 2000). On the contrary, studies performed with intensive exercise indicate that acute vigorous exercise may have negative effects on blood pressure regulation by causing oxidative stress (Witt et al., 1992, Bergholma et al., 1999). However, information on the effect of intensive exercise on urinary dopamine levels has not been found in the literature. In this study, the intensive exercise program alone did not affect the urinary dopamine levels, the combination with LNNA caused an increase in blood pressure and significantly decreased urinary dopamine levels. Since these two applications together increase sodium excretion in the urine, although it is not significant, it suggests that the increased blood pressure cannot be associated with sodium retention. In this case, the negative effects of oxidative stress formation as a result of the combination of intensive exercise and LNNA may have played a role in the increase in blood pressure. Oxidative stress has negative effects on both sodium retention and vascular tone increase. In this study, it can be thought that oxidative stress causes vasoconstriction with negative effects of O_2^- and H_2O_2 on vascular tissues, especially as a result of oxidative stress rather than its effects on sodium retention.

In intensive exercise with $VO_{2\ max}$ higher than 75%; metabolic rate, energy and oxygen consumption are increasing. Free radicals appear as byproducts of normal exercise metabolism and the production of reactive oxygen radicals (ROS) increases. Oxidative stress develops as a result of the significant increase in ROS and insufficiency of protective systems, deterioration of the prooxidant antioxidant balance (Alessio, 1993, Loperena and Harrison 2017). It has been demonstrated that intensive exercise causes increased free radical production, depletion of antioxidant agents, and disruption of endothelium dependent relaxation with in vivo studies (Bergholma et al., 1999). In this study, it was determined that high salt diet application alone significantly increased TAS, TOS and OSI levels and caused oxidative stress. While intensive exercise or LNNA application alone did not affect the measured TAS, TOS and OSI levels; combined application of them increased the oxidative stress index. In addition, the application of high salt diet with LNNA and / or intensive exercise also increased OSI values. The antioxidant system could not prevent the increase in oxidant status and oxidative stress developed (Table 4).

High salt diet causes an increase in the activity of oxidant enzymes such as NADPH oxidase in the arteries, veins and kidneys, and thus ROS production. Previous studies showed that especially when O_2^- increases, as a result of the interaction of O_2^- with NO, the bioactivity of NO decreases, endothelium-dependent vasodilator response is impaired, vasoconstrictor response dominates, vascular growth factors are activated and/or antioxidant enzymes are reduced and thus oxidative stress develops. (Kopkan and Majid, 2005, Wilcox, 2005, Lenda et al.,2000). It has been reported that oxidative stress is effective in the pathology of hypertension both in hypertensive animal models and humans (Champlain et al., 2004). In addition, hydrogen peroxide (H_2O_2), which is a free radical, is also known as an endothelium-derived hyperpolarizing factor, which is important in maintaining vascular tone. It has been shown that increased ROS when oxidative stress develops causes vasoconstriction by causing vascular Ca^{+2} increase (Tabet et al., 2004).

5. CONCLUSION

In this study, it has been shown that the combination of intensive exercise, LNNA and high salt diet, which have no effect on blood pressure at the dose and time applied alone, increases urinary sodium excretion and renal dopamine synthesis, but significantly increases blood pressure, the antioxidant system cannot prevent the increase in oxidant status, and oxidative stress develops.

The findings suggest that the reason for hypertension developed by the combination of intensive exercise, LNNA and high salt diet may be due to the vascular resistance increasing effect of oxidative stress rather than water-salt retention.

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Research Article

MORPHOMETRIC ANALYSIS OF THE WHITE MATTER STRUCTURES IN FEMALE MIGRAINE PATIENTS

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ABSTRACT

Migraine is a multifactorial neurovascular syndrome characterized by typical headache attacks that occur with internal and external triggering factors in individuals with genetic susceptibility. It affects more than 12% of the general population. The aim of the present study is to compare the morphometric measurements of the determined white matter structures with the control group to investigate whether there is a structural difference in white matter structures in female patients with migraine.

The width of the internal capsule parts (anterior limb, posterior limb and genu) and genu angle was evaluated through MRI. Corpus callosum related measurements were determined in the sagittal section. It was manually traced following its edge on the midsagittal slice of T1 images, where its structure appeared most remarkable. The right and left internal capsule related measurements were compared with migraine and control groups. Except the genu angle, there were statistically significant difference between all measurements and widths in the migraine group were greater than controls. No significant difference was found between the corpus callosum related measurements in the comparison of both groups.

Internal capsule consists of several essential white matter fiber bundles of the brain, and is strongly connected between a range of cortical and subcortical anatomical structures. It has a crucial importance for brain functions and it can be affected by a variety of pathologies. The corpus callosum is the main fiber tract connecting two hemispheres with extensive connections and is topographically organized. It has been investigated in several neurodegenerative diseases as a marker for cortical pathology.

Knowing the white matter structure in migraine patients, determining its prevalence, and its correlation with the severity, type and duration of migraine can give an idea to clinicians.

Keywords: *Migraine, Internal Capsule, Corpus Callosum, Morphometry, MR.*

1. INTRODUCTION

Migraine is a multifactorial neurovascular syndrome characterized by typical headache attacks that occur with internal and external triggering factors in individuals with genetic susceptibility. It affects more than 12% of the general population (Lipton *et al.*, 2001). Migraine headache does not always occur in the same clinical form. Due to different characteristics and accompanying symptoms, migraine symptoms may vary between patients and sometimes even in the same person over time (Russel *et al., 1994*). Structural lesions of unknown clinical significance are frequently observed in brain Magnetic Resonance Images

(MRI) in migraine patients (Del Zotto *et al., 2008*). Migraine is more common in men before puberty and in female during and after adolescence (Lipton and Bigal 2005; Lipton *et al., 2007*). It is most common in the 20-50 age range (Russel *et al., 1994*). The incidence of migraine in Turkey was found to be 16.4%. It has been reported that the female to male ratio was found to be 3: 1 and it was seen in 24.6% of female (Ertas *et al., 2012*).

The corpus callosum is composed of thick myelinated fibers that connect the relevant centers in the right and left brain hemispheres. This is the biggest and most important way that plays a role in the transmission of information reaching one hemisphere to the other. Corpus callosum atrophy can be seen in various neurological and psychiatric diseases ranging from Alzheimer's disease to depression (Gazzaniga, 2000; Quigley, 2003).

The internal capsule is a white matter composed of efferent and afferent fibers that extend vertically to connect some of the cortical centers to the spinal cord. It is divided into five parts which are anterior limb, genu, posterior limb, retrolentiform limb (retrolenticular part) and sublentiform limb (sublenticular part). Different fibers pass through each part of the internal capsula. Anterior thalamic radiation and frontopontine tracts from anterior limb, corticonuclear tracts from genu; retrolentiform parts and sublentiform parts are pass through the posterior limb. It is an important indicator place to identify neurological diseases at all stages of life (Turamanlar *et al., 2020*).

The aim of the present study is to compare the morphometric measurements of the determined white matter structures with the control group to investigate whether there is a structural difference in white matter structures in female patients with migraine.

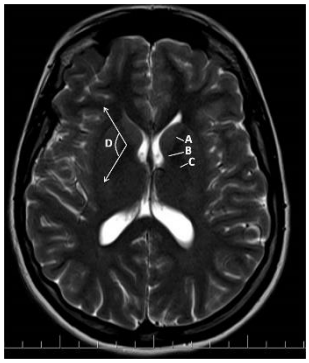
2. MATERIALS AND METHODS

Our study was carried out retrospectively in the Department of Radiology and Department of Anatomy, Faculty of Medicine, Afyonkarahisar Health Sciences University. The patient group was formed with 40 female patients between the ages of 18-65 who were admitted to the neurology outpatient clinic and diagnosed with migraine. A control group was formed from 79 female individuals in the same age range who had normal findings and had not been diagnosed with migraine, who had previously undergone Magnetic Resonance Imaging (MRI) for any reason. Those who were outside the specified age range, male patients and patients with any pathology other than migraine on MRI were excluded from the study.

Images were obtained from T1 weighted both transverse and sagittal brain sections through 1.5 T MRI (Philips Medical Systems, The Netherlands). MR protocol was as follows: TR = 500 ms; TE = 15 ms; FOV = 23 cm, slice thickness 5 mm; number of exitations = 2; matrix size = 256 × 256. Anatomical measurements were conducted on the Aquarius Workstation using the Aquarius NET program (TeraRecon Inc., USA).

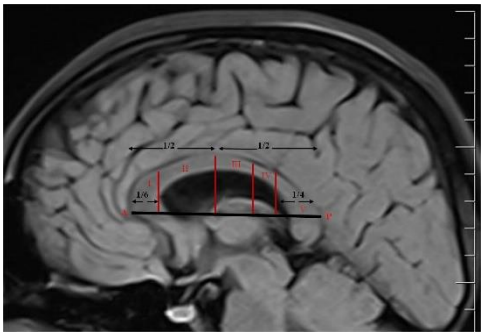
The width of the internal capsule parts (anterior limb, posterior limb and genu) and genu angle was evaluated through MRI. The internal capsula related measurements were made at the level where the caudat nucleus, lentiform nucleus and thalamus were seen in the same transverse section. Measurements made bilaterally in the same section. The angle of the lateral opening of the internal capsule in the genu of the line passing through the middle of the anterior and posterior limb was evaluated. The widest parts of the internal capsule between the thalamus and the lentiform nucleus were measured (Figure 1).

Figure 1



Corpus callosum related measurements were determined in the sagittal section. It was manually traced following its edge on the midsagittal slice, where its structure appeared most remarkable. Morphometric measurements were conducted according to the same methods reported previous studies (Hofer and Frahm, 2006; Unlu *et al.,2014*). After drawing the transverse line (AP line) that connects the extreme point of the genu with the extreme point of the splenium in the sagittal plane, a total of 5 areas were measured and evaluated through MRI (Figure 2).

Figure 2



The data obtained from the study were evaluated using IBM SPSS 20.0 (IBM Statistical Package Social Science) Means, standard deviations and percentages of the data were given in statistical analysis. The distribution of the data was analyzed using the Kolmogorov-Smirnov test. Mann- Whitney U test was used for independent two group comparisons, and Kruskal Wallis test was used in multiple group comparisons. The results were evaluated at the 95% confidence interval and the significance at the $p < 0.05$ level.

3. RESULTS

40 female patients with migraine and 79 female individuals without a diagnosis of migraine were included in the study. The mean age of the migraine group was 40.65 year and the controls was 40.24.

The mean widths of the anterior limb, genu and posterior limb were measured as 3.99 mm, 7.29 mm and 5.17 mm on the left side and 4 mm, 7.28 mm and 5.17 mm on the right side in migraine group, respectively. The mean widths of the anterior limb, genu and posterior limb were measured as 3.29 mm, 5.93 mm and 4.26 mm on the left side and 3.37 mm, 5.98 mm and 4.06 mm on the right side in controls, respectively.

The measurements of the right and left internal capsule structures of the controls were compared, statistical difference was found only in the posterior limb width (Table 1).

Table 1. Comparison of right and left internal capsula related measurements in controls ($p<0.05$).

Controls	**Left**			**Right**			**p**
	Min.	Max.	Mean±SD	Min.	Max.	Mean±SD	
Anterior limb (mm)	1.42	9.37	3.29±1.30	1.96	9.60	3.37±1.31	0.63
Genu (mm)	3.21	7.69	5.93±0.84	3.20	7.63	5.98±0.78	0.67
Posterior limb (mm)	2.25	7.12	4.26±0.88	1.96	6.76	4.06±0.94	**0.01**
Genu Angle (x/180)	104.00	137.00	123.22±5.27	105.00	135.50	123.03±5.41	0.81

When the same measurements were made for migraine group, no significant difference was detected (Table 2). The right and left internal capsule related measurements were compared with migraine and control groups. Except the genu angle, there were statistically significant difference between all measurements and widths in the migraine group were greater than controls (Table 3).

Table 2. Comparison of right and left internal capsula related measurements in patient group ($p<0.05$).

Migraine Patients	**Left**			**Right**			
	Min.	Max.	Mean±SD	Min.	Max.	Mean±SD	**p**
Anterior limb (mm)	2.02	6.15	3.99±0.82	2.19	6.27	4±0.88	0.67
Genu (mm)	5.71	9.39	7.29±1.02	5.62	9.56	7.28±1.02	0.67
Posterior limb (mm)	3.01	8.21	5.17±1.25	2.95	8.05	5.17±1.24	0.81
Genu Angle (x/180)	106.00	138.00	121.95±8.05	104.80	137.00	122.13±7.99	0.4

Table 3. Comparison of right and left internal capsula related measurements patient group with migraine group ($p<0.05$).

		Patients	**Controls**	
		Mean±SD	Mean±SD	**p**
Left	Anterior limb (mm)	3.99±0.82	3.29±1.30	**0.001**
	Genu	7.29±1.02	5.93±0.84	**0.001**
	Posterior limb (mm)	5.17±1.25	4.26±0.88	**0.001**
	Genu Angle (x/180)	121.95±8.05	123.22±5.27	0.22
Right	Anterior limb (mm)	4±0.88	3.37±1.31	**0.001**
	Genu	7.28±1.02	5.98±0.78	**0.001**
	Posterior limb (mm)	5.17±1.24	4.06±0.94	**0.001**
	Genu Angle (x/180)	122.13±7.99	123.03±5.41	0.4

No significant difference was found between the corpus callosum related measurements in the comparison of both groups (Table 4).

Table 4. Comparison of Corpus Callosum related measurements patient group with migraine group ($p<0.05$). (CC: Corpus Callosum)

	Migraine	**Control**	
	Mean±SD	Mean±SD	**p**
CC 1 (mm^2)	115.51 ± 19.34	169.54 ± 38.23	0.12
CC 2 (mm^2)	148.54 ±21.52	147.95 ± 28.81	0.54
CC 3 (mm^2)	72.68 ± 14.30	68.51 ± 15.22	0.14
CC 4 (mm^2)	35.29 ± 10.17	35.21 ± 9.42	0.78
CC 5 (mm^2)	211.49 ± 29.76	217.29 ± 39.41	0.5
CC lenght (mm)	67.27 ± 5.43	68.08 ± 5.07	0.72

4. DISCUSSION

Understanding the pathophysiology of migraine has provided scientists with significant advances in recent years. According to the data of the World Health Organization (WHO), migraine is among the first 20 diseases that cause severe disability in the world (Leonardi *et al., 2005*). Migraine is a familial disease and is often characterized by a unilateral, throbbing headache and may begin in childhood, adolescence or early adulthood (Allan and Martin, 2011). As a result of recent researches, only vascular theory has been moved away from the pathophysiology of migraine and integrated neurovascular theory has been accepted (Evans *et al., 2020*).

Internal capsule consists of several essential white matter fiber bundles of the brain, and is strongly connected between a range of cortical and subcortical anatomical structures (Hyett *et al., 2018*). It has a crucial importance for brain functions and it can be affected by a variety of pathologies. It is important to know the morphological structure of this structure, which is affected differently by various diseases. It has been examined using various methods in many diseases (Chowdhury *et al., 2010*). In the present study, when the right and left internal capsule related measurements of both groups were compared, all measurements except the genu angle were found to be significantly higher in migraine patients.

The corpus callosum is the main fiber tract connecting two hemispheres with extensive connections and is topographically organized (Frazier and Hardan, 2009). It has been investigated in several neurodegenerative diseases as a marker for cortical pathology (Wiltshire *et al., 2005*). In this study, there was no significant difference in corpus callosum related measurements between the migraine and controls.

There are many studies examining different brain structures conducted to investigate the pathophysiology of migraine. In the study performed with Voxel-Based morphometry, it was stated that there was no significant difference between migraine patients and controls in terms of white matter volume or concentration (Matharu *et al., 2003*). In a 9-year prospective study to investigate the relationship between migraine and brain structure and function, it was shown that only female patients had higher prevalence and deeper white matter hyperintensities than controls, not male patients (Palm-Meinders *et al., 2012*). In a study evaluating the risk of infarction in certain regions of the brain, it was found that infarctions in the cerebellum were more common in female with migraine with aura than controls, with no

difference between men with migraine and controls (Scher *et al., 2009*). One meta-analysis demonstrated that migraine patients were more likely having white matter abnormalities on MRI than controls (Swartz and Kern, 2004).

Recent studies have shown a direct relationship between chronic strokes and white matter lesions in patients with migraine (Over *et al., 2007*). Under the age of 40, migrainous infarcts are estimated at 1.4-14%. According to studies, the incidence of stroke due to brain infarctions increases in migraine (Chang *et al., 1999*). Additionally, sex differences seem to play an important role because progression of deep white matter hyperintensities was only found in women. The relationship between migraine and ischemic stroke has been known for many years, based on the results of many studies on this subject, and migraine has been accepted as a risk factor for stroke (Buring *et al., 1995; Longstreth et al., 1996*). The brain damage seen in patients with migraine is related to the frequency of migraine attacks and the duration of the disease, and it is claimed that this damage occurs about fifteen years after the onset of the disease (Schmitz *et al., 2008*).

Some limitations of this study should be considered when interpreting our findings. For example, we did not divide patients with migraine into subgroups and our sample was relatively small. Knowing the white matter structure in migraine patients, determining its prevalence, and its correlation with the severity, type and duration of migraine can give an idea to clinicians. In our study, the capsula interna structures were found to be more enlarged in patients with migraine than in normal individuals. Does this enlargement have clinical and diagnostic significance in patients with migraine? Can this enlargement be explained by inflammatory mechanisms? Actually, this is the main question to be answered. We could not find any study in the literature that could answer this question. Additional research is needed to describe in more detail the association between migraine and white matter structures.

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Conflict of Interest: None

The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee.

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Research Article

HOW ARE CARDIAC FUNCTIONS ALTERED IN PEDIATRIC PATIENTS RECEIVING ORAL IRON SUPPLEMENTATION DUE TO ANEMIA?

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ABSTRACT

Objective: This study aims to investigate whether oral iron supplementation improves structural and functional alterations by evaluating the echocardiography and electrocardiography findings of the pediatric patients with iron deficiency anemia.

Methods: This is a prospective review of 30 pediatric patients who were diagnosed with iron deficiency anemia and received 12-week-long iron supplementation at the study center. All children underwent hematological workup, detailed echocardiography examination and electrocardiography evaluation at the time of diagnosis for iron deficiency anemia and the end of 12-week-long iron treatment.

Results: After 12-week-long iron treatment, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, red blood cell count, Mentzer index, serum iron, serum iron binding capacity, serum ferritin and transferrin increased significantly (p=0.001, p=0.001, p=0.001, p=0.036, p=0.001, p=0.002, p=0.001 and p=0.001 respectively). Pulse rate, respiration rate, and diastolic left ventricle wall thickness decreased significantly while tricuspid E and A wave velocities increased significantly at the end of iron supplementation (p=0.004, p=0.033, p=0.009, p=0.003 and p=0.021 respectively). As for the tissue Doppler echocardiography findings, only interventricular septum isovolumetric contraction time decreased significantly after iron treatment (p=0.002). The PR interval shortened significantly, p dispersion decreased significantly and T-peak to T-end interval shortened significantly following iron supplementation (respectively p=0.013, p=0.033 and p=0.029).

Conclusion: Oral iron supplementation seems to contribute to the reversal of cardiac remodeling, elimination of compensatory hemodynamic mechanisms and inhibition of sympathetic activation within cardiac tissues.

Keywords: *Child, Echocardiography, Electrocardiography, Iron Deficiency Anemia*

1. INTRODUCTION

Anemia is defined as a decrease in the number of red blood cells and hemoglobin (Hb) concentration in blood (DeLoughery, 2017). Iron deficiency is the most common cause of anemia which occurs due to the lack of iron intake and/or depletion of iron stores in the body (Camaschella, 2015). Being the most abundantly found trace element in human body, iron is required to produce Hb and transport oxygen (Subramaniam & Girish, 2015). Iron deficiency is a global health problem and a common medical condition which is frequently encountered in daily clinical practice (Lopez et al., 2016). It has been reported that iron deficiency affects 30–40% of pre-school children in industrialized countries, and nearly all children at pre-school age in developing countries (Wang, 2016).

World Health Organization estimated that nearly half of the children worldwide aged 0–5 years between 1993 and 2005 had anemia (McLean et al., 2009). In order to make a diagnosis of iron deficiency anemia in children, lowered Hb concentrations, ferritin levels and/or reticulocyte counts should be measured depending on age, sex, pregnancy, altitude, and smoking (Powers & Buchanan, 2014). The physiologic response to anemia is a compensatory elevation in cardiac output by increasing blood volume, preload, heart rate, and stroke volume. These alterations can be identified with the augmentation in sympathetic nervous activity (Yokusoglu et al., 2007).

The symptoms and signs of iron deficiency anemia that result from hypoxic functioning include fatigue, breathlessness at rest, exertional dyspnea, vertigo, syncope, headache, tachycardia, and cardiac systolic flow murmurs (Anand & Gupta, 2018). In severe cases, patients might have dyspnea at rest, angina pectoris, and hemodynamic instability (Anand & Gupta, 2018; Yokusoglu et al., 2007). Accordingly, it has been speculated that ongoing acceleration in sympathetic nervous activity of the heart might even lead to cardiomyopathy (Hegde et al., 2006).

This study aims to investigate whether oral iron supplementation improves structural and functional alterations by evaluating the echocardiography and electrocardiography findings of the pediatric patients with iron deficiency anemia.

2. METHODS

This is a prospective review of 30 pediatric patients who were diagnosed with iron deficiency anemia and received 12-week-long iron supplementation at the Department of Pediatrics in Afyonkarahisar Health Sciences University Hospital between January 2019 and March 2019. The study cohort consisted of 9 boys (30%) and 21 girls (70%) and their mean age was 14.2 ± 3.1 years (range: 7-18 years). This study was approved by the Institutional Review Board and Ethical Committee of the study center.

The inclusion criteria were being a pediatric patient aged less than 19 years and having a diagnosis of iron deficiency anemia. The pediatric patients with other types of anemia, other hematologic diseases, infections, cardiac diseases, renal diseases, gastrointestinal system diseases, endocrinopathies and malignancies were excluded. The children who used drugs impairing iron absorption, pediatric patients who had iron treatment or anti-inflammatory treatment previously and children who failed to get regular iron treatment were excluded.

Data related with age, height, body weight, pulse rate, respiration rate, peripheral capillary oxygen saturation, systolic and diastolic blood pressures were recorded. Body mass index was computed as follows: Body mass index= Weight (kg) / Height^2 (m^2)

2.1. Laboratory Studies

Complete blood count was made, serum concentrations of iron, ferritin and transferrrin were measured and total iron binding capacity (TIBC) was specified at the time of diagnosis for iron deficiency anemia and the end of 12-week-long iron treatment.

Complete blood count was made by using daily calibrated hemocytometer (LH-780, Beckman Coulter, USA). Measurements of Hb, mean erythrocyte volume (MCV), mean corpuscular Hb concentration (MCHC), red blood cell distribution width (RDW), red blood cell count, white blood cell count and platelet count were made. In order to distinguish iron deficiency anemia from beta-thalassemia, Mentzer index was calculated as MCV per red blood cell count.

A calibrated Dimension RxL Max Integrated Chemistry System was used to measure serum concentrations of iron, ferritin and transferrin (Siemens Healthineers, Erlangen, Germany). TIBC was directly determined by IL TestTM TIBC Sample Pretreatment Kit (Instrumentation Laboratory SpA, Milano, Italy; Cat. No 181730–00).

2.2. Echocardiography Examination

Echocardiography examination was performed by means of a commercially available machine with 3-5 MHz transducers (Vivid I, GE Healthcare, Chicago, IL, USA). The patients were made to rest for 5 minutes before the measurements and breathe slowly throughout the procedure. Recordings were performed with subjects in the supine or left lateral positions. All children underwent M-mode, two-dimensional, color, Doppler-continuous, and pulse wave-echocardiography examination at the time of diagnosis and the end of iron treatment. The mean values were recorded by averaging the results of three consecutive measurements.

M-mode tracings were supplied at the level of the tips of mitral leaflets in the parasternal long-axis position, and measurements of the left ventricular end-systolic and end-diastolic dimension were performed according to the recommendations of the American Society of Echocardiography (Nagueh et al., 2016). Left ventricular end-systolic and end-diastolic dimensions as well as aorta and interventricular septum dimensions were measured from the parasternal long-axis window. Left ventricular ejection fraction and fractional shortening were provided using Teichholtz in M-mode echocardiography.

Tissue Doppler measurements were performed to designate the myocardial velocities during systole, early diastole and late diastole. The isovolumic contraction time (IVCT) was the time period between the end of the myocardial wave during late diastole (Am) and the beginning of the myocardial wave during systole (Sm). The isovolumic relaxation time (IVRT) was the time period between the end of the Sm wave and the beginning of the myocardial wave during early diastole (Em). Ejection time was the duration of ventricular outflow. Myocardial performance index (MPI) was the sum of IVCT and IVRT values, divided by ejection time.

2.3. Electrocardiography Evaluation

All children had electrocardiography (ECG) at the time of diagnosis and the end of iron treatment. The 12-lead ECG was saved at a paper speed of 50 mm/hour and gain of 10 mm/mV (Cardiofax V; Nihon Kohden Corporation, Tokyo, Japan) in the supine position. The patient was allowed to breathe spontaneously, but speaking was not permitted during the recording. All measurements were done manually by using magnifying glass and the mean values were recorded by averaging the results of three consecutive measurements.

The electrical axis of the heart in the frontal plane was represented by the QRS-axis. P-wave duration was measured in lead II, from the beginning to the end of P-wave. PR interval was also measured in lead II, from the beginning of P-wave to the beginning of R-wave. QRS complex duration was measured in lead V, from the beginning of Q wave to the end of the S wave. The measurement of the QT interval was started from the onset of the QRS complex until the end of the T-wave. Corrected QT interval was specified by Bazett's formula (Bazett, 1920). P-wave dispersion was calculated by subtracting minimum P-wave duration from maximum P-wave duration. QRS dispersion was the difference between maximum and minimum QRS complex durations. Corrected QT dispersion was found by subtracting minimum corrected QT interval from maximum corrected QT interval.

2.4. Statistical Analysis

Collected data were analyzed by Statistical package for Social Sciences version 25.0 (SPSS Inc., SPSS IBM, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (range: minimum-maximum) and categorical variables were denoted as numbers or percentages. Kolmogorov-Smirnov test was used to test the distribution of variables. Paired samples t-test and Wilcoxon test were used for the comparisons. Two-tailed p values less than 0.05 were accepted to be statistically significant.

3. RESULTS

Table 1 shows the clinical characteristics of the patients before and after 12-week-long iron administration. The height and weight of the patients increased significantly but the pulse rate and respiration rate decreased significantly following iron supplementation ($p=0.002$, $p=0.014$, $p=0.004$ and $p=0.033$ respectively).

Table 1. Clinical characteristics before and after iron supplementation

	Before treatment (n=30)	After treatment (n=30)	p
Height (m)	1.56±0.16	1.58±0.15	0.002*
Weight (kg)	49.0±15.7	50.3±15.2	0.014*
Body mass index (kg/m^2)	19.67±4.13	19.77±3.90	0.631
Arterial oxygen saturation (%)	97.0±2.0	97.5±1.4	0.340
Pulse rate (beats/min)	97.6±17.5	86.6±10.3	0.004*
Respiration rate (breaths/min)	23.8±2.5	22.4±2.1	0.033*
Systolic blood pressure (mmHg)	108.3±9.9	105.3±8.5	0.177
Diastolic blood pressure (mmHg)	69.3±8.7	66.3±6.7	0.163

*p<0.05 was accepted to be statistically significant.

After 12-week-long iron treatment, hemoglobin, MCV, MCHC, red blood cell count, Mentzer index, serum iron, TIBC, ferritin and transferrin values increased significantly (p=0.001, p=0.001, p=0.001, p=0.036, p=0.001, p=0.002, p=0.001 and p=0.001 respectively). On the contrary, RDW, Mentzer index and platelet count decreased significantly (p=0.001, p=0.004 and p=0.006 respectively) (Table 2).

Table 2. Hematological parameters before and after iron supplementation

	Before treatment (n=30)	After treatment (n=30)	p
Hemoglobin (g/dl)	9.97±1.28	12.7±1.54	0.001*
Mean corpuscular volume (fl)	72.45±7.83	82.15±8.25	0.001*
Mean corpuscular hemoglobin concentration (g/dl)	29.31±1.67	31.5±1.42	0.001*
Red blood cell count (x10^3/mm^3)	4645.0±549.5	4836.7±434.2	0.036*
Red cell distribution width (%)	17.28±2.56	15.26±2.60	0.001*
Mentzer index (MCV/RBC count)	15.84±2.69	17.18±2.63	0.004*
White blood cell count (/mm3)	6942.3±2292.5	7334.7±2179.6	0.277
Neutrophil/Lymphocyte	2.32±1.13	2.17±1.10	0.430
Platelet count (x10^3/mm3)	339.570±105.827	302.370±62.924	0.006*
Serum iron (mg/dl)	27.50±9.33	66.58±38.85	0.001*
Serum iron binding capacity (µg/dl)	419.64±60.32	383.73±53.33	0.002*
Serum ferritin (ng/ml)	6.42±5.24	23.62±24.34	0.001*
Serum transferrin	6.76±2.71	17.69±10.0	0.001*

*p<0.05 was accepted to be statistically significant

Table 3. Echocardiography findings before and after iron supplementation

	Before treatment (n=30)	After treatment (n=30)	p
Systolic interventricular septum thickness (mm)	1.58±0.79	1.40±0.33	0.243
Diastolic interventricular septum thickness (mm)	0.97±0.19	0.95±0.20	0.655
Systolic left ventricle internal diameter (mm)	2.30±0.52	2.43±0.58	0.257
Systolic left ventricle posterior wall thickness (mm)	1.66±0.35	1.69±0.35	0.658
Diastolic left ventricle internal diameter (mm)	4.12±0.61	4.24±0.56	0.208
Diastolic left ventricle posterior wall thickness (mm)	1.12±0.21	0.99±0.23	0.009*
Systolic volume (mm^3)	53.83±20.92	59.73±18.11	0.103
End-diastolic volume (mm^3)	75.23±25.60	81.63±24.66	0.112
Fractional shortening (%)	44.40±7.61	44.43±8.47	0.985
Ejection fraction (%)	76.50±8.67	75.13±8.88	0.492
Systolic left ventricle mass (g)	138.45±45.38	146.66±62.0	0.254
Systolic left ventricle mass index (g/m)	98.78±28.16	100.08±39.12	0.860
Diastolic left ventricle mass (g)	163.73±58.98	157.46±73.26	0.462
Diastolic left ventricle mass index (g/m)	116.68±34.30	106.71±37.97	0.241
Systolic left ventricle outflow tract (m/sec)	1.32±0.20	1.33±0.24	0.597
Diastolic left ventricle outflow tract (m/sec)	1.20±0.55	1.25±0.68	0.157
Ascending aorta (mm)	1.39±0.20	1.34±0.27	0.243
Descending aorta (mm)	1.44±0.17	1.46±0.38	0.761
Mitral E velocity	0.99±0.21	0.99±0.23	0.919
Mitral A velocity	0.67±0.16	0.63±0.17	0.379
Mitral E/A	1.50±0.28	1.85±1.79	0.297
Tricuspid E velocity	0.67±0.15	0.81±0.25	0.003*
Tricuspid A velocity	0.43±0.10	0.50±0.16	0.021*
Tricuspid E/A	1.62±0.47	1.66±0.4	0.599
Deceleration time (sec)	83.40±30.38	85.93±15.54	0.669
Tricuspid annular plane systolic excursion	3.86±0.81	3.92±0.79	0.618
Mitral annular plane systolic excursion	3.19±0.63	3.36±0.59	0.188

*$p<0.05$ was accepted to be statistically significant.

Table 3 summarizes the echocardiography findings of the patients before and after iron treatment. Diastolic left ventricle wall thickness decreased significantly while tricuspid E and A wave velocities increased significantly at the end of iron supplementation (p=0.009, p=0.003 and p=0.021 respectively). As for the tissue Doppler echocardiography findings, only interventricular septum IVCT decreased significantly after 12-week-long iron administration (p=0.002) (Table 4).

Table 4. Tissue doppler echocardiography findings before and after iron supplementation

	Before treatment (n=30)	After treatment (n=30)	p
Left ventricle			
Em	0.1993±0.0378	0.2067±0.0706	0.543
Am	0.0830±0.0177	0.0887±0.0391	0.491
Sm	0.1140±0.0275	0.1360±0.1264	0.359
Interventricular relaxation time	62.77±11.72	57.90±9.38	0.055
Interventricular contraction time	60.63±8.38	58.97±11.34	0.365
Myocardial performance index	48.42±7.99	45.74±7.93	0.230
Interventricular septum			
Em	0.1403±0.0281	0.1417±0.0232	0.830
Am	0.1027±0.1512	0.0723±0.0148	0.288
Sm	0.0857±0.0119	0.0850±.0128	0.827
Interventricular relaxation time	60.07±13.19	63.0±10.48	0.290
Interventricular contraction time	67.03±10.61	58.13±8.9	0.002*
Myocardial performance index	50.52±10.78	47.76±7.67	0.227
Right ventricle			
Em	0.1747±0.0386	0.1720±0.0295	0.677
Am	0.1230±0.0295	0.1210±0.0264	0.774
Sm	0.1507±0.0393	0.1483±0.0231	0.798
Interventricular relaxation time	61.07±14.28	58.47±14.16	0.396
Interventricular contraction time	64.03±13.62	63.47±1.50	0.832
Myocardial performance index	52.45±10.95	49.41±11.40	0.218

*p<0.05 was accepted to be statistically significant.

Table 5 displays the ECG findings of the patients before and after 12-week-long iron treatment. The PR interval shortened significantly, p dispersion decreased significantly and T-peak to T-end interval shortened significantly following iron supplementation (respectively p=0.013, p=0.033 and p=0.029).

Table 5. Electrocardiography findings before and after iron supplementation

	Before treatment (n=30)	After treatment (n=30)	p
Heart rate (beats/min)	86.57±16.49	77.47±14.21	0.480
Heart axis (°)	48.50±25.08	34.27±19.38	0.050
P wave (sec)	110.67±31.40	94.0±20.44	0.928
PR interval (sec)	188.0±38.09	171.33±26.09	0.013*
QRS interval (sec)	108.0±27.04	79.33±24.34	0.118
QT interval (sec)	364.67±32.24	377.33±35.12	0.612
Corrected QT interval (sec)	436.67±47.74	424.73±32.31	0.682
P dispersion	62.66±22.73	40.33±20.40	0.033*
QRS dispersion	64.67±44.47	35.33±15.25	0.340
Corrected QT dispersion	62.07±30.18	66.10±22.95	0.337
T-peak to T-end interval (sec)	80.33±23.85	56.33±22.97	0.029*

*p<0.05 was accepted to be statistically significant.

4. DISCUSSION

Anemia is a consequence of long term iron deficiency which leads to compensatory changes in circulation (Hegde et al., 2006). At the beginning of iron deficiency anemia, cardiac output increases and circulation is enhanced. Long-lasting hyperdynamic circulation increases the load on the heart and causes myocardial ischemia and hypoxia (Hegde et al., 2006; Jankowska & Ponikowski, 2010).

In case iron deficiency anemia is not treated, myocardium cannot overcome the high load and, thus, ventricular walls are thickened and ventricles become hypertrophic and dilated (Cohen-Solal et al., 2014; Jankowska & Ponikowski, 2010). Remodeling of cardiac muscles is a long term complication of iron deficiency anemia. This complication usually ends up with the development of mechanisms for hemodynamic compensation (Cohen-Solal et al., 2014; Jankowska & Ponikowski, 2010). These mechanisms include (i) reduced afterload due to a decrease in systemic vascular resistance, (ii) increased preload due to acceleration in venous return and (iii) increased left ventricular function triggered by increased sympathetic activity and inotropic factors. In addition, heart rate is increased in anemic patients due to hypoxia-stimulated chemoreceptors (Cohen-Solal et al., 2014; Hegde et al., 2006; Jankowska & Ponikowski, 2010).

It has been reported that left ventricle volume index is decreased, cardiac index is reduced, left ventricle end diastolic pressure is increased, left atrium is enlarged and left ventricle diastolic filling parameters are extended significantly in patients with iron deficiency anemia (Simsek et al., 2010). A Turkish study also pointed out significantly elevated myocardial performance indices of left ventricle, right ventricle and interventricular septum in infants with iron deficiency anemia. Additionally, ejection time was significantly lowered in left ventricle, right ventricle and interventricular septum in these infants (Alioglu et al., 2013).

Anemia may cause abnormalities in sympathetic nerve activity due to the perception of hypoxia in the carotid body (Kobak et al., 2019). Turner et al. established a model of iron deficiency in mice and found that the increase in cardiac output triggered the activation of sympathetic nervous system within the heart and ultimately resulted in left ventricular hypertrophy (Turner et al., 2002). Similarly, Yokusoglu et al. demonstrated the impairment in autonomic nervous system of the heart in patients with iron deficiency anemia (Yokusoglu et al., 2007). It has been hypothesized that hypoxia inhibits mitochondrial respiratory chain or potassium channels and, thus, intracellular calcium accumulates. The accumulation of calcium in myocardial cells subsequently impairs the myocardial functions (Kobak et al., 2019; Turner et al., 2002; Yokusoglu et al., 2007).

The PR interval is defined as the time interval from the onset of the p wave to the start of the QRS complex. This interval reflects the conduction through the atrioventricular node (Schumacher et al., 2017; Simsek et al., 2010). P wave dispersion is described as the difference between the widest and the narrowest p wave duration recorded from all of the ECG leads. P wave dispersion has been addressed as a marker for atrial remodeling and predictor for atrial fibrillation (Okutucu et al., 2016; Pérez-Riera et al., 2016). Increased p wave dispersion designates the delay in intra-atrial and inter-atrial conduction time which can be attributed to the lack of a well-coordinated conduction system within the atrial muscles (Okutucu et al., 2016; Pérez-Riera et al., 2016; Schumacher et al., 2017). T-peak to T-end interval is depicted as an index for transmural dispersion of ventricular repolarization. Any prolongation in this interval has been considered as a risk factor for ventricular tachyarrhythmia (Dinshaw et al., 2018; Tse et al., 2018). A Turkish study determined that heart rate was significantly higher and maximum P wave duration and P wave dispersion were

significantly longer in patients with iron deficiency anemia when compared to healthy controls (Simsek et al., 2010).

Previously published studies have revealed the effects of iron treatment on exercise capacity and life quality of the adults with heart failure (Anker et al., 2009; Ponikowski et al., 2015; van Veldhuisen et al., 2017). In the FAIR-HF study, intravenous ferric carboxymaltose treatment significantly improved clinical symptoms, functional capacity and quality of life in patients with heart failure (Anker et al., 2009). The CONFIRM-HF study attested that intravenous iron treatment over a 1-year period helped to maintain the improvements in functional capacity, clinical symptoms, and quality of life in heart failure patients (Ponikowski et al., 2015). The EFFECT-HF study designated the beneficial effects of intravenous iron supplementation on mixed venous oxygen tension of the adults with heart failure (van Veldhuisen et al., 2017). A meta-analysis confirmed that intravenous iron supplementation is a safe and efficient treatment approach which is associated with a recovery in quality of life parameters, reduction in hospitalizations, and prolongation of six minute walk distance (Avni et al., 2012).

To the best of our knowledge, this is the first study to investigate how the echocardiography and electrocardiography findings are altered in pediatric patients who received oral iron supplementation for the treatment of iron deficiency anemia. In this study, diastolic left ventricle wall thickness decreased significantly, tricuspid E and A wave velocities increased and interventricular septum IVCT decreased significantly in children who received oral iron supplementation for 12 weeks. Moreover, The PR interval shortened significantly, p dispersion decreased significantly and T-peak to T-end interval shortened significantly following iron supplementation.

5. LIMITATIONS OF THE STUDY

The power of the present study is limited by several factors such as relatively small cohort size, relatively short follow up time and absence of a healthy control group.

6. CONCLUSION

The findings of the present study imply that oral iron supplementation contributes to the reversal of cardiac remodeling, elimination of compensatory hemodynamic mechanisms and inhibition of sympathetic activation within cardiac tissues. Further research is warranted to clarify the effects of iron supplementation on cardiac functions of the pediatric patients diagnosed with iron deficiency anemia.

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